Parasitic infection and anaemia during pregnancy in Sierra Leone

Harriet Torlesse

Thesis presented for the degree of Doctor of Philosophy
to the University of Glasgow

WHO Collaborating Centre for Soil-Transmitted Helminthiases
Institute of Biomedical and Life Sciences
University of Glasgow

February 1999

Summary

Anaemia in pregnancy is widely recognised as a risk factor for maternal mortality and morbidity. In developing countries, parasitic infections and anaemia frequently coincide during pregnancy. Intestinal nematode infections may contribute to anaemia by causing blood loss and by affecting the supply of nutrients for erythropoiesis. The role of intestinal nematode infections in the aetiology of iron deficiency and anaemia in pregnant women was investigated in peri-urban and rural areas of Western Sierra Leone. A randomised placebo-controlled field trial was carried out to evaluate the efficacy of a single course of albendazole (400 mg) and daily iron-folate supplements (36 g iron and 5 mg folate), administered after the first trimester, as control interventions for intestinal nematode infections and anaemia during pregnancy. The study population comprised 184 pregnant women aged 15-40 years, gravidity 1-11. Fifty-nine women were lost to follow-up during pregnancy, including eight subjects who were withdrawn from the study due to severe anaemia.

At baseline, in the first trimester of pregnancy, the prevalence (and geometric mean intensity) of intestinal nematodes was as follows: *Ascaris lumbricoides* 21.1% (267 eggs per gram); *Necator americanus* 66.5% (191 epg); and *Trichuris trichiura* 71.9% (93 epg). Chronic undernutrition (height <150 cm) and chronic energy deficiency (body mass index <18.5 kg/m^2) was found in 5.4% and 8.2% of women respectively. Anaemia (Hb <110 g/l) was diagnosed in 58.7% of women and associated with iron deficiency (serum ferritin <20 μg/l) in 21.2% of women. Iron deficiency is likely to have a dietary basis in these women. The dietary iron intake was predominantly non-haem, and was estimated to supply less than 1 g of absorbable iron daily. High fertility and closely spaced pregnancies placed additional stress on iron stores.

Albendazole was highly effective in eliminating infection and reducing the baseline egg counts of *A. lumbricoides* and *N. americanus*. Albendazole was less effective in clearing *T. trichiura* infections but reduced the geometric mean egg counts considerably. The protection afforded by a single dose of albendazole, in terms of reducing the intensities below the level associated with morbidity, extended for the duration of pregnancy for all intestinal nematode infections.

Intervention with daily iron-folate supplements and/or albendazole was not effective in improving Hb or SF concentration or in decreasing the prevalence of iron deficiency, anaemia or iron-deficiency anaemia between baseline and the third trimester. However, these values did not change significantly in the group of women who received both iron-folate supplements and albendazole. After controlling for baseline Hb concentration and season, the mean benefit of

iron-folate supplements and albendazole, relative to their respective controls, on the change Hb concentration between baseline and the third trimester was 13.7 g/l Hb and 6.6 g/l Hb respectively. The effects of iron-folate supplements and albendazole treatment were additive. Women who received iron-folate supplements also experienced less decline in baseline SF concentration. Albendazole did not appear to influence the change in baseline SF concentration, which may indicate that iron savings associated with treatment were not sufficient to allow net storage of iron.

Intestinal nematode infections do not appear to pose a threat to maternal body size and growth during pregnancy in these communities. This may reflect the mild degree of underlying chronic undernutrition and energy deficiency in these women. However, it is feasible that the impact of infection on anthropometric measures of nutritional status may have been concealed by physiological or behavioural adaptations to infection.

Measures of weight, length, gestational age at delivery and Hb concentration were obtained from 105 neonates. Exploratory analyses indicated that neonatal weight was inversely related to the decline in baseline maternal Hb concentration during pregnancy, after controlling for maternal and background factors. These findings justify further investigations to establish the role of maternal anaemia in the aetiology of low birth-weight in these communities. None of the stool samples obtained from 101 neonates was found to contain intestinal nematode eggs. However, vertical transmission of *A. lumbricoides* infection is not precluded in this setting, as the diagnostic technique did not detect prepatent infections.

Most women were seropositive to past markers of toxoplasmosis (76.0%) and cytomegalovirus (82.7%). The high carrier rate for hepatitis B virus (HBV) (11.3%) indicates that this infection is highly endemic among the antenatal population, and integration of the HBV vaccine into the Expanded Programme on Immunisation in Sierra Leone is recommended. None of the pregnant women was found to be infected with human immunodeficiency virus, but the relatively high prevalence of past or present syphilis infection (7.8%) indicates a high incidence of risky sexual behaviour. Adequate control of maternal iron deficiency and anaemia during pregnancy is essential in Western Sierra Leone, given that this setting is highly infectious for these deleterious microparasitic infections. If the iron or anaemia status of women is neglected during pregnancy, severe anaemia may be treated with parenteral iron or blood transfusion. Both forms of treatment carry a risk of these microparasitic infections, either from contaminated syringes or via transfusion of blood products from an infected donor.

Ethnographic research among the Temne ethic group highlighted several traditional beliefs and practices that may affect compliance with interventions to control intestinal nematode infections and anaemia during pregnancy. These include the concealment of pregnancy, which delays access to Western antenatal clinics, and low confidence in the efficacy and safety of Western pharmaceuticals.

The findings of this study indicate that anthelminthic treatment should be included in strategies to control maternal anaemia in Western Sierra Leone. It is recommended that pregnant women routinely receive a single course of anthelminthics after the first trimester, alongside daily iron-folate supplements, to minimise the decline in maternal Hb concentration during pregnancy. These interventions could be implemented through the existing primary health care system, although the use of traditional birth attendants should be investigated as a means of improving compliance and coverage. The implications of these findings for public health policy in other antenatal populations will depend on the local epidemiology of intestinal nematode infections and on the extent of underlying maternal iron deficiency and anaemia.

Declaration

I declare that the work presented in this thesis was carried out by myself unless otherwise cited or acknowledged. It is of my own composition and has not, in whole or in part, been presented for any other degree.

[redacted]

Harriet Torlesse

5 February, 1999

Acknowledgments

I am grateful to the great number of individuals and institutions who have contributed to the research presented in this thesis. First and foremost, I wish to thank my supervisors, Prof. D.W.T. Crompton and Dr. M. Hodges for their constructive advice, support and encouragement. Many thanks also to Dr. M. Kamara (Princess Christian Maternity Hospital, Freetown, Sierra Leone) and I.M. Wurie (Ramsy Medical Laboratories, Freetown, Sierra Leone) for their collaboration. I am grateful to Vice-Principal A.C. Allison whose financial support enabled me to begin this research and to the Institute of Biomedical and Life Sciences for the award of a Research Studentship.

I am indebted to all members of the field research team, M.M. Koroma, M.B. Juana and E. Nyorkor, who worked diligently, often under difficult field conditions. I also thank the staff members of St. Andrew's Clinics for Children for their logistical support. I am grateful for the assistance of Paramount Chief Bai Shebura Kumkanda II, and also the chiefs, headmen, medical staff, traditional birth attendants and motivators of Kaffu-Bollom Chiefdom, Lokomasama Chiefdom, Portee, Kuntola and Goderich. Many thanks to Mr. I. McCluney and the British High Commission (Freetown, Sierra Leone), who kindly donated £1000 to provide the study clinics with equipment.

The assistance of the following institutions is acknowledged: the serum ferritin analysis was conducted at Stobhill Hospital (Glasgow, UK); the Hemocue Photometer was validated against the QBC Centrifugal Hematology System by B. Ginorlei at the Joint Health Facility, British High Commission (Freetown, Sierra Leone); the serological assays for syphilis and cytomegalovirus were performed by laboratory technicians at Ramsy Medical Laboratories (Freetown, Sierra Leone), who also assisted in the assays for hepatitis B virus and toxoplasmosis; the serological assays for human immunodeficiency virus were conducted at the HIV Laboratory, Connaught Hospital (Freetown, Sierra Leone). Many thanks also to: Dr. J. Reilly (Yorkhill Hospital, Glasgow) and Prof. M.E. Lean (Royal Infirmary, Glasgow) for advice on the analysis of dietary intake and anthropometric data; Dr. J. Opala (Freetown, Sierra Leone) for advice on ethnographic methodology; and Dr. J. Currell, Dr. A. Bowman and Dr. G. Ruxton (Glasgow University) for advice on statistical analysis.

Final thanks go to all my friends and colleagues in the UK, Sierra Leone and elsewhere. I am eternally grateful to my family and to Tom, Gabby, Ross, Sam, Fatorma and John for their support, friendship and tolerance at all times.

The work described in this thesis has been published in scientific journals and presented at conferences as oral papers or posters as indicated below:

Publications

Torlesse, H., Crompton, D.W.T., Savioli, L. & Albonico, M. (1995). Hookworm disease and pregnancy in tropical Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 595.

Torlesse, H., Wurie, I.M. & Hodges, M. (1997). The use of immunochromatography test cards in the diagnosis of hepatitis B surface antigen (HBsAg) among pregnant women in West Africa. *British Journal of Biomedical Science* **54**, 256-259.

Torlesse, H., Wurie, I.M. & Hodges, M. (1997). Diagnosis of hepatitis B surface antigen (HBsAg) among pregnant women in Sierra Leone: the use of immunochromatography test cards. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 506-507.

Torlesse, H., Crompton, D.W.T. & Hodges, M. (1998). Intestinal nematode infections among pregnant women in Sierra Leone: the efficacy of albendazole treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 477.

Oral papers

Torlesse, H. (1996). Hookworm anaemia during pregnancy in Sierra Leone: an interim report. Paper presented at the Sierra Leone Medical and Dental Association 23rd Annual National Congress, 8 November, 1996, Freetown, Sierra Leone.

Torlesse, H. (1996). Toxoplasmosis antibody prevalence in pregnancy in Sierra Leone and it's significance. Paper presented at the Sierra Leone Medical and Dental Association 23rd Annual National Congress, 7 November, 1996, Freetown, Sierra Leone.

Posters

Torlesse, H., Crompton, D.W.T., Savioli, L. & Albonico, M. (1995). Hookworm disease and pregnancy in tropical Africa. Poster presented at the Royal Society of Tropical Medicine and Hygiene Non-Thematic Meeting, May, 1995.

Torlesse, H., Crompton, D.W.T., Savioli, L. & Albonico, M. (1995). Hookworm disease and pregnancy outcome. Poster presented at the British Society for Parasitology Spring Meeting, 10-12 April, 1995, Edinburgh.

Torlesse, H., Wurie, I.M. & Hodges, M. (1997). Diagnosis of hepatitis B surface antigen (HBsAg) among pregnant women in Sierra Leone: the use of immunochromatography test cards. Poster presented at the Royal Society of Tropical Medicine and Hygiene Non-Thematic Meeting, 7 May, 1997.

Torlesse, H., Crompton, D.W.T. & Hodges, M. (1998). Intestinal nematode infections among pregnant women in Sierra Leone: the efficacy of albendazole treatment. Poster presented at the Royal Society of Tropical Medicine and Hygiene Non-Thematic Meeting, 13 May, 1998.

Contents

List of tables

List of figures

Chapter 1

Parasitic infections and anaemia during pregnancy

1.1 INTRODUCTION

Pregnancy is probably the most hazardous normal physiological state for women. Alterations in maternal immunity may increase the susceptibility of pregnant women to parasitic infections and disease (Brabin, 1985; Weinberg, 1987). Pregnant women are also more vulnerable to nutritional disorders, as the growth of maternal and fetal tissues elevates the requirements for energy and nutrients (Hytten & Leitch, 1971). The risks of parasitic infection and undernutrition are particularly great in developing countries, where maternal ill-health is aggravated by frequent pregnancies, dietary deficiencies and inadequate maternal health services. In these countries, parasitic infections and anaemia frequently coincide during pregnancy.

Many parasitic infections have been shown to influence the outcome of pregnancy (Brabin & Brabin, 1992). Parasites that inhabit the human intestinal tract are in a good physiological position to interfere with host nutrition (Crompton, 1986). Intestinal parasitic infections may impair host nutritional status by affecting one or more facets of nutritional physiology, including the intake, intestinal absorption, metabolism, excretion or loss of nutrients (Stephenson, 1987a). Several of these infections are implicated in the aetiology of iron deficiency, anaemia and poor growth. The extent to which they affect maternal health is poorly understood, although the association between poor maternal nutritional status and adverse pregnancy outcomes is well established (Kramer, 1987; de Onis *et al.*, 1998b).

Parasites are grouped into two categories according to a number of key characteristics. Macroparasites, which include helminths and arthropods, are comparatively larger than microparasites and have a longer generation time (Anderson & May, 1991b). Macroparasitic infections tend to be chronic and persistent in humans, as full protective immunity is not developed following initial exposure (Anderson & May, 1991b). In endemic areas, continual reinfection with macroparasites is the norm. Microparasites include the majority of bacterial, protozoal and fungal parasites. Most microparasitic infections are transient, as the host is usually able to mount an effective immune response to invasion (Anderson & May, 1991c).

The phylum Nematoda includes the most important human intestinal macroparasites in terms of world-wide prevalence and potential for inducing debilitating disease. Approximately 60 nematode species have been found in the human alimentary tract and ducts of its associated organs (Coombs & Crompton, 1991). *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms, *Ancylostoma duodenale* and *Necator americanus*, collectively infect over one billion of the world's population (Montresor *et al.*, 1998). These infections prevail in regions of the world where undernutrition is a major public health problem. Their life histories, epidemiology and population biology are well described (Bundy & Cooper, 1989; Crompton *et al.*, 1989; Schad & Warren, 1990; Anderson & May, 1991a).

This review examines how intestinal nematode infections may contribute to iron deficiency, anaemia and subnormal growth in pregnant women and their fetuses. The significance of microparasitic infections in relation to maternal iron deficiency and anaemia is also discussed. It considers the complications that may arise if intestinal nematodes migrate within the mother during the course of the pregnancy. Alternative approaches to the control of maternal iron deficiency and anaemia in areas endemic for intestinal nematode infections are then outlined. Finally, the conceptual framework for the work presented in this thesis is described.

1.2 IRON DEFICIENCY AND ANAEMIA

1.2.1 Maternal iron deficiency and anaemia

Anaemia is a disorder in which the blood haemoglobin concentration is lower than normal. It has several causes, which may occur in isolation or simultaneously. In Africa, where 52% of pregnant women are estimated to be anaemic (WHO, 1992a), the most common causes include iron deficiency, folate deficiency, malaria, acquired immune deficiency syndrome (AIDS) and sickle cell disease (WHO, 1989).

Iron deficiency is the most prevalent human nutritional deficiency and the predominant cause of anaemia in pregnant women (ACC/SCN, 1997). Iron is required for the synthesis of haemoglobin and myoglobin, but is also an essential component of proteins, cofactors and enzymes involved in cellular metabolism. Deficiency occurs when the intake of absorbable iron is insufficient to meet the body's requirements. The body responds to iron lack by drawing on the body iron stores and by increasing intestinal iron absorption. Body iron stores exist as ferritin and haemosiderin in the liver, spleen and bone marrow. When these stores become depleted, and there is insufficient supply of iron to the bone marrow for the synthesis of haemoglobin, the concentration of blood haemoglobin declines. Iron-deficiency anaemia is characterised by hypochromic microcytic erythrocytes (red blood cells).

Pregnant women are more susceptible to iron deficiency than any other adult population group because their iron balance is disrupted by the increased requirements for iron during pregnancy. The median amount of iron required for a single pregnancy is 790 mg: basal iron needs over nine months are 190 mg, the growth and development of the fetus and placenta requires 350 mg iron, and losses during delivery and in the puerperium amount to 250 mg (Hallberg, 1988). A further 450 mg iron is used for the expansion of the maternal red blood cell mass, but this iron is retained at parturition and returned to the maternal iron stores. Although the cessation of menstruation reduces iron losses during pregnancy, the daily iron need increases from the pre-pregnant requirement of 2-3 mg to 5-6 mg during the second and third trimesters (Bothwell *et al.*, 1989; Hallberg & Rossander-Hulten, 1991). These requirements are met in part through mobilisation of the maternal iron stores (Taylor *et al.*, 1982b) and increased intestinal iron absorption (Whittaker *et al.*, 1991; Barrett *et al.*, 1994). Despite these adaptations, negative iron balance frequently occurs, as the intake of dietary iron is rarely sufficient to meet the requirements, even in Western populations (Bentley, 1985). In developing countries, pre-pregnancy iron stores are low or depleted from frequent pregnancies, inadequate dietary iron intake and infections (WHO, 1993), and are inadequate to protect against iron-deficiency anaemia during pregnancy.

Maternal anaemia is widely recognised as a risk factor for poor pregnancy outcomes. Anaemia is associated with 40% of maternal perinatal deaths (WHO, 1996b). Severe anaemia can lead to cardiac failure in pregnancy, while moderate anaemia may indirectly contribute to maternal deaths by decreasing tolerance to haemorrhage (McFee, 1973) or by depressing immunity against infections (Chandra, 1981; Prema *et al.*, 1982). Observational studies indicate that maternal anaemia is associated with increased risks of preterm delivery, low birth-weight and perinatal mortality (Allen, 1997). Maternal iron deficiency and iron-deficiency anaemia are also specifically linked to outcomes (Goepel *et al.*, 1988; Ulmer *et al.*, 1988; Bhargava *et al.*, 1991; Scholl *et al.*, 1992; Allen, 1993). In addition, iron deficiency is associated with alterations in cellular growth and metabolism, which may affect immunocompetence and gastrointestinal function (Hercberg & Galan, 1989; Prasad & Prasad, 1991). Both anaemia and iron deficiency reduce physical working capacity and may diminish the ability of a mother to engage in economic activities and household duties including child care (Fleming, 1989; Allen, 1997).

Newborns are rarely anaemic themselves unless maternal anaemia is very severe (Viteri, 1994) because iron is actively transported across the placenta regardless of maternal iron status (Bentley, 1985). However, studies indicate that the size of the infant iron stores at birth correlate with maternal iron stores (Kaneshige, 1981; Milman *et al.*, 1987; Daouda *et al.*, 1991). Thus, infants of mothers with iron deficiency usually have lower iron stores, and may develop iron deficiency during infancy when high growth rates elevate iron requirements (Colomer *et al.*, 1990). Iron deficiency in infancy and childhood is associated with impaired psychomotor development and cognitive performance, which may persist after correction of anaemia and may even be irreversible (Dallman, 1989).

1.2.2 Impact of intestinal nematode infections

In developing countries, the most important cause of iron deficiency and iron-deficiency anaemia is poor dietary iron intake, aggravated by chronic intestinal blood loss in areas where intestinal nematode infections are endemic (ACC/SCN, 1991; 1997).

Hookworm infection is the single most important cause of pathological blood and iron loss in the topics and sub-tropics (Fleming, 1989), and is perceived to be a major contributor to iron deficiency and iron-deficiency anaemia in women of child-bearing age (WHO, 1996b). An estimated 44 million pregnant women were infected with hookworms in 1990, of whom 7.5 million resided in sub-Saharan Africa (Bundy *et al.*, 1995). Hookworms affect haemoglobin concentration most directly by causing intestinal blood loss and thus disturbing iron balance. The fourth-stage larvae and adult worms attach to the jejenal mucosa and feed from ruptured blood vessels. They secrete an anticoagulant, and so the ulcers created by the feeding activity continue to bleed after the worms move to new sites. A single *A. duodenale* and *N. americanus* adult worm is estimated to cause a mean daily blood loss of 0.20 ml and 0.04 ml respectively (Pawlowski *et al.*, 1991). Up to two-thirds of the haem iron lost through bleeding is reabsorbed, and although this proportion increases as iron stores become depleted, this reabsorption may be insufficient to prevent the disruption of iron balance (Areekul *et al.*, 1970).

The haemophagus activity of *T. trichiura* also causes intestinal blood loss. The blood loss is lower than hookworms on a per worm basis (0.005 ml blood per worm daily) (Layrisse *et al.*, 1967). However, there is no reabsorption of haem iron lost through bleeding, as the adult worms reside in the large intestine, while the majority of iron absorption occurs in the duodenum and upper jejenum (Flanagan, 1989). Substantial blood loss may also occur as a consequence of gross haemorrhage resulting from dysentery or rectal prolapse (Bundy & Cooper, 1989). The total blood loss due to the *T. trichiura* dysentery syndrome can be substantial, and the resultant anaemia severe (Bundy & Cooper, 1989).

A. lumbricoides does not have a direct impact on iron balance, but appears to reduce the utilisation of vitamin A (Nesheim, 1989), a vitamin that is reported to improve iron metabolism and blood haemoglobin levels (Mejia & Chew, 1988; Suharno *et al.*, 1993). Hookworms, *T. trichiura* and *A. lumbricoides* may all deteriorate iron status further by reducing appetite and decreasing the intake of dietary iron and by increasing the excretion of dietary iron through vomiting or diarrhoea (Holland, 1987a; Holland, 1987b; Stephenson, 1987c; see also section 1.3.2). It is also noteworthy that anorexia, vomiting and diarrhoea may reduce the availability of other haematopoietic nutrients for erythropoiesis, including folate, vitamin B_{12} and riboflavin.

The extent to which intestinal nematode infections contribute to the development of iron deficiency and anaemia in pregnant women will depend on the host iron status and the species, intensity and duration of infection (Gilles *et al.*, 1964; Roche & Layrisse, 1966). Hookworm burden is positively related to the magnitude of the intestinal blood loss (Gilles *et al.*, 1964; Roche & Layrisse, 1966; Martinez-Torres *et al.*, 1967), and inversely related to both host haemoglobin concentration (Roche & Layrisse, 1966; Areekul *et al.*, 1979; Bauerfeind *et al.*, 1992) and iron stores (Pritchard *et al.*, 1991; Bakta *et al.*, 1993a; Bakta *et al.*, 1993b). In individuals whose iron stores are low or absent from inadequate dietary iron intake and concurrent infections, relatively small hookworm burdens can exacerbate iron deficiency and precipitate iron-deficiency anaemia. Pregnancy is a period of elevated nutritional requirements (FAO/WHO, 1977; 1985; 1988), when the consequences of suboptimal nutrition are more likely to be observed. There is increased demand on body iron stores during pregnancy, and it is possible that iron storage depletion may occur at lower worm burdens in pregnant women (Crompton & Whitehead, 1993).

1.2.3 Impact of microparasitic infections

When microparasitic infections are considered in the context of pregnancy, attention is usually focused on their contribution to pregnancy wastage and adverse sequelae in live-born infants. Maternal microparasitic infection may increase the risk of both short- and long-term morbidity and mortality in both mother and fetus. Fetal consequences are particularly severe if infection crosses the placental barrier and affects development (Dickinson & Gonick, 1990). These infections are rarely considered in the differential diagnosis of anaemia during pregnancy, with the exception of malaria and more recently, human immunodeficiency virus (HIV) (WHO, 1989). However, many microparasitic infections are accompanied by some degree of anaemia due to changes in host iron metabolism (Weatherall & Wasi, 1984). When infection occurs, serum iron is removed from the host's circulation by macrophages and is incorporated into ferritin molecules (Arthur & Isbister, 1987). The resultant 'hypoferraemia of infection' does not represent true iron deficiency, but instead the redistribution of iron, which is suggested to protect against the proliferation of microroganisms (Kent *et al.*, 1994). As this iron is not available for haemoglobin synthesis, there is reduced erythropoiesis. A moderate degree of anaemia develops, which is characterised by normochromic or slightly hypochromic red blood cells (Weatherall & Wasi, 1984). 'Anaemia of infection' is therefore indicated by low serum iron, low haemoglobin concentration and normal or elevated serum ferritin concentration (Kent *et al.*, 1994).

Microparasitic infections may also produce specific haematological manifestations that are superimposed on the anaemia of infection (Weatherall & Wasi, 1984). For example, the anaemia associated with malaria is predominantly caused by haemolysis of red blood cells, which may lead to the development of folate deficiency (WHO, 1993). In addition, microparasites may interfere with food intake thereby decreasing the intake of haematopoietic nutrients, and those that inhibit the gastrointestinal tract, such as *Giardia lambia* (Hall, 1994), may interfere with the absorption of haematopoietic nutrients.

1.3 BODY SIZE AND GROWTH

1.3.1 Maternal body size and fetal growth.

Maternal body size and composition, both prior to and during pregnancy, are key determinants of pregnancy outcomes for the mother and fetus (Krasovec & Anderson, 1991a; 1991b). Low maternal height is a risk factor for obstetric complications including cephalopelvic disproportion and prolonged labour (Martorell, 1991). Arguably the most important fetal outcome is birth-weight. In developing countries, the predominant cause of low birth-weight is intrauterine growth retardation, followed by preterm delivery (Villar & Belizan, 1982). Mothers who are short or thin at conception or who gain inadequate weight relative to their body size during pregnancy are more likely to deliver growth retarded infants (Kramer, 1987; Krasovec & Anderson, 1991a). These fetuses are at greater risk of intrauterine complications and perinatal and postneonatal mortality (Ashworth, 1998). Among those who survive, the short- and long-term health sequelae include postnatal growth retardation, impaired immunocompetence and increased morbidity (Ferro-Luzzi *et al.*, 1998).

1.3.2 Impact of intestinal nematode infections

Hookworms, *Trichuris trichiura* and *Ascaris lumbricoides* may reduce the supply of nutrients for growth by decreasing host food intake, reducing intestinal absorption and by increasing nutrient excretion or loss (Stephenson, 1987a).

The symptoms associated with infection, which include abdominal and epigastric pain, fever, nausea, vomiting and diarrhoea, may suppress appetite and reduce nutrient intake (Crompton, 1984). Cytokines such as tumour necrosis factor alpha, which are produced by the host in response to infection, may precipitate physiological anorexia by affecting the control of appetite by the brain (see Stephenson *et al.*, 1993b). Iron deficiency, which is associated with hookworm infection and to a lesser extent *T.trichuria* infection, is also associated with poor appetite and growth (Hercberg & Galan, 1989; Prasad & Prasad, 1991). Decreased food intake during parasitic infection has been demonstrated in animals (Crompton, 1984). There are also reports of reduced appetite during intestinal nematode infection in humans (Holland, 1987a; Holland, 1987b; Stephenson, 1987c). Improved appetite has been reported four months after anthelminthic treatment in Kenyan school boys infected with intestinal nematodes (Stephenson *et al.*, 1993b). However, owing to the difficulties in accurately measuring human dietary intake, conclusive empirical evidence to support the impact of infection on appetite is lacking.

Ascaris lumbricoides is associated with abnormal fat digestion, disturbed nitrogen balance, lactose maldigestion and malabsorption of vitamin A, although overall these mechanisms are probably less important than reduced food intake in the aetiology of poor growth (Nesheim, 1989). Hookworm infection has also been linked with malabsorption, but it is now thought that concurrent protein-energy malnutrition accounts for this association (Holland, 1987a). This protein-energy malnutrition may arise indirectly from intestinal nematode infection through reduced food intake (Castro *et al.*, 1990) or from concomitant nutrient deficiency or other infections such as tropical sprue (Holland, 1987a). Hypoproteinaemia with hypoalbuminaemia is sometimes observed in patients with hookworm anaemia, but the relative contribution of inadequate protein intake and protein-losing enteropathy to these conditions is unknown (Variyam & Banwell, 1982). In severe *T. trichiura* infection, chronic diarrhoea or dysentery may be a cause of abnormal nutrient excretion (Gilman *et al.*, 1983).

The public health significance of intestinal nematode infections has frequently been evaluated in terms of the impact of infection on human body size, composition and growth. Previous work has focused almost entirely on children, who tend to the harbour the highest worm loads in the community during the period of life when the nutritional requirements for physical development are proportionately greatest. The association between intestinal nematode infection and poor growth in children (Gupta, 1990; Thein-Hlaing *et al.*, 1991; Robertson *et al.*, 1992) is supported by improvements in growth following anthelminthic intervention (Gupta *et al.*, 1977; Willett *et al.*, 1979; Stephenson *et al.*, 1980; Gilman *et al.*, 1983; Stephenson *et al.*, 1985; Stephenson *et al.*, 1989b; Cooper *et al.*, 1990; Stephenson *et al.*, 1993a; Stephenson *et al.*, 1993b; Koroma *et al*, 1996).

The impact of intestinal nematodes infections on maternal and fetal growth is poorly understood (Villar *et al.*, 1989a). It has been suggested that pregnant women may experience undesirable changes in body composition, such as inadequate pregnancy weight gain, and deliver underweight infants if their nutritional requirements are not met as a result of infection (Durnin, 1993, Nesheim, 1993).

1.4 MIGRATION OF INTESTINAL NEMATODE WORMS

Ectopic migration of adult *A. lumbricoides* worms within the human host can result in serious life-threatening sequelae. Worms may migrate to many organs including the appendix, bile duct, pancreas and female genital tract (Pinus, 1985; MacLeod, 1988). Invasion of these organs may result in severe complications such as appendicitis, cholangitis, liver abscesses and pancreatis (Pinus, 1985; MacLeod, 1988). Although these complications are more frequently observed in children, ectopic migration may be induced in pregnant women by the stress of labour or inhalation anaesthetics (MacLeod, 1988). Migration of a single adult worm from the intestine into the trachea and bronchi was reported as the cause of asphyxiation and death in a pregnant woman under anaesthesia during a caesarean section (MacLeod & Lee, 1988).

Nematode species with obligatory migratory tissue stages, including *A. lumbricoides* and hookworms, may be transmitted from the mother to the fetus or suckling infant. There is direct evidence that non-human mammalian hosts acquire related species by transplacental (Loke, 1982) and transmammary routes (Miller, 1981; Burke & Roberson, 1985), but the evidence in humans hosts is circumstantial. Congenital infection with *A. lumbricoides* (Chu *et al.*, 1972; da Costa-Macedo & Ray, 1990) and *A. duodenale* (Nwosu, 1981; Yu & Shen, 1990) is indicated by the recovery of adult worms or eggs from infants younger than the pre-patent period of the nematodes. There is also evidence to implicate a transmammary route of infantile *A. duodenale* infection (Schad, 1990; Schad, 1991), but larvae have yet to be identified in human breast-milk (Schad, 1994). Vertical transmission of either *T. trichiura* or *N. americanus* is not expected as the life history of *T. trichiura* is not known to involve systemic migration and *N. americanus* does not enter a period of parental hypobiosis, a prerequisite for vertical transmission (Schad, 1991).

In view of their small body size, infants are more likely to be affected by intestinal obstruction due to infestation with adult *A. lumbricoides* worms, and fatal haemorrhage due to severe *A. duodenale* infection (Yu & Shen, 1990; Sen-Hai, 1994). In China, neonatal and infantile hookworm infection is associated with diarrhoea, anorexia, listlessness, oedema, anaemia and, in severe cases, death due to intestinal haemorrhage or cardiac failure (Yu & Shen, 1990; Sen-Hai, 1994).

1.5 APPROACHES TO THE CONTROL OF MATERNAL ANAEMIA IN AREAS ENDEMIC FOR INTESTINAL NEMATODE INFECTIONS

The most common factor contributing to maternal anaemia is iron deficiency. Approaches to the control of iron deficiency and anaemia during pregnancy may include strategies to increase the intake of iron and/or interventions to prevent pathological iron loss. As the requirements for iron during pregnancy cannot be met by the diet alone, the most appropriate means of improving iron intake within the time-frame of pregnancy is through oral iron supplementation. Dietary modification is also an important way of improving the intake and bioavailability of dietary iron, but may be difficult to implement due to limited purchasing power and long-established dietary habits and customs (WHO, 1993). In areas where intestinal nematodes are endemic, anthelminthic treatment could replace or complement iron supplements by reducing intestinal iron loss and lowering iron requirements.

Iron supplementation has been a key strategy for the short term control of maternal iron deficiency and anaemia in developing countries (Viteri, 1997). In areas where the prevalence of anaemia is severe, mass treatment of pregnant women with iron is recommended (ACC/SCN, 1991). Controlled field trials have indicated that iron supplements can induce rapid improvements in iron status and haemoglobin concentrations (Yip, 1996; Allen, 1997; Viteri, 1997). It has even been suggested that iron supplements may obviate the need to treat intestinal nematode infections during pregnancy (Roche & Layrisse, 1966; MacLeod, 1988), particularly where low intensity infections predominate. However, iron supplementation only provides a short term improvement in iron status, and iron stores and haemoglobin levels will decline once supplementation is discontinued if the source of the blood loss is not targeted (Gilles *et al.*, 1964; Gilles, 1975). This is an important concern in developing countries, where the supply and distribution of iron supplements to pregnant women is often inadequate (Galloway & McGuire, 1994). In these countries, where many women do not receive a continuous supply of iron supplements during pregnancy, the effectiveness of iron supplementation programmes has rarely repeated the efficacy of controlled field trials (ACC/SCN, 1991; Pappagallo & Bull, 1996).

It has been suggested that these iron supplementation programmes were not successful in normalising haemoglobin levels in pregnant women because they did not address pathological causes of chronic blood and iron loss, such as hookworm infection (Charoenlarp *et al.*, 1988; Santiso, 1997). Iron treatment may not be adequate if the iron loss due to infection is substantial and cannot be replaced by supplementary iron alone. In fact, Hibbard (1988) suggests that in communities where intestinal parasites are rife, anthelminthic treatment of pregnant women may be more beneficial than iron-folate supplements.

Anthelminthic treatment is regarded as the most effective means of controlling the mortality and morbidity associated with intestinal nematode infections (WHO, 1996a). A number of countries have included intestinal nematode control as part of their anaemia control programmes in school children (ACC/SCN, 1997), but very few countries have implemented similar programmes in pregnant women. The main obstacles against the integration of anthelminthic treatment into maternal anaemia control programmes has been the fear that the drugs may have teratogenic effects on the fetus and the lack of information on the contribution of intestinal nematodes to maternal iron deficiency and anaemia (Santiso, 1997). In 1994, the World Health Organization reassessed the issues surrounding the administration of anthelminthic treatment during pregnancy, and concluded that the risks of delayed anthelminthic treatment outweigh the teratogenic risks to the fetus in areas where hookworm infection is endemic (prevalence greater than 20-30%) and anaemia is prevalent (WHO, 1996b). In these areas, WHO now recommends that treatment with any one of four anthelminthics (albendazole, mebendazole, levamisole or pyrantel) be included in strategies to improve the nutritional status and health of women after the first trimester of pregnancy (WHO, 1996b).

There is a lack of information on the haematological benefits of anthelminthic treatment during pregnancy. One study in Sri Lanka reported that a single course of mebendazole treatment after the first trimester of pregnancy in addition to daily iron-folate supplements increased the haemoglobin concentration and iron status of pregnant women in Sri Lanka (Atukorala *et al.*, 1994). This study is frequently quoted in support of anthelminthic treatment during pregnancy. Unfortunately, neither the prevalence or intensity of intestinal nematode infections were measured. Therefore, it is difficult to extrapolate the results of this study to other communities where the prevalence and intensity of intestinal nematode infections are known. It has been suggested that the findings from this study may encourage the use of anthelminthics during pregnancy in areas where intestinal nematodes do not pose a threat to maternal iron deficiency and anaemia (Pawlowski, 1995).

In developing countries, the financial and human resources for maternal health are extremely limited (Walker *et al.*, 1994). It is therefore important to identify the minimum interventions that are consistent with acceptable maternal and fetal health. The World Health Organization recognises that intestinal nematode infections can contribute to poor iron status and anaemia in pregnant women (WHO, 1996b). However, there is need to document this contribution more adequately with unequivocal evidence from intervention studies if convincing arguments are to be constructed for the integration of anthelminthics into maternal anaemia control programmes.

1.6 THEORETICAL FRAMEWORK AND STUDY AIMS

1.6.1 Theoretical framework

The lack of information on the impact of intestinal nematode infections on the health of pregnant women and their fetuses probably reflects the ethical barriers to research in this vulnerable sector of the community. The recommendation made by the World Health Organization regarding anthelminthic treatment during pregnancy has, however, opened up research possibilities in pregnant women. The study reported in this thesis was designed to improve the understanding of the role of intestinal nematode infections in the aetiology of maternal iron deficiency and anaemia, with a view to determining priorities for intervention during pregnancy. It incorporated research topics that were suggested by the World Health Organization in relation to the control of severe anaemia (WHO, 1993) and to hookworm infection and anaemia (WHO, 1996b) during pregnancy.

The theoretical framework underlying the design of the research strategy is shown diagrammatically in Figure 1.1. This framework comprises factors that are related to iron stores and haemoglobin concentration during pregnancy, some of which were not measured in the study but are known to be important determinants. Maternal socio-demographic factors, socio-economic status and cultural background of the population may directly or indirectly determine the exposure to intestinal nematode infections (Sanjur, 1989; Rauyajin *et al.*, 1995), the quality and quantity of the dietary iron intake (Sanjur, 1982) and consequently, the susceptibility to anaemia. Seasonal variation in rainfall may indirectly affect nutritional status by altering the transmission of infections including intestinal nematodes (Tomkins, 1993; Pawlowski *et al.*, 1991), the local availability of foods in the diet (Sanjur, 1982) and the demands for physical activity associated with agriculture (Roberts *et al.*, 1982; Lawrence & Whitehead, 1988). Maternal iron stores and haemoglobin concentration prior to delivery are a function of these values at conception, iron intake during pregnancy and nutrient losses due to intestinal nematode infections. Intestinal nematode infections may induce anorexia thus reducing the dietary iron intake and impairing energy balance. It is also important to consider other factors that affect maternal haemoglobin concentration, including: nutritional deficiencies of folate, vitamin B_{12}, vitamin A and riboflavin; maternal infections such as malaria and AIDS; and haemoglobinopathies including sickle cell disease (WHO, 1989). Both maternal anthropometry and maternal iron deficiency and anaemia are important predictors of neonatal outcomes, including size at birth. Microparasitic infections may also contribute to maternal anaemia and neonatal outcomes. Intervention with anthelminthics will curtail the loss of nutrients due to intestinal nematode infection and relieve the symptoms that precipitate anorexia, while iron-folate supplements will increase the supply of these nutrients for cellular growth (including erythropoiesis) and metabolism. Traditional beliefs and practices relating to dietary intake, intestinal worm infections and anaemia may have important influences on the effectiveness of interventions to control intestinal nematode infections and anaemia during pregnancy by determining the risk of these disorders and the level of compliance with control interventions (WHO, 1993).

1.6.2 Study aims

The central aim of the work described in this thesis was to demonstrate the efficacy of anthelminthic treatment and iron-folate supplements as control interventions for maternal iron status and anaemia during pregnancy in an area endemic for intestinal nematode infections.

The general aims were as follows:

1. To obtain social, demographic and reproductive data from women in the first trimester of pregnancy in the study area, which may identify groups at special risk of parasitic infection, poor nutritional status or adverse pregnancy outcomes.
2. To examine the prevalence, intensity and distribution of intestinal nematode infections among women in the first trimester of pregnancy.
3. To characterise the nutritional status of the women in the first trimester of pregnancy, as reflected by anthropometric indicators of chronic undernutrition and chronic energy deficiency and biomedical indicators of iron deficiency and anaemia.
4. To examine the adequacy of the dietary intake of the women during pregnancy, and assess the contribution of poor dietary iron intake to iron deficiency and anaemia.
5. To investigate the efficacy of a single course of albendazole (400 mg) and daily iron-folate supplements (36 mg iron and 5 mg folic acid), administered after the first trimester of pregnancy, as interventions to control intestinal nematode infections and improve maternal growth, iron stores and haemoglobin concentration during pregnancy.
6. To follow the birth outcomes of the women in terms of neonatal weight, length, gestational age, haemoglobin concentration and infection with intestinal nematodes, and to examine the impact of maternal iron stores, haemoglobin concentration and intestinal nematode infections on these outcomes.

7. To examine the sero-prevalence and sero-epidemiology of immunological markers to a sample of microparasitic infections (syphilis, toxoplasmosis, cytomegalovirus, hepatitis B virus and human immunodeficiency virus) in the pregnant women, and to assess the impact of these infections with respect to the control of maternal anaemia.
8. To assess the influence of traditional beliefs and practices relating to dietary intake, intestinal worm infections and anaemia on compliance with interventions to control intestinal nematode infections and anaemia during pregnancy.

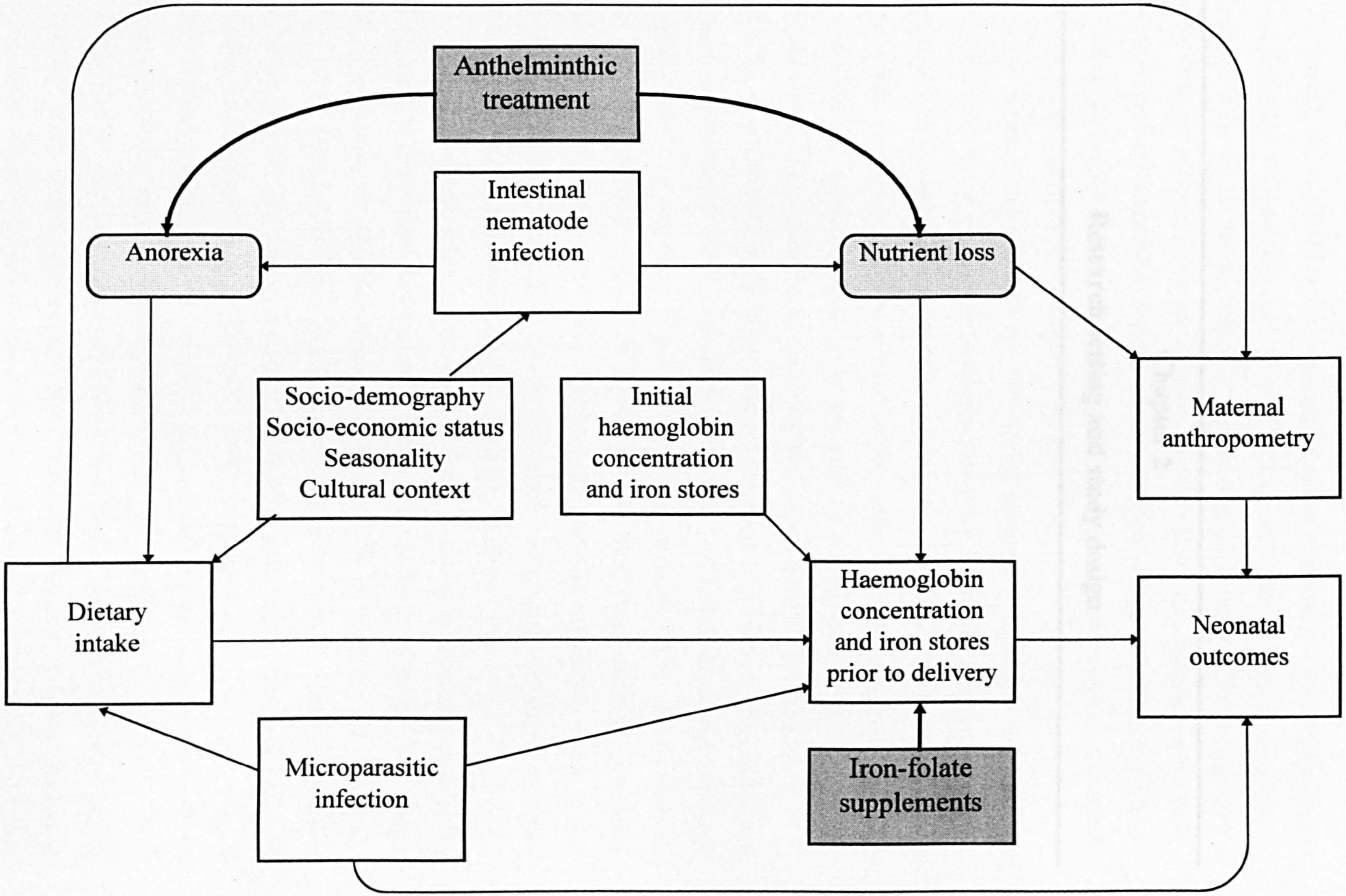

Figure 1.1 Theoretical framework underlying the research strategy.

Chapter 2

Research setting and study design

2.1 RESEARCH SETTING

2.1.1 Sierra Leone

Sierra Leone is located on the west coast of Africa, bordering Guinea to the north and east and Liberia to the south (Figure 2.1). With an area of 72,000 sq. km and population of 4.9 million, it is one of the most densely populated areas of Africa. The administrative divisions comprise one area (Western) and three provinces (Northern, Eastern and Southern), which are divided into a total of 12 districts and further subdivided into 146 Chiefdoms. There are 17 ethnic groups, the dominant groups being the Temne and Mende, which each constitute about 30% of the population (Fyle, 1976).

The climate is tropical and has two distinct seasons, a wet season from May to October followed by a hot dry season from November to April. The yearly average temperature is 26°C, and the annual rainfall ranges between 2000 and 3500 mm. Agricultural activity, which employs about three-quarters of the working population, is dominated by subsistence farming, although coffee, cocoa and palm oil are exported (MNDEP/UNICEF, 1989). The mining of diamonds, bauxite and rutile is a major source of hard currency.

Despite substantial agricultural, fishery and mineral resources, the social and economic infrastructure of Sierra Leone is poor (UNDP, 1993). A disproportionately large proportion of the rural population live in absolute poverty, while urban living conditions remain extremely difficult, with many barely able to meet their minimum nutritional requirements (UNDP, 1993). The situation has been aggravated by the recent civil war, which has diverted government funds from health to military, suppressed the major economic activities of the country and created a large displaced population.

The maternal and infant health record in Sierra Leone is amongst the worst in the world (Table 2.1). Each year, an estimated 1,000 women in Sierra Leone die from causes related to pregnancy and childbirth (Daoh, 1995) and 17% of newborns die in infancy (UNDP, 1995). Maternal mortality and morbidity may result directly from complications during labour, delivery or postnatal period, or indirectly from the aggravation of pre-existing malnutrition and infections (MNDEP/UNICEF, 1989). In Sierra Leone, these conditions are frequently encouraged by traditional beliefs and practices that are strongly endorsed by respected elders and so remain resilient to change.

The high priority conditions among pregnant women in Sierra Leone include maternal malnutrition, haemorrhage, obstructed labour, toxaemia, acquired immune deficiency syndrome and anaemia. Forty-five percent of pregnant women are anaemic (WHO, 1977), and anaemia is estimated to account for 20% of maternal deaths (MNDEP/UNICEF, 1989). There is no programme for the prevention or control of maternal anaemia in pregnant women.

However, the use of iron preparations to treat anaemia is common practice, and iron supplements are distributed by the primary health care outlets throughout the country. Pregnant women are routinely offered these supplements at a cost recovery price when they attend antenatal clinics. Severe anaemia is treated with parenteral iron or blood transfusion. The management of severe anaemia is compromised by the absence of a functional blood transfusion service outside Freetown and by the lack of facilities that are equipped to detect very low haemoglobin levels. Investigation for haemoglobin concentration during pregnancy is only conducted at the major hospitals that have laboratory facilities. Only a small proportion of pregnant women receive screening, as access is hindered not only by distance to the nearest facility, but by the high cost of screening relative to income.

The iron status of a pregnant woman in Sierra Leone may be compromised by frequent pregnancies (high fertility), poor dietary intake and parasitic infections. Haemoglobinopathies, predominantly sickle cell disease (Wurie, *et al.*, 1996), compound the problem of anaemia.

(i) High fertility

Sierra Leone is a society in which women are valued primarily for child-bearing. Childlessness is regarded with suspicion and a childless woman may be accused of witchcraft (MNDEP/UNICEF, 1989). The young age at marriage, the low use of modern contraceptive methods and the poor literacy level of women contribute to the high total fertility rate (Bailey, 1989; MNDEP/UNICEF, 1989).

(ii) Poor dietary intake

The majority of women eat only one or two meals a day, and yet they work an average of 16 hours per day on domestic, farming and cash-earning activities (MNDEP/UNICEF, 1989; Sierra Leone Government, 1992). Poor dietary intake is attributed to low purchasing power, nutritional ignorance and adverse cultural practices (MNDEP/UNICEF, 1989).

A study in the Western Area and rural areas of the Northern and Southern Provinces indicated that one in twenty families cannot afford three meals a day (MNDEP/UNICEF, 1989). The main staple is rice, which is believed to be essential for life and is virtually synonymous with food. Indeed, an individual will often claim she has not eaten if she has not had a meal containing rice, irrespective of any other dietary intake (Aitken, 1990). Many individuals believe that their nutritional needs are met once they have eaten rice and feel satiated. Flesh foods are valued components of the diet, but intake is limited by their high cost. The intra-family distribution of food favours adult male members of the household, who receive a disproportionate amount of food, particularly meat. The nutritional intake of women is further limited by cultural or religious food proscriptions, which limit access to certain foods (Thompson-Clewry, 1972; Pratt, 1983).

Quantitative data on the dietary iron intake of the typical Sierra Leonean diet is unavailable. However, the low iron bioavailability of the main staple rice (Bothwell *et al.*, 1989), and the low intake of flesh foods, would suggest a poor dietary iron intake. Pregnant women may be at greater risk of suboptimal iron nutriture because of their increased requirements.

(iii) Intestinal nematode infections

The public health significance of intestinal nematode infections in Sierra Leone has only been recognised in recent years (Williams, 1996). These infections are highly prevalent in Sierra Leone, where the tropical climate, the dependence on agriculture and widespread poverty facilitate transmission (Alghali *et al.*, 1990; Ewald, 1994; Bayoh & Hodges, 1994). Previous community and school based surveys have consistently reported a heavy burden of infection in the population (see Appendix 2). Hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* are most widespread. The prevalence and intensity of these infections varies with age, gender and geographical location of the hosts (Ewald, 1994). *Strongyloides stercoralis* and *Strongyloides fuelleborni* are less frequent and more focal in distribution. The range of prevalences reported for recent surveys that included individuals of all ages and both sexes were: *A. lumbricoides*, 21% to 67%; hookworms, 21% to 77%; *T. trichiura*, 0% to 59%; and *S. stercoralis*, 0% to 3% (Alghali *et al.*, 1990; Ewald *et al.*, 1993; Bayoh & Hodges, 1994). The national prevalence of *A. lumbricoides*, hookworms and *T. trichiura* is estimated to be 28.5%, 20.4% and 16.4% respectively (Williams, 1996).

No studies have specifically investigated the pattern of infection in pregnant women in Sierra Leone. A cross-sectional survey in 1990 examined the distribution of intestinal nematodes among mothers (16 to 40 years) in urban Freetown and rural Koindagu District (Wilson *et al.*, 1991). The prevalence of *A. lumbricoides*, *N. americanus* and *T. trichiura* was 38.5%, 11.5%, 46.9% respectively in the urban area (n=60) and 40.6%, 59.0%, 10.0% respectively in the rural areas (n=129). The high prevalence and intensity of common intestinal nematode infections among the general population and women of child-bearing age suggest that pregnant women may be at risk of infection and associated disease.

Anthelminthics are not routinely prescribed during pregnancy. Diagnostic facilities for stool analysis are available at major hospitals. At the main maternity hospital in Freetown, pregnant women are screened for intestinal infections if their haemoglobin concentration at the first visit to the antenatal clinic is below 100 g/l, and are advised on treatment accordingly.

2.1.2 Study sites

The poor maternal health background and high prevalence of anaemia and intestinal nematode infections in Sierra Leone provided the appropriate location for the present study. In addition, the existing collaboration between Glasgow University and Saint Andrew's Clinics for Children (STACC) in Sierra Leone provided field assistance and facilitated access to pregnant women. STACC is a non-government organisation that supports and develops child and maternal primary health care.

Due to the volatile security situation caused by the ongoing civil war in the provinces, the study was forced to locate in areas close to the capital city, Freetown. Nine antenatal clinics were established by the research team, six at rural sites in Kaffu Bollom and Lokomasama Chiefdoms (Port Loko District, Northern Province) and three in peri-urban Freetown (Western Area) (Table 2.2; Figure 2.2). These clinics were either set-up for the sole purpose of the study, or operated alongside existing government or non-government organisation clinics. Prior to the present study, there had not been an iron-folate supplementation or anthelminthic programme among the adult population within the study area.

(i) Kaffu-Bollom and Lokomasama Chiefdoms

These coastal rural Chiefdoms had not been directly affected by the rebel war. The houses were usually of clay framework with thatched or corrugated iron roofs. Almost all households had pit latrines but they tended to be poorly utilised. Most of the study communities were supplied with household or communal wells, although Tintafor and Pewulay had a piped water supply. A small proportion relied on stream or swamp water. Agriculture (market gardening) and fishing were the main sources of economic income. The crops grown included rice, cassava, groundnuts and fruits (orange, banana, plantain, mango and pineapple).

There were five clinics operating within the rural study area. The medical staff at these clinics reported poor attendance at the antenatal clinics. Most medical staff were not fluent in Temne (the predominant ethnic language in the community) and medicinal supplies were frequently low.

(ii) Peri-urban Freetown

Kuntola and Portee are located in Eastern Freetown. This area has undergone significant uncontrolled expansion in recent years, due to the rapid influx of displaced people from rebel-controlled areas in the provinces. A significant proportion of the community lived in overcrowded conditions, often in makeshift shacks, although the original residents usually possessed brick or clay house with corrugated iron roofs. The sanitary facilities were inadequate to suffice the population, and access to water from the small streams or communal wells was poor, particularly during the dry season. The community was poorly serviced by government health facilities, although STACC operated weekly mobile clinics. Petty trading was the predominant source of income, but there was also some small scale market gardening.

Goderich is a fishing community in Western Freetown. Although there was a displaced population in the community, there was less overcrowding. The community tapped water directly from the gravity fed water pipe that supplies Freetown from the Guma Dam. There was a government health clinic in close proximity, but it was underutilised. Fishing and trading were the main economic activities.

2.1.3 Subjects

As the study was essentially experimental rather than observational, it was not a priority to ensure that the study population was representative of the pregnant population of Sierra Leone. However, by avoiding locations that may have introduced bias, such as hospitals or refugee camps, an effort was made to ensure that the subjects were not markedly atypical. The study population comprised pregnant women residing within walking distance of the study clinics.

A prospective subject was invited to enrol on the study, provided the following selection criteria were met: (1) the subject was in the first trimester of pregnancy (<14 weeks gestation) (2) the subject was not enrolled at any other antenatal clinic (3) the subject intended to stay within the study area for the duration of the pregnancy and for one month post-partum (4) the subject's haemoglobin concentration was greater or equal to 80 g/l at baseline (first trimester of pregnancy). Any subject with Hb <80 g/l at a later stage of the study was withdrawn from the study and treated immediately with appropriate therapy.

2.2 STUDY DESIGN

2.2.1 Preliminary investigations and orientation

The present study was designed to examine the effect of iron-folate supplements and albendazole treatment during pregnancy on various outcome variables including maternal infection with intestinal nematode infections, maternal anthropometry, maternal iron deficiency and anaemia and neonatal outcomes.

Between July 1995 and November 1995, prospective study sites were visited and assessed to determine their suitability as research locations. Two cross-sectional pilot studies were conducted in rural sites in Kaffu-Bollom Chiefdom and peri-urban sites in Freetown.

These pilot studies provided an opportunity to test the research methodology, as well as to assess the prevalence and intensity of intestinal nematode infections and the prevalence of anaemia among the pregnant women. The results indicated a high prevalence of intestinal nematode infections and anaemia (Appendix 3). The intensities of the intestinal nematode infections were rather low, but the security situation prohibited investigations further afield, where higher intensities were expected on the basis of previous research in the provinces (see Appendix 2).

Permission to conduct the study was obtained from the relevant authorities at the village, community and district level. Orientation meetings were held between the members of the research team and Paramount chiefs, village chiefs and headmen, local clinic staff and pregnant women. These meetings motivated and educated the community as to the nature and purpose of the study.

2.2.2 Study protocol

Enrolment commenced in December 1995 and was closed in June 1996. Gestational age was determined from the date of the last menstrual period, if known, but otherwise by fundal height. At enrolment, a questionnaire was administered to obtain information on the demographic, social and reproductive background of each subject. Five detailed assessments were conducted during the course of the study, three during pregnancy ('trimester' assessments), one at or closely after delivery ('birth assessment') and one at 4-6 weeks post-partum ('post-partum' assessment). The neonate was also examined at delivery and at 4-6 weeks post-partum. Intervention began during the first two weeks of the second trimester. A summary of the study protocol for pregnant subjects and their neonates is given in Table 2.3.

The subjects were asked to attend the antenatal clinics fortnightly. At each visit the subjects received routine antenatal and clinical procedures including abdominal palpation and the measurement of weight, fundal height and blood pressure. The subjects were examined for any clinical symptoms of ill health, such as dietary deficiencies and infections. The majority of subjects were examined between 0900 and 1200 hours and before the midday meal.

(i) Trimester assessments

An assessment was conducted during each of the three trimesters of pregnancy. The first trimester results constitute the baseline measurements.

The following measurements were taken at each of these assessments:

- 24-hour dietary recall.
- stool sample for diagnosis of intestinal nematode infections.
- urine sample for diagnosis of *Schistosoma haematobium* (first trimester only[1]).
- capillary blood sample for measurement of blood haemoglobin concentration.
- 5 ml venous blood sample for measurement of haematocrit, serum ferritin concentration, and detection of antigens and/or antibodies to human gonadotrophin hormone, toxoplasmosis, syphilis, cytomegalovirus (CMV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV).
- anthropometrical measures of body size and composition.

An additional stool sample was collected two to four weeks after treatment with the anthelminthic or anthelminthic placebo, to determine the efficacy of the anthelminthic.

(ii) Birth assessment

Immediately following delivery, the subjects were asked to report to a local health representative who contacted a member of the research team. Both parturient and neonate were clinically examined, weighed and the haemoglobin concentration measured. The length of the neonate was also measured.

(iii) Post-partum assessment

A maternal and infant post-partum assessment was made approximately four weeks after delivery. The procedures carried out for the mother and infant were identical to the trimester assessments and birth assessment respectively. In addition, an infant stool sample was obtained for diagnosis of intestinal nematode infections.

(iv) Non-pregnant women survey

As no pre-pregnancy data was available for the pregnant subjects, data was obtained from non-pregnant women who were matched for household with the pregnant women. Each pregnant subject was asked to nominate one non-pregnant women of child-bearing age from the same household. The following measurements were taken:

- stool sample for diagnosis of intestinal nematode infections.
- capillary blood sample for measurement of haemoglobin concentration.

---

[1] Only a first trimester urine sample was examined for evidence of *S. haematobium* infection, as the results of these samples indicated that this parasite was not of public health importance in any of the study sites.

2.2.3 Intervention groups

A randomised placebo-controlled factorial design was applied in order that the two interventions, daily iron-folate supplements (Fe) and a single dose anthelminthic treatment (A), be simultaneously compared with each other and with the placebo alternatives (P_{Fe} and P_A). The four intervention groups thus comprised:

- Iron-folate supplements and anthelminthic (FeA)
- Iron-folate supplements and anthelminthic placebo (FeP_A)
- Iron-folate placebo and anthelminthic ($P_{Fe}A$)
- Iron-folate placebo and anthelminthic placebo ($P_{Fe}P_A$)

2.2.4 Choice of iron-folate supplements, anthelminthic treatment and placebos

(i) Iron-folate supplements

Iron preparations vary in intestinal absorption capacity, gastric tolerance and cost. The preparation chosen, ferrous gluconate (Kerfoot Pharmaceuticals, Aston-under-Lyne, England), has similar absorption to ferrous sulphate (Harju, 1989) and is well-tolerated (Masawe, 1981).

There is still a lack of agreement on the appropriate dosage of iron supplements during pregnancy. The World Health Organization (WHO) recommends daily supplementation with 120 mg iron in areas where there is a high prevalence of iron-deficiency anaemia (ACC/SCN, 1991). However, theoretical calculations based on iron requirements and the absorptive capacity during pregnancy suggest that this level may be unnecessarily high (Viteri, 1997). The efficiency of iron absorption falls with increasing iron dose, and a substantial proportion of high iron doses is not absorbed (Hahn *et al.*, 1951; Viteri, 1997). Furthermore, recent animal studies in iron-supplemented rats indicate that iron blockage of the intestinal mucosal cells following a recent iron dose limits further absorption until these cells are replaced (Wright & Southon, 1990; Viteri *et al.*, 1995). These findings suggest that the efficiency of iron absorption and utilisation could be increased by lowering the daily supplementary dose or by applying the usual supplementary dose less frequently (Viteri, 1997). Low dose or less frequent supplementation is desirable as it is economically advantageous and may improve patient compliance by reducing the dose-dependant gastro-intestinal side-effects that are associated with oral iron supplements (Yip, 1996). The adverse interactive effect of high iron doses on the absorption of other micronutrients such as zinc may also be reduced (Hambridge *et al.*, 1987). Although recent studies have indicated that once weekly and twice weekly supplementation schedules are as equally efficacious as daily supplementation in pregnant women (Lui *et al.*, 1995; Chew *et al.*, 1996; Ridwan *et al.*, 1996), less attention has been given to the use of lower daily dose regimens. A daily dose of

30-60 mg is associated with minimal gastrointestinal side effects, and the incremental gain in haematological improvement with increasing dose is small (Chanarin & Rothman, 1971). To investigate the efficacy of lower daily dose regimens, a daily dose of 300 mg ferrous gluconate was supplied in the present study, which provided 36 mg of elemental iron.

Folic acid, 5 mg daily (Cox Pharmaceuticals, Widdon Valley, England), was also administered to prevent folic acid deficiency limiting erythropoiesis under iron repletion. The administration of folic acid was particularly important, as the sickle cell trait is prevalent in Sierra Leoneon populations, and subjects with sickle cell disorders are at increased risk of folate deficiency due to elevated erythropoietic activity (Lindenbaum & Klipstein, 1963).

(ii) Anthelminthic treatment

WHO (1996b) recommends the use of either albendazole, mebendazole, levamisole or pyrantel pamoate for women after the first trimester of pregnancy. We chose to use albendazole (proprietary name Zentel, Smithkline-Beecham Pharmaceutical Laboratories Nanterre, France) administered as a single dose (2 x 200 mg). This benzimidazole derivative has been approved for human use since 1982 (WHO, 1996a). It has distinct advantages over other anthelminthics: (1) it has the broadest spectrum of activity of the common anthelminthics (active against *Ancylostoma duodenale*, *Necator americanus*, *A. lumbricoides*, *T. trichuria*, *Enterobius vermicularis*, *S. stercoralis and Taenia* species) (2) it is the only anthelminthic effective against all stages of the life-cycles (ova, larvae and adult worms) thereby providing 'longer' protection (Cline *et al.*, 1984; Maisonneuve *et al.* 1985) (3) it is well tolerated with minimal side-effects similar to a placebo (Rossingnol & Maisonneuve, 1983) (4) it is administered as a single dose (5) although it has been reported to be embryotoxic and teratogenic in rats and rabbits (Dollery, 1992), no deleterious effects were observed among 10 women who were accidentally exposed to high doses during the first trimester of pregnancy and followed-up to term (Horton, 1993) (6) studies in Sierra Leonean school children have demonstrated relatively high cure rates and a high reduction in the intensity of infection (Koroma *et al.*, 1995) (7) it is recommended by local health professionals in Sierra Leone (Z.Bahsoon[2] & M.Hodges[3], personal communication) (8) a physically similar placebo treatment was available.

(iii) Anthelminthic and iron-folate placebos

A study design that fails to incorporate a concurrent control is highly unlikely to produce results that are reliable or even interpretable (Altman, 1991). Indeed, such a study may be regarded as unethical by unjustifiably exposing patients, by wasting resources and time and by the publication of misleading results (Altman, 1982). The use of concurrent

---

[2] Pharmacologist, Ph.D. (Bristol University).
[3] Paediatrician, MB BS, MRCP (Bristol University).

placebo group provides an essential control for changes occurring in pregnancy, seasonal variation in food availability, infection transmission, nutritional status and resultant health (Stephenson, 1987b).

An attempt was made to manufacture placebos that physically resembled the iron preparations and anthelminthic and contained non-active ingredients, but the necessary raw materials were unavailable. Two calcium with vitamin D tablets (Regent Laboratories Ltd, London, England), which were similar in appearance to the albendazole tablets, were used as the placebo for the anthelminthic. White calciferol tablets (1.25 mg calciferol equivalent), one daily (Regent Laboratories Ltd, London, England), were chosen as the placebo for the iron-folate supplements.

While these interventions will be referred to as placebos, it is acknowledged that they are not true placebos because they contain active ingredients. We were unable to avoid the use of these placebo interventions as compliance would have been severely compromised if no medication had been given. The use of calcium and vitamin D as a placebo for albendazole was considered acceptable as both calcium and vitamin D are not known to have any anthelminthic activity. Calcium inhibits iron absorption (Hallberg *et al.*, 1991), but it is unlikely that the dose of calcium and vitamin D, taken only once at the clinic and not with a meal, would have made a significant impact on the long term iron status of the women receiving this placebo. Calciferol has not, to my knowledge, been linked to anthelminthic activity, iron metabolism or erythropoiesis.

2.2.5 Intervention allocation system

(i) Randomisation

In the ideal clinical trial, the characteristics of the individuals in each intervention group should be similar. This avoids a biased comparison of the intervention effects. By randomising the intervention allocation to the subjects, an attempt is made to control for confounding variables that may affect the outcome variables of interest. In the present study, the intervention groups were allocated using random number tables to generate the random number sequence. The subjects were allocated an intervention group at enrolment. Some of these subjects were later found not to be pregnant and were excluded from the study[4]. Coincidentally, a disproportionate number of these non-pregnant women had been allocated the $P_{Fe}A$ intervention group. Four months after the start of the field work, it was clear that there was an imbalance in the number of pregnant women in each intervention group. To

[4]A clinical examination of fundal height was used to determine the gestational age if the date of the last menstrual period was unknown. This technique was not always accurate for disqualifying non-pregnant women, as the fundus is not palpable during most of the first trimester. Consequently some non-pregnant women were inadvertently enrolled, but were later excluded.

rectify this, a list of the intervention groups that had been allocated to these non-pregnant women was made, in chronological order of their enrolment. Women joining the study thereafter were allocated an intervention group from this list.

(ii) Degree of blindness

A randomised double-blind controlled trial is often regarded as the 'gold standard' design for clinical trials (Altman, 1991). A double blind trial, in which both the subject and the person assessing the subject are unaware of the intervention being received, eliminates the possibility that the response of the subject and the observations of the assessor are biased as a result of knowledge of the intervention group.

It was not possible to incorporate blinding into the design of the study, as we were unable to provide 'placebo' medications that were physically identical to the active form, and we were unable to assign one field assistant solely to the administration of intervention. While the subjects were not led to believe that either of the intervention groups were superior, the red colour of the iron tablets was more highly valued by the subjects than its white placebo. The administration of the anthelminthic placebo was of less concern because it was physically similar to albendazole. Furthermore, it was administered in a single dose under the presence of an field assistant and the subjects did not have any opportunity to compare their medication. The field assistant who administered the iron-folate supplements and albendazole treatment also conducted the 24-h dietary recalls and analysis of stool samples. The failure to incorporate blinding is a limitation in the study design, but was unavoidable under the conditions of limited material and human resources.

(iii) Set-up of the intervention allocation system

The intervention allocation system was organised in such a way that the field assistants did not know in advance which intervention group would be administered to each new subject. This avoided bias that may have been introduced if the enrolment of a prospective subject was affected as a result of knowing in advance the intervention group that would be allocated.

Following enrolment, the subjects were allocated an intervention group. Intervention was commenced during the first two weeks of the second trimester. The albendazole or albendazole placebo was taken at the clinic in the presence of a field assistant. The subjects received two-weekly supplies of daily iron-folate supplements or the iron-folate placebo at each fortnightly visit to the clinic. Subjects failing to attend a clinic were visited at home and, if absent, the supplements were left with a responsible member of the household. Medication other than that indicated by the study intervention group was not routinely given. Subjects were asked to report the use of any other medication during the study.

2.2.6 Compliance

The present study was semi-supervised in that all subjects were given two weeks supplies of iron-folate or placebo supplements, regardless of whether or not they attended the antenatal clinic on a particular fortnight, but actual consumption was dependent on compliance by the subjects. We had no assurance that the subjects took the daily supplements. Subject compliance was not quantitatively measured, but subjects were provided with tablet boxes and were asked to bring them each time they attended a clinic so that the field assistants could observe whether the previous supply of tablets had been finished. In addition, subjects were asked to report if they experienced any side-effects associated with intervention that may have affected compliance. Only two subjects complained of side-effects that they associated with the iron-folate supplements, and both these women continued to take the supplements following reassurance that the symptoms were harmless and were likely to subside over time. On the basis of these observations, there was little evidence for non-compliance, although it is possible that the medication was discarded, distributed among family members or sold. The intake of albendazole or albendazole placebo was fully supervised, and no subject expressed concern over treatment. There is the possibility that some subjects may have taken iron, folate or albendazole independently.

An attempt to control for non-compliance may result in bias, as it would be difficult to accurately quantify and would involve subjective decisions in deciding whom to exclude from the analysis. The recommended approach under these circumstances is 'intention to treat analysis' in which the analysis is based on the groups as initially randomised, thereby neither changing the intervention group or excluding any of randomised subjects from the analysis (Altman, 1991). The only exception to this approach was the exclusion of non-pregnant women who had been incorrectly accepted on the study and subjects who dropped out of the study.

2.2.7 Sample size

(i) Sample size determination

In order to determine the approximate sample size necessary to detect a statistically significant difference of given magnitude for a particular hypothesis, an *a priori* test is required before field-work commences. The primary research objective on which the determination of sample size was based was the effect of intervention (iron-folate supplements and/or anthelminthic treatment) on the change in haemoglobin concentration between baseline (the first trimester) and the third trimester. Details of the methods and assumptions used to determine the sample size are given in Appendix 4. Assuming a dropout rate of 20%, significance level α=0.05, power level β=0.8 and a medium strength of association between intervention and outcome, 49 subjects were required in each of the four intervention groups. This number is reduced to 20 if a strong strength of association is assumed.

(ii) Actual sample size, drop-outs and missing data

The rate of accrual of subjects onto the study was less than anticipated, largely due to the selection criteria that excluded those exceeding three months' gestation from joining the study. Most women in Sierra Leone are reluctant to attend antenatal clinics during their first or even second trimesters. The antenatal clinic is not regarded as a necessity in early pregnancy, particularly before the abdomen becomes noticeably distended and the fetal movements are obvious. Some are embarrassed or afraid to reveal they are pregnant and conceal the pregnancy for as long as possible. If financial resources are limited, they prefer to delay attendance until the later stages of pregnancy. Although the study's antenatal clinics were provided free of charge, thereby releasing financial constraints, the loss of working hours deterred some pregnant women from joining the study.

One hundred and eighty five pregnant women were enrolled on the study. One subject was excluded from all analysis as she was clinically obese (body mass index >30 kg/m^2) and her nutritional status was highly atypical of her peers. Thus 184 subjects were included in the baseline analysis. Six subjects were withdrawn at baseline due to a low haemoglobin (Hb) concentration (Hb <80 g/l). Of the remaining 178 individuals, 43 were allocated to receive FeA, 46 were allocated FeP_A, 44 were allocated $P_{Fe}A$ and 45 were allocated $P_{Fe}P_A$. Eighty-three (46.4%) women dropped-out of the study between intervention allocation and one-month post-partum. The predominant cause for these drop-outs was anaemia (Hb <80 g/l). Drop-outs due to anaemia were particularly large at the third trimester assessment, and as these drop-outs were biased with respect to intervention group, those remaining in the study thereafter were a biased subset of the sample at baseline. To minimise the effect of biased drop-outs, and maximise the sample size in the statistical analyses, the longitudinal analyses presented in this thesis are based on data collected between the baseline assessment (first trimester) and the third trimester assessment. This data is of greatest clinical relevance, as (1) pregnancy outcomes are determined by events occurring *during* the pregnancy (2) events occurring at delivery, which are independent of intervention group (such as heavy blood loss due to poor obstetric management), are likely to confound the effects of intervention on haematological status at delivery and in the post-partum period. A total of 125 women were followed-up to the third trimester and provided all measurements and samples, 32 in intervention group FeA, 35 in intervention group FeP_A, 29 in intervention group $P_{Fe}A$ and 29 in intervention group $P_{Fe}P_A$. The proportion of dropouts was similar for each intervention group (Multiple logistic regression: iron-folate Wald=1.46, p=0.23; albendazole Wald=0.02,

p=0.88; interaction Wald=0.05, p=0.82). The reasons for the 53 (29.8%) drop-outs occurring between intervention allocation and the third trimester assessment are given in Table 2.4.

Exploratory analysis was also conducted on the neonatal outcomes for pregnant women who remained in the study until delivery, but there are problems attached to this data due to intervention-related drop-outs at the third trimester assessment (see Chapter 9 for further details).

2.2.8 Ethical considerations

Ethical approval was obtained from Research and Ethics Committees in both Sierra Leone (Ministry of Health, Research and Ethics Committee, Medical Entomology Division, New England, Freetown) and the United Kingdom (Glasgow University, Glasgow) (Appendix 5). Informed consent was obtained from each subject and, where possible, her partner or close relative. All subjects were made aware that alternative interventions would be allocated to different individuals and that they were free to withdraw from the study without disadvantage at any stage.

Following ethical recommendations issued by WHO (1981), any subject with Hb <80 g/l at any stage of the study was treated immediately with appropriate therapy and withdrawn from the study, but continued to attend the clinic as a non-study case. On completion of the study, iron-folate supplements were administered to any parturient with Hb <100 g/l in accordance with WHO ethical guidelines (WHO, 1981), and all mothers received albendazole (400 mg) irrespective of whether they had received albendazole during the course of the study.

2.3 RESEARCH TEAM AND TRAINING

In a multi-disciplinary study of this nature, the establishment of a research team with collaboration between persons of relevant disciplines is essential. The research team and the respective responsibilities are given in Table 2.5. In addition, the assistance of the following institutions is acknowledged: the serum ferritin analysis described in Chapter 8 was conducted at Stobhill Hospital (Glasgow, United Kingdom); the B-Haemoglobin Photometer for haemoglobin concentration analysis was validated against the QBC Centrifugal Hematology System at the Joint Health Facility, British High Commission (Freetown, Sierra Leone); laboratory technicians at Ramsy Laboratories (Freetown, Sierra Leone) performed the serological assays for syphilis and cytomegalovirus and assisted in the assays for hepatitis B virus and toxoplasmosis (Chapter 10); the serological assays for human immunodeficiency virus (HIV) diagnosis were conducted at the HIV Laboratory, Connaught Hospital (Freetown, Sierra Leone).

The principal investigator received training at the following institutes: stool analysis and haematocrit measurement at the World Health Organization Collaborating Centre for Soil-Transmitted Helminthiases, Glasgow University; haemoglobin concentration measurement at the Blood Transfusion Centre, Glasgow; anthropometrical measurements and 24-h dietary recall at Cambridge University; abdominal palpation at Queen Mother's Hospital, Glasgow. Prior to the field research, the principal investigator trained the field assistants in the techniques appropriate to their responsibilities.

Table 2.1 Statistics relevant to maternal health in Sierra Leone.

Indicator		Reference
Total fertility rate	6.4	UNICEF, 1996
Median age of women at marriage (years)	18	MNDEP/UNICEF, 1989
Literacy among women (%)	15	UNICEF, 1996
Contraceptive prevalence (%)	4	UNICEF, 1996
Births attended by trained health personnel (%)	25	UNICEF, 1996
Number of people per each		DHSS, 1993
obstetrician/gynecologist	160,000	
state-registered nurse	21,000	
maternal and child health aide	11,000	
Pregnant women with anaemia (%)	45	WHO, 1977
Daily calorie supply *per capita*	1,695	FAO, 1994
Maternal mortality rate (per 100,000 deliveries)	450	UNICEF, 1996
Cause of maternal mortality:		
eclampsia (%)	25	MNDEP/UNICEF, 1989
septicaemia (%)	25	
ruptured uterus (%)	20	
anaemia (%)	20	
haemorrhage (%)	11	
Infant mortality rate (per 1,000 live births)	166	UNDP, 1995
Infants born with low birth weight (<2500 g) (%)	13	WHO, 1992

Table 2.2 Study sites.

Site	Village	Type of clinic[†]	Number of subjects at baseline
Kaffu-Bollom Chiefdom (rural)	Tagrin	Study	13
	Yongoro	NGO (New Life Church)	23
	Kasongha	Study	9
	Tintafor	Study	16
	Pewulay	NGO (Christian Children's Fund)	39
Lokomasama Chiefdom (rural)	Menika	Government	17
Freetown (peri-urban)	Portee	NGO (St. Andrew's Clinics for Children)	32
	Kuntola	NGO (St. Andrew's Clinics for Children)	15
	Goderich	Study	20

[†] NGO = non-government organisation

Table 2.3 Study protocol for the pregnant women and neonates.

		Gestation (weeks)							Post-partum (weeks)
		≤12	10-14	14	16-18	22-26	34-38	Birth	4-6
Pregnant women	Demographic, social and reproductive questionnaire	*							
	Intervention commenced			*					
	24-hour dietary recall		*			*	*		*
	Stool examination		*		*	*	*		*
	Urine examination		*						
	Haemoglobin (capillary blood sample)		*			*	*	*	*
	Serum ferritin (venous blood sample)		*			*	*		*
	Anthropometry		*			*	*		*
Neonates	Stool examination								*
	Haemoglobin (capillary blood sample)							*	*
	Anthropometry							*	*

Table 2.4 Reason for the drop-outs from the study occurring between allocation of intervention group and the third trimester assessment

Cause of drop-outs	Number of subjects (%) in each intervention group[†]				Total
	FeA	FeP_A	$P_{Fe}A$	$P_{Fe}P_A$	
Miscarriage	3 (7.0)	0 (0.0)	3 (6.8)	4 (8.9)	10 (5.6)
Missing data[‡]	4 (9.4)	6 (10.0)	4 (9.1)	7 (15.6)	20 (11.8)
Left area	4 (9.3)	4 (8.7)	5 (11.4)	1 (2.2)	14 (7.9)
Hb <80 g/l at second trimester assessment	0 (0.0)	1 (2.2)	3 (6.8)	4 (8.9)	8 (4.5)
Total drop-outs	11 (26.6)	11 (23.9)	15 (34.1)	16 (35.6)	53 (29.8)

[†] Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.
[‡] Includes subjects who failed to continue the clinic regularly and those who delivered prematurely.

Table 2.5 Research team.

Member	Responsibilities
H. Torlesse Biological anthropologist, M.A.	Principal investigator Anthropometry Stool and urine samples: collection and analysis Ethnographic interviews Serology
M. M. Koroma Parasitologist, B.Sc.	Non-clinical field assistant Demographic, social and reproductive questionnaire. 24-h dietary recall Administration of intervention medications Stool and urine samples: analysis Ethnographic interviews Serology
J.B. Juana and E.Yorkor State-Enrolled Community Health Nurse	Clinical field assistants Collection of capillary and venous blood samples Measurement of haemoglobin concentration and haematocrit Clinical and obstetric examination Ethnographic interviews
Dr. M. Kamara Senior government specialist obstetrician (MD, FWACS)	Obstetric consultant
Dr. M. Hodges Paediatrician, MB BS, MRCP Medical director of St. Andrew's Clinics for Children, Sierra Leone.	Co-ordinate project (Sierra Leone)
Prof. D.W.T. Crompton Parasitologist, FRSE	Co-ordinate project (United Kingdom)

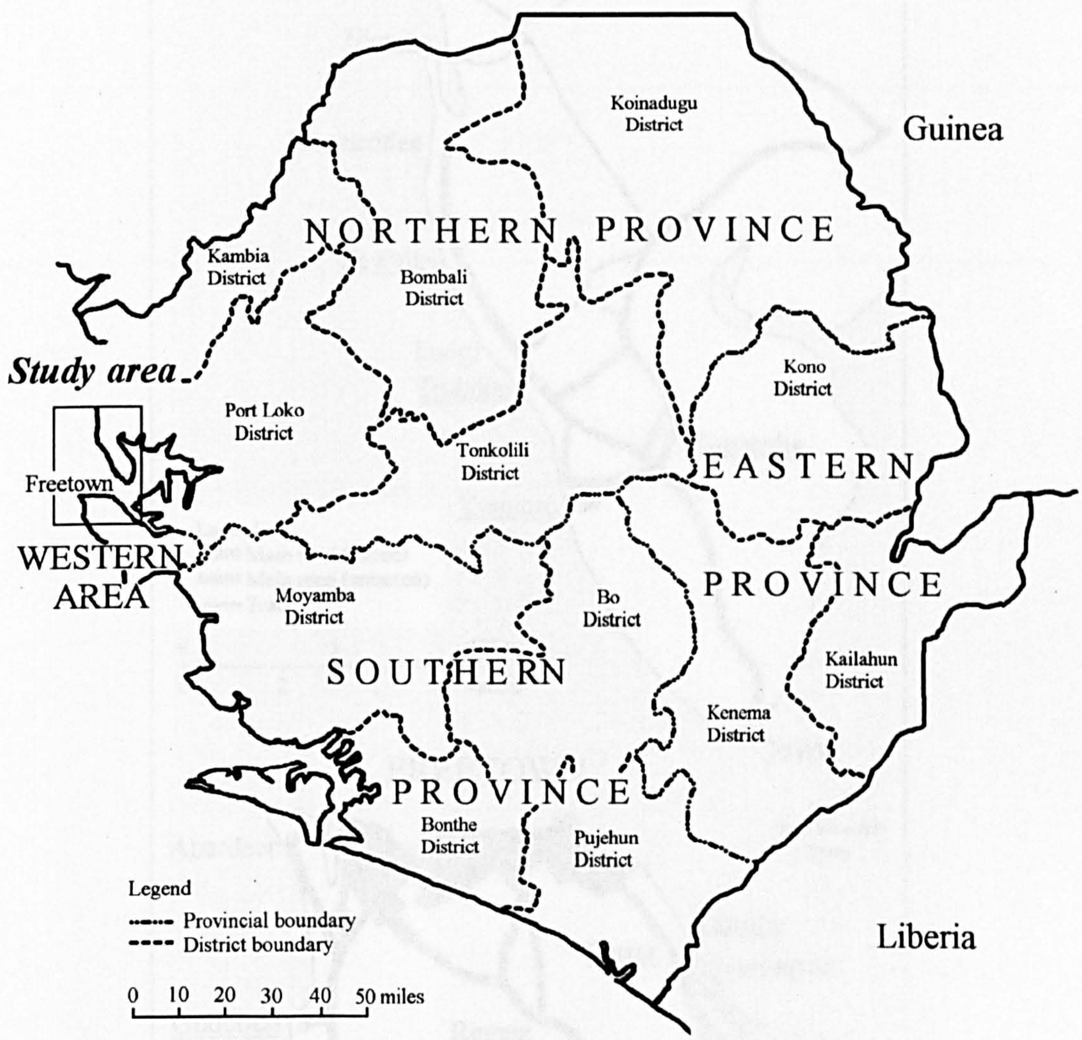

Figure 2.1 Map of Sierra Leone showing the study area.

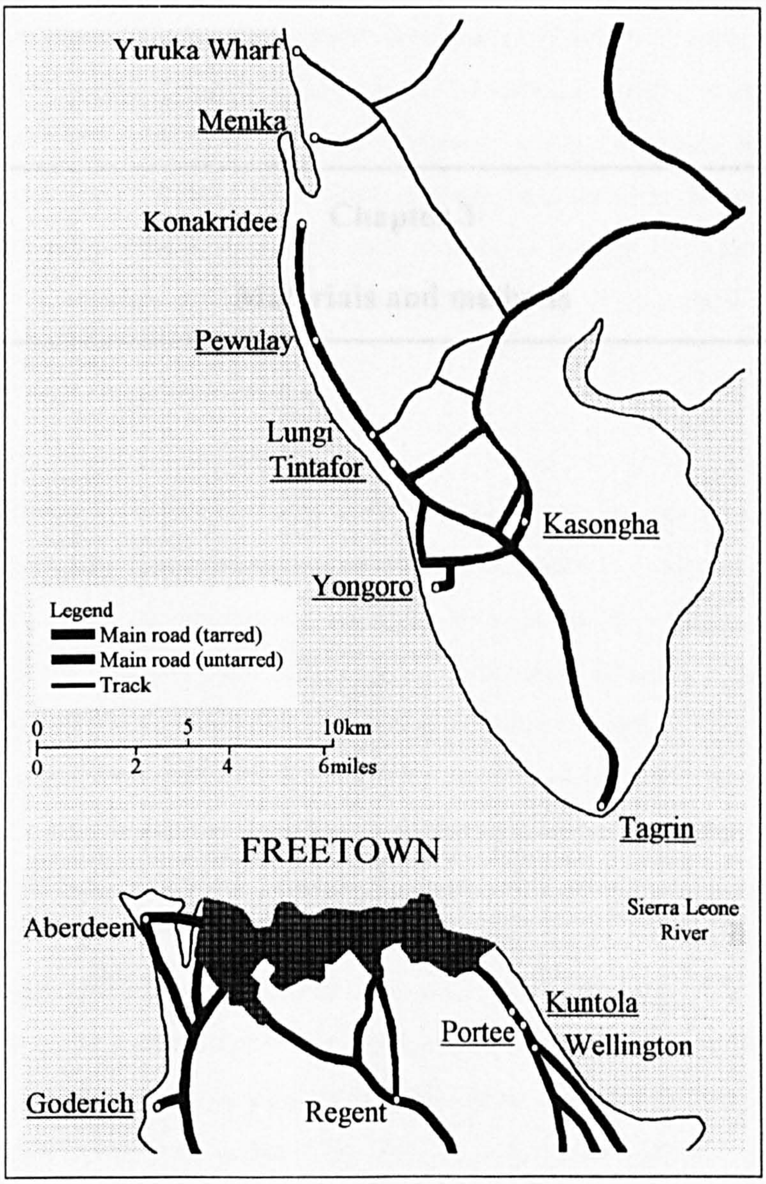

Figure 2.1 Map of study area showing the location of the study Clinics (underlined)

Chapter 3

Materials and methods

3.1 DEMOGRAPHIC, SOCIAL AND REPRODUCTIVE CHARACTERISTICS

Information on the demographic, social and reproductive characteristics of the subjects was obtained by a structured questionnaire at enrolment (Appendix 6). Demographic and social data included maternal age, place of residence, marital status, ethnic group, maternal education, maternal and paternal occupation, sanitary facilities, source of drinking water and possession of a radio. Reproductive history data included the number of children, previous pregnancies, miscarriages, stillbirths, number of months since previous pregnancy, use of modern methods of contraception and duration of menstrual bleeding during menstruation.

3.1.1 Construction of a socio-economic scale

A socio-economic scale is used to classify individuals into one or more categories of socio-economic status (SES). There are no set variables used to construct a socio-economic scale, and the choice of appropriate variables depends on the indicators that will best illuminate different levels of SES within a given community (Sanjur, 1982). In the present study, information from previous studies in Sierra Leone (Alcorn, 1993) and ethnographic observations during the course of the field-work were used to select appropriate variables. The SES scale was devised by assigning ordinal scores to the level of maternal education, maternal and paternal occupation, sanitary facilities, water supply and possession of a radio (Table 3.1). This scale reflects the SES variation within the study population in a way that encompasses economic resources, economic income and social status. The individual scores were summed to give a total score with possible values ranging from 0 to 13, where 0 and 13 represent the lower and upper extremes of the SES scale respectively. The SES scores had a non-Normal distribution with median (QR) 7 (5, 8). Individuals with SES scores less than 7 were arbitrarily assigned 'low SES' while individuals with SES scores greater or equal to 7 were assigned 'high SES'. The 'low' and 'high' SES categories were used throughout the data analysis and related to the epidemiology of infection and nutritional status.

3.2 DIETARY METHODS: 24-HOUR RECALL

Dietary intake was estimated by the 24-hour dietary recall method. The subjects were asked to recall all foods and beverages consumed during the previous day. The food/beverage type, quantity and daily frequency were recorded. The quantities of food eaten were estimated using local measures, such as serving implements, the standard cups used to measure out food items for purchase and the monetary value of food items. These quantities were subsequently converted to metric units.

To maintain rapport with the respondents, and thus encourage valid responses, the same field assistant conducted all 24-h recall interviews. This interviewer had knowledge of the local foods and their methods of preparation, and was fluent in both Temne, the predominant ethnic language in the study areas, and Krio, the *lingua franca*. The interviewer was trained to anticipate and recognise potential sources of distortion or bias, such as the over-estimation of intake to give socially desirable answers. Interview techniques were standardised and the data quality was periodically assessed to ensure that full and detailed descriptions of food preparation techniques were obtained.

Information on the ingredients and preparation of local dishes was collected during arranged cooking sessions at which ingredient portions were weighed and the preparation steps recorded. Additional information was provided by interviewing the respondents and other members of the community, by participating in meals in the community, by observing the availability of food items in the markets across the seasons and from a recipe booklet compiled by a Sierra Leonean nutritionist (Greene, 1978).

A food-composition database was compiled of all the food and beverage items consumed by the subjects throughout the study period. As no food composition data is available for the Sierra Leonean diet, values were obtained from food composition tables for the African continent (Wu Leung, 1968), Mali (Nordeide, 1997), Ghana (Barreau, 1993) and the United States (USDA, 1998). Estimates of the mean daily intake of total energy (MJ), carbohydrate, protein, fat, calcium, iron, Vitamin A, Vitamin C and folate were made.

3.3 STOOL EXAMINATION

Subjects were provided with labelled plastic containers at the clinic for the collection of a fresh stool sample. Within 30 min of collection, the stool sample was mixed and a portion fixed in 10% formalin. The samples were later transferred to the laboratory in Freetown and examined using a modified Kato-Katz method (WHO, 1991a; Robertson *et al.*, 1989). This technique enables the diagnosis and quantification of intestinal nematode infections using minimal laboratory equipment. Sub-samples of stool were spun for 3-4 min using a bench centrifuge. The stool pellet was passed through a steel mesh sieve to remove large particles of debris. A stainless steel template was used to transfer approximately 50 mg of sieved stool material to a microscope slide. Two to three drops of 3% malachite green in 50% glycerol were mixed with the sieved stool material, and the preparation covered with a glass cover-slip.

After 30 min, but within 150 min, the slides were examined microscopically at x40 and x100 magnification. The species and number of intestinal nematode eggs and larvae were recorded. Three slides were prepared for each sample and the mean of the three measurements was used in subsequent analysis. A second observer re-examined 10% of slides for quality control. In 88% of re-examinations, the egg counts were within 10% of the first examination. Concentration techniques were not employed as it is unlikely that the very low intensity infections identified using these techniques were of clinical significance.

To confirm the species of hookworm in the study areas, 50 unfixed stool samples were cultured using a modified version of the Harada and Mori faecal culture method (Pawlowski *et al.*, 1991), and the larvae examined using a hand lens (x4 magnification) and compound microscope (x10 and x40 magnification). The larvae were identified using identification keys (see Pawlowski *et al.*, 1991).

3.4 URINE EXAMINATION

Where possible, urine samples were collected at the clinic between 11 p.m. and 2 p.m. as studies have indicated that *Schistosoma haematobium* egg counts are highest during this period (Pugh 1979). The fresh urine sample was collected into a clean dry container and immediately fixed in 10% formalin (approximately 1 ml 10% formalin per 50 ml urine) and later transferred to the laboratory. The samples were shaken and a 10 ml subsample was transferred to a test-tube and left to stand for 60 min. The supernatant was decanted and the remaining urine spun for 2-3 min using a bench centrifuge. The deposit was examined for *S. haematobium* eggs under a light microscope.

3.5 COLLECTION OF BLOOD SAMPLES

A capillary blood sample for measurement of haemoglobin concentration was taken from the left index finger in pregnant women and the left heel in infants. A 5 ml venous blood sample was obtained by venipuncture and transferred to a gel separator serum collection tube. A sample of venous blood was used to determine the haematocrit. The remaining venous blood was spun at 1500 *g* for 10 min. The serum was decanted into two separate microcentrifuge tubes and stored at -20°C until analysis for serum ferritin concentration and antigens and/or antibodies to human chorionic gonadotrophin, syphilis, toxoplasmosis, cytomegalovirus (CMV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

3.6 ANAEMIA AND IRON STATUS INDICATORS

3.6.1 Haemoglobin concentration

Haemoglobin concentration was measured using a portable B-Haemoglobin Photometer (HemoCue®, Angelholm, Sweden). The skin was punctured with a sterile lancet and a single drop of capillary blood collected into a disposable microcuvette. The microcuvette controls the volume of sample and acts as a reaction vessel in containing the dry reagents. Haemolysis of the erythrocytes by sodium deoxycholate releases haemoglobin which reacts with sodium nitrite and then sodium azide to produce azide methaemoglobin. The microcuvette was placed in the photometer, and the concentration of haemoglobin was measured at two wavelengths (570 nm and 880 nm) as azide methaemoglobin.

The Hemocue Photometer was validated against the QBC Centrifugal Hematology System (Becton Dickinson, New Jersey, USA). The blood haemoglobin concentration of 30 subjects was measured using both techniques: 90% of measurements were within ±1 g/l and all measurements were within ±4 g/l.

3.6.2 Haematocrit

Heparinised microhaematocrit tubes containing venous blood were spun (MSE Haemocentaur) for 10 min, and the haematocrit value calculated as the length of the column of erythrocytes divided by the total length of the column of blood.

3.6.3 Serum ferritin concentration

The concentration of serum ferritin was measured by immunoradiometry (^{125}I) using the ICN Ferritin Mab Immunoradiometric Assay kit (ICN, High Wycombe, UK). Each assay was controlled internally by a commercial tri-level control (Lyocheck Levels 1, 2 and 3) run in duplicate at the beginning of each run and singly at the end of each run. All results for the controls were within the specification.

3.7 SEROLOGY

3.7.1 Human gonadotrophin hormone

Within 4 weeks of enrolment, 44 subjects dropped out of the study as they moved from the area or claimed to have miscarried. It was not possible to be sure these women were ever pregnant on the basis of abdominal palpation alone, as the fundus is only just palpable in the latter stages of the third month of pregnancy and is not a reliable indicator of pregnancy status. To verify the pregnancy status of these women, the baseline serum sample was examined for antibodies to human chorionic gonadotrophin (hCG) using a qualitative one-step assay: Babycheck-1 hCG Dipstick Pregnancy Test (Vedalab, Alenconm France). The assay uses both monoclonal-dye conjugate and polyclonal-solid phase antibodies to selectively identify hCG. A positive pregnancy status was confirmed in 22 of these 44 subjects.

3.7.2 Microparasitic infections

The following serological tests were performed according to the manufacturer's instructions using a serum sample obtained at one of the trimester assessments from 179 subjects unless otherwise stated.

(i) Syphilis

Antibodies to *Treponema pallidum*, indicating a past or active infection, were detected by passive particle haemagglutination using the Serodia®-TP·HA Test (Fujirebio Inc., Tokyo, Japan).

(ii) Toxoplasmosis

Antibodies to *Toxoplasma gondii* were detected by direct agglutination using the Toxo-Screen DA IgG test (BioMerieux, Lyons, France).

(iii) Cytomegalovirus

A randomly selected sample of 139 sera were analysed for anti-IgG CMV antibodies by passive particle-agglutination using the Serodia®-CMV Test (Fujirebio Inc., Tokyo, Japan).

(iv) Hepatitis B virus

Serum samples were analysed for the surface antigen of HBV (HBsAg) by reverse passive haemagglutination (R-PHA) using the Serodia®-HBs kit (Fujirebio Inc., Tokyo, Japan). The presence of HBsAg was confirmed by immunochromatography (IC) using the ICT Hepatitis B surface antigen test (ICT Diagnostics, Brookvale, Australia). Samples positive for HBsAg were analysed for the envelope antigen of HBV (HBeAg) by R-PHA using the Serodia®-HBe kit (Fujirebio Inc., Tokyo, Japan). Samples negative for HBeAg were analysed for the antibody to HBeAg (anti-HBe) by passive haemagglutination (PHA) using the Serodia®-Anti HBe kit (Fujirebio Inc., Tokyo, Japan).

(v) Human immunodeficiency virus

All sera were screened for antibodies to HIV-1 and HIV-2 using the HIV-SPOT kit (Genelabs Diagnostics, Singapore) and the Capillus^R HIV-1/HIV-2 (Cambridge Biotech Ltd., Galway, Ireland).

3.8 ANTHROPOMETRY

3.8.1 Anthropometric measures

The following anthropometric measures were taken from the subjects at each assessment during pregnancy and at the postnatal assessment, unless otherwise stated. Standard anthropometric techniques, as suggested by Frisancho (1990), were employed.

(i) Height

Height was measured at the first trimester assessment only using a stadiometer (CMS Weighing Equipment Ltd, London, UK), and recorded to the nearest 1 mm. An adjustment was made to account for notably thick hair.

(ii) Weight

Weight was measured at each fortnightly visit to the clinics using balance scales (Healthometer Inc., Bridgeview, Illinois, USA), and recorded to the nearest 0.1 kg. No corrections were made for clothes worn, but subjects were asked to remove all but light clothing.

(iii) Mid-upper arm circumference

Mid-upper arm circumference (MUAC) of the left arm was measured using a standard tape measure, and recorded to the nearest 1 mm.

(iv) Skinfolds

Skinfold measures of subcutaneous fat were obtained at the triceps (TSF) and biceps (BSF) sites on the left-hand side of the body using Harpenden callipers (St. Albans, UK). Each reading was repeated three times and the mean of the three measurements was recorded to the nearest 0.1 mm.

(v) Fundal height

Fundal height was measured at each fortnightly visit to the clinic, using a standard tape measure, and recorded to the nearest 0.5 cm.

The following anthropometric measures were taken from the neonate at the birth and postnatal assessment:

(i) Recumbent length

Recumbent length was measured using an infantiometre, and recorded to the nearest 1 mm.

(ii) Weight

Weight was measured using hanging scales (Salter), and recorded to the nearest 0.01 kg. All clothes were removed from the neonate before measurement.

3.8.2 Standardisation of anthropometric procedures

Anthropometric measurements are known to be sensitive to both intra- and inter-observer error. The great majority of anthropometric measurements were made by the principal investigator (H.T.). When the principal investigator was absent, a second observer (J.B.J.) performed these measurements. Standardised procedures were employed to minimise measurement errors, including (1) training in the proper methods of use of the measuring apparatus (2) scale adjustment of balance scales and skinfold callipers before each measuring session (3) statistical checks for measurement error. To determine the measurement error, the two observers measured 10 pregnant women twice during the same antenatal visit to the clinic. The significance of the intra-observer and intra-observer error was calculated using two-way analysis of variance (ANOVA) for repeated measures. The error measurement was calculated as the square root of the sum of the mean square for intra-observer, inter-observer and the interaction (intra-observer x inter-observer) from the two-way ANOVA for repeated measures (Villar *et al.*, 1989b). The error values were within published maximum acceptable error measurements for a reliability coefficient of 0.99 for women aged 18-40 years (Ulijaszek, 1992).

3.9 ETHNOGRAPHY

An ethnographical study was carried out to examine the traditional beliefs and practices relating to dietary intake, intestinal worm infections and anaemia during pregnancy in the Temne ethnic group in Western Sierra Leone.

3.9.1 Site and sample

The research was carried out between November 1996 and February 1997 in all areas of the core project. Two non-random samples were carefully selected. The first group comprised the key informants, and included medically trained members of the community who worked and resided within the study area. Due to a lack of Temne medical personnel in the area, individuals of the Mende ethic group were also chosen. The second group comprised a cross-section of the community, both males and females above the age of reproductive maturity of varying educational backgrounds and occupations. Only individuals of the Temne ethnic group were selected.

3.9.2 Interviewers

The interviewers included the principle investigator and two field assistants of the research team. All had experience in the field of hookworm control in West Africa. H.Torlesse (biological anthropologist) is fluent in Krio and had taken a continuing interest in the traditional beliefs of the communities. M.M.Koroma (parasitologist) is half-Temne, fluent in Temne and Krio, and is familiar with the Temne world view, thus able to verify the findings and place them in their appropriate cultural context. E. Nyorkor (state-enrolled community health nurse: SECHN) is fluent in Krio, and is familiar with lay perceptions of nutrition and ill health in Sierra Leone.

3.9.3 Data collection procedures

Anthropologists favour qualitative research, using participant observation over several months or years, as the most valid and reliable means of elucidating the systematics of cultural behaviour (Sanjur, 1982). Unfortunately, the use of this methodology in large-scale interdisciplinary studies is limited by the requirement for a high level of training and the allocation of much time for field research. To produce highly focused sets of information within short time frames, rapid ethnographic techniques, which utilise both qualitative and quantitative procedures, have been developed (Bentley *et al.*, 1988; Herman & Bentley, 1992; Manderson, & Aaby, 1992; Cassidy, 1994).

As no single method could adequately address all the research objectives, several ethnographic techniques were employed in the present study, including the traditional anthropological methodology of participant observation, and more rapid procedures such as key informant interviews, in-depth interviews and case studies.

(i) Key informant interviews

Nine medical personal were interviewed using an open-ended interview guide to obtain general information on the prevailing traditional beliefs and practices relating to dietary intake, intestinal worm infections and anaemia among pregnant women of the Temne ethnic group in the communities.

(ii) In-depth interviews

An ethnographic field guide was formulated with closed and open ended questions. The guide was based on culturally specific observations made during the previous 12 months of field-work in the area. Additional information was provided by the key informants, social anthropologists and previous ethnographical studies. The guide was pretested and continually revised after each interviewing session to rephrase unclear questions, replace inappropriate vocabulary with familiar local terms and concepts and adjust sensitive questions. The interview consisted of sections relating to: socio-demographic data; dietary restrictions and proscriptions during pregnancy; lay definitions, symptoms and causes of intestinal worm infections and maternal anaemia; treatment-seeking behaviour for intestinal worm infections and maternal anaemia (Appendix 7). The interviewers were encouraged to allow the respondents to give their opinions spontaneously, and to follow-up concepts expressed by the respondents in more depth. The interviews were conducted in familiar surroundings (for example, the respondent's compound) to place the respondent at ease and maintain confidentiality. Krio was the preferred language as it is easier to translate to English without loss of meaning, but in cases where the respondent was not fluent in Krio, it was more beneficial to conduct the interview in Temne. Forty-two interviews, of approximately one hour's duration each, were conducted.

(iii) Participant observation and case studies

The field-work team spent 15 months living and working in the study area, observing behaviours relating to dietary intake during pregnancy, intestinal worm infections and anaemia. By residing within the Temne community, the interviewers were able to maximise exposure to such behaviours. During this period, qualitative information regarding the Temne notions of illness and the health care systems was also acquired. Individual case studies of relevance to the investigation were compiled from pregnant women participating in the intervention study.

3.10 DATA ENTRY AND STATISTICAL ANALYSIS

The data analysis was conducted by the principal investigator using Statistical Package for Social Scientists for Windows (SPSS Inc. ©) Version 6.1.3. Data entry was checked and frequency and descriptive statistics were employed to identify implausible data. A variety of statistical methods were employed, including Chi-square, Chi-square for trends, analysis of variance, linear regression and logistic regression. Unless otherwise stated, DF=1 (degrees of freedom), p-values are two-sided, and a p-value was considered to be significant if $p<0.05$. To satisfy the assumption of Normality upon which parametric tests are based, continuous variables were examined for departures from normality using Normal Plots and the Kolmogorov-Smirnov test. Where appropriate, non-Normal data was transformed to bring them acceptably close to Normal. The effects of intervention were analysed by intention to treat.

The analyses presented in Chapters 5 to 8 are split into two sections (1) 'baseline (first trimester) analysis' for all 184 women who completed the initial assessment in the first trimester of pregnancy (2) 'longitudinal analysis' for the subset of 125 women who completed all three trimester assessments during pregnancy.

Table 3.1 Score values applied to the components of the socio-economic status scale.

Component of scale	Category	Score
Maternal education	none	0
	primary	1
	secondary	2
	tertiary	3
Occupation (maternal and paternal)	housewife	0
	farmer, fisherman	1
	carpenter, cook, driver, hairdresser, labourer, mason, miner, praise singer, trader, tailor	2
	chief, civil servant, student, Imam, mechanic, pastor, policeman, soldier, teacher	3
Sanitation	no latrine	0
	pit latrine	1
Water supply	stream or swamp	0
	communal well	1
	stand pipe	2
Radio possession	no	0
	yes	1

Chapter 4

Demographic, social and reproductive characteristics

4.1 INTRODUCTION

4.1.1 Use of demographic, social and reproductive information

Information on the demographic, social and reproductive characteristics of the study population contextualises the research setting. This information is relevant to the present investigation as previous studies have indicated that demographic, social and reproductive variables are frequently related to nutritional status, infection status and pregnancy outcome. The overlapping distribution of poor socio-economic status (SES), intestinal nematode infections and malnutrition is widely recognised (Sanjur, 1989). Low SES and poor obstetric history are often associated with adverse pregnancy outcomes (West Suitor, 1994).

Randomisation ensured that the allocation of the intervention groups to subjects was left purely to chance, but did not guarantee that the intervention groups were comparable with respect to baseline characteristics. There was a chance possibility that the intervention groups were dissimilar with respect to one or more of the demographic, social and reproductive variables. As it was possible that the outcome variables of interest were a function of these variables, it was important to assess the homogeneity of the four intervention groups at baseline. In this way, adjustments could be made for imbalanced variables of suspected prognostic importance in subsequent statistical analyses.

4.1.2 Objectives

The objectives of the work described in this chapter were:

1. To describe the demographic, social and reproductive characteristics of the study sample of women in the first trimester of pregnancy.
2. To assess the homogeneity of the four intervention groups with respect to demographic, social and reproductive characteristics.

4.2 METHODS

4.2.1 Data analysis

Two-way analysis of variance (ANOVA) was used to assess the homogeneity of the intervention groups with respect to continuous socio-demographic variables. The main intervention effects (iron-folate supplements and albendazole treatment) and the interaction effect for the interventions were entered into the ANOVA model simultaneously. Where the assumptions of two-way ANOVA were not met, the Scheirer-Ray-Hare extension of the Kruskal-Wallis Test (Scheirer *et al.*, 1976) was employed. This nonparametric method is essentially a two-way ANOVA on the ranked data, in which the ratio of sum of squares of the ranks (*SS*) divided by the total mean sum of squares of the ranks (MS_{total}), SS/MS_{total}, is a χ^2-distributed variable with degrees of freedom pertaining to SS.

Multiple logistic regression was used to assess the homogeneity of the intervention groups for bivariate socio-demographic and socio-economic variables. The main intervention effects and the interaction effect for the interventions were entered into the multiple logistic regression model simultaneously. For categorical variables with more than two categories, the frequency in each category was compared to the sum frequencies of the other categories, as logistic regression requires that the dependant variable is bivariate.

The homogeneity of the intervention groups at baseline was assessed for two groups of subjects (1) all 184 subjects enrolled at baseline (2) the longitudinal cohort of 125 subjects who completed all three trimester assessments during pregnancy.

The intervention groups are defined as follows: FeA = iron-folate supplements and albendazole treatment; FeP_A = iron-folate supplements and albendazole placebo; $P_{Fe}A$ = iron-folate placebo and albendazole treatment; $P_{Fe}P_A$ = iron-folate placebo and albendazole placebo.

4.3 RESULTS

4.3.1 Demographic, social and reproductive characteristics at baseline

The baseline demographic, social and reproductive characteristics of the 184 subjects enrolled at baseline are shown in Table 4.1. The season and gestational age of the subjects at the first trimester assessment are given in Table 4.2. The characteristics of the subjects withdrawn at baseline due to low haemoglobin concentration (Hb<80 g/l), who were not allocated an intervention group, are shown separately.

(i) Age

Maternal age to the nearest birthday at enrolment ranged from 15 to 40 years, mean 25.0 years (SD 5.8). Thirty-eight (20.7%) women were less than 20 years of age.

(ii) Rural/peri-urban location

Rural subjects (Kaffu-Bollom and Lokomasama chiefdoms) comprised 63.6% of the study population. The remainder (36.4%) were residents of peri-urban Freetown. The civil war had recently displaced 5.4% of subjects from rebel-controlled districts. All subjects were living at sea level.

(iii) Ethnic group

Twelve of the 17 ethnic groups in Sierra Leone were represented in the study population. Three ethnic groups, the Temne, Susu and Mende accounted for 87.0% of the subjects with smaller numbers from the remaining groups (Creole, Fullah, Kissi, Koranko, Limba, Loko, Madingo, Sherbro and Wolof). Peri-urban Freetown was more cosmopolitan than the rural areas with eleven tribes represented as compared to eight, but the Temne was the predominant group in both areas (58.2% and 59.8% in the peri-urban and rural areas respectively).

(iv) Marital status

The majority of subjects (90.2%) were married. Most marriages were polygamous, particularly in the rural areas. Only 8.2% of subjects were single, and these women tended to be younger women who resided with their parents. The remaining subjects (1.6%) were widows.

(v) Maternal education

Almost two-thirds of subjects (65.2%) were without formal education, 13.6% had received primary education only and 20.7% had reached secondary level. One subject had received tertiary education.

(vi) Sanitation and water supply

All but 10.3% of subjects had access to a pit latrine in their household compound. Only 4.9% of subjects relied entirely on rivers, streams or swamps for drinking water. Almost all those who had access to pumped water (58.2%) or a communal well (37.0%) also used natural sources of water, as pumps were not open at all times, and wells often dried up during the dry season. Rain water was collected and stored during the wet season.

(vii) Maternal and paternal occupation

Most subjects were involved in some form of income generating activity, although 18.5% worked only in the home. Petty trading (62.5%) was the most common form of income generating activity followed by farming (13.6%). The majority of husbands (n=166) were fisherman (28.3%), farmers (17.5%) or traders (13.3%).

(viii) Radio ownership

The households belonging to 67.9% of subjects possessed a radio.

(ix) Reproductive characteristics

Gravidity ranged from one to eleven (median 3). Primigravidae accounted for 16.3% of the study population and grandmultigravidae (gravidity ≥6) for 25.0%. Only 12.0% of subjects had used modern methods of contraception. Among multigravidae (n=154), the median interval since the previous pregnancy was 28 months, although 15.6% of multigravidae had been pregnant within 12 months of the present pregnancy and 7.1% within

6 months. Previous miscarriage, stillbirth and child death was reported by 27.9%, 8.4% and 42.9% of multigravidae respectively.

(x) Other medical history

Only 8.2% subjects reported to be currently smoking. In most cases, smoking habits amounted to an occasion cigarette taken once or twice a week. Nine subjects (4.9%) believed that they had sickle cell disease.

(xi) Season and gestational age at first trimester assessment

The proportion of subjects enrolled the early dry season (November to January), late dry season (February to April) and early wet season (May to July) was 43.5%, 35.3% and 21.2% respectively. The mean gestational age at the first trimester assessment was 12.3 weeks (SD 2.6).

4.3.2 Comparison of intervention groups

(i) Subjects enrolled at baseline

The statistical analysis indicated that for all subjects enrolled at baseline, the intervention groups differed significantly at baseline with respect to only two demographic, social and reproductive characteristics. The proportion of women who received the iron-folate placebo was significantly greater among women whose husbands were fishermen as compared to women whose husbands were engaged in non-fishing occupations (Multiple logistic regression, n=161: iron-folate Wald=5.25, p=0.020; albendazole Wald=0.06, p=0.80; interaction Wald=0.38, p=0.54). The proportion of multigravidae who received the iron-folate placebo was significantly greater for those who had a history of stillbirth (Multiple logistic regression, n=150: iron-folate Wald=4.01, p=0.045; albendazole Wald=0.08, p=0.77; interaction Wald=0.90, p=0.34).

The proportion of subjects whose first trimester assessment was in the early wet season (May to July) was not similar for all intervention groups (Table 4.2). The logistic regression analysis indicated a significant interaction between the interaction effects (iron-folate supplements and albendazole treatment), and therefore pairs of intervention groups were compared. These results indicated that the proportion of subjects whose first trimester assessment was in the early wet season was greater for intervention group $P_{Fe}A$ than for intervention group FeA (Chi-square, n=89, χ^2=4.90, p=0.027) and intervention group $P_{Fe}P_A$ (Chi-square, n=87, χ^2=7.03, p=0.008). The intervention groups were similar with respect to the gestational age of the pregnant women at the first trimester assessment.

(ii) Longitudinal cohort.

The baseline demographic, social and reproductive characteristics of the 125 subjects in the longitudinal cohort are shown in Table 4.3. There were no significant differences in baseline demographic, social and reproductive characteristics between the intervention groups.

The proportion of subjects whose first trimester assessment was in the early wet season was higher for intervention group $P_{Fe}A$ than for intervention group FeA (Chi-square, n=61, χ^2=8.72, p=0.003) and intervention group $P_{Fe}P_A$ (Chi-square, n=58, χ^2=5.76, p=0.016) (Table 4.4). The gestational ages of the subjects at each of the three trimester assessments during pregnancy, and the time interval between first and third assessments, was similar for all the intervention groups.

4.4 DISCUSSION

The baseline questionnaire was designed to contextualise the research setting with respect to the demographic, social and reproductive characteristics of the study population. The study population comprised pregnant women living in rural areas in Kaffu-Bollom and Lokomasama Chiefdoms and peri-urban areas of Freetown. These communities appeared to be well supplied with sanitary and water facilities, with only one in ten women without a pit latrine and one in twenty without access to a well or water pump. However, the presence of a pit latrine did not guarantee utilisation, as the code of social conduct controlled access for some women, who were prohibited to use the latrine if their mother-in-law was residing within the same household (J.B. Juana[1], personal communication). Most women were often forced to use natural sources of water, as the water pumps were only open at restricted times, and wells often dried up during the dry season.

The Temne was the predominant ethnic group in both rural and peri-urban communities, followed by the Susu and Mende. Almost all the women were married, two-thirds had no formal education and three-quarters were engaged in petty trading or farming. The reproductive histories of the study population was characterised by high gravidity and high pregnancy wastage. One fifth of the study population was below 20 years of age. Grandmultigravidae accounted for one quarter of the study population. Over one quarter of multigravidae had miscarried during at least one previous pregnancy, almost one-tenth had experienced one or more stillbirths and over two-fifths had lost at least one child. The reported frequency of adverse pregnancy outcomes is likely to be an underestimate of the true value. Women prefer to conceal abortions, miscarriages, stillbirths and child deaths, as these events are frequently attributed to supernatural causes such as witchcraft (see Chapter 11).

---

[1] State-enrolled community health nurse

Abstinence from sexual intercourse remains the predominant means of birth spacing, with only one in eight women reporting to have used any modern methods of contraception. Even so, less than one in six women had been pregnant during the 12 months prior to the present pregnancy. A cultural taboo[2] forbids lactating mothers to have sexual intercourse, and since infants are usually breast-fed for at least one year, deliveries tend to be well spaced. However, if the pregnancy results in miscarriage or stillbirth, or if the child dies, a woman will immediately attempt to become pregnant again (Bailey, 1989). Eleven subjects had completed the previous pregnancy within six months of the present pregnancy. Frequent pregnancies may contribute to maternal nutritional depletion (Merchant & Martorell, 1990) if the mother is unable to replenish nutrient stores within a short inter-birth interval.

The prevalence of smoking during pregnancy was low, and the smoking habits reported by smokers are unlikely to pose a significant threat to pregnancy outcome[3] in the study population.

Only one in twenty subjects claimed to suffer from sickle cell disease. This number is unlikely to reflect the prevalence of sickle cell disease in the study population as the heterozygous sickle cell trait (HbAS), which accounts for the majority of sickle cell disease in a population, is usually subclinical. It is possible that symptoms of heterozygous sickle cell disease were not recognised amidst the plethora of other illnesses occurring in the population. A previous study in Sierra Leone reported that 22.1% (n=3,524) of patients attending the Ramsy Medical Laboratory in Freetown had sickle cell disease. A sub-sample (n=344) were separated into their haemoglobin variants by electrophoresis and 5% found to have homozygous sickle cell anaemia (HbSS) (Wurie *et al.*, 1996). However, this sample was not random, and included subjects requiring routine pre-employment and pre-emigration health examinations, antenatal screening and those presenting with symptoms of anaemia.

The distribution of the baseline social, demographic and reproductive characteristics among the intervention groups was statistically similar for all patients enrolled at baseline apart from two variables. Given that over 30 comparisons were made and we would expect one in twenty comparisons to be significant by change alone using a significance level of 5%, the intervention groups can be considered statistically homogenous at baseline with respect to these variables. The distribution of these variables was also similar for the longitudinal cohort of women. This suggests that the drop-outs from the study were not markedly atypical with respect to these variables.

---

[2] Sexual intercourse is believed to spoil breast milk and thus cause diarrhoea in the infant (MNDEP/UNICEF, 1989)

[3] The effects of smoking during pregnancy include intrauterine growth retardation, premature delivery and increased perinatal mortality (Meyer *et al.*, 1976).

A certain amount of caution must be excerised in making the assumption of homogeneity using hypothesis testing. The use of randomisation in the allocation of intervention group means that any differences between the groups are due to chance alone (Altman, 1985). A non-significant result simply infers that there is no gross violation of the hypothesis; any differences would have to be very large in order to reject the hypothesis. In essence, hypothesis testing merely verifies that the randomisation process has been performed fairly (Altman, 1991). Furthermore, the size of the trial affects the significance of a difference, and for smaller trials is it possible to have an imbalance that is not statistically significant but which could be clinically important (Altman, 1991). Closer inspection of the four intervention groups was therefore required to identify demographic, social and reproductive variables that were imbalanced clinically rather than statistically. This involves a degree of subjectivity because a difference that is considered to be of clinical importance must be set for each variable. This is not a simple matter, particularly for variables whose effect on the outcome variables is unknown.

Most variables appear to be clinically balanced among the intervention groups, although there are some exceptions, all involving the $P_{Fe}P_A$ and FeA intervention groups: the proportion of peri-urban subjects in intervention group $P_{Fe}P_A$ was almost double that in intervention group FeA; the proportion of subjects with secondary or tertiary education in intervention group $P_{Fe}P_A$ was over double that in intervention group FeA; and the proportion of subjects who had used modern contraceptive methods in intervention group $P_{Fe}P_A$ was almost double that in intervention group FeA (or over double in the longitudinal cohort). It is likely that these differences were inter-related, and centred on the higher proportion of peri-urban residents in the $P_{Fe}P_A$ intervention groups: peri-urban residents have better access to schools and modern family planning treatment, which may underlie the longer intervals between deliveries.

Notable differences were also found in the proportion of subjects whose first trimester assessment was in the early wet season. Season is an important variable to consider, as seasonality is also closely linked with the incidence of infections and nutritional status in tropical settings (Ferro-Luzzi & Branca, 1993; Tomkins, 1993). The proportion of subjects in intervention group $P_{Fe}A$ whose first trimester assessment was in the early wet season was significantly higher than for intervention groups FeA and $P_{Fe}P_A$. This disparity was due to the reallocation of intervention groups from non-pregnant women who were excluded from the study to new subjects enrolled from May 1996. A disproportionate number of the non-pregnant women had been allocated intervention group $P_{Fe}A$ (see Section 2.2.5 (i)). Inspection of Tables 4.2 and 4.4 clearly indicates some variation among the other seasons that, although not significant, may interfere with the analyses of the intervention effects.

As the effect of the imbalances is unclear, the analyses in the following chapters were conducted with and without these variables and other variables of known or suspected prognostic importance with respect to the outcome variables of interest. If the effect of adding the variable as a covariate was negligible, it was ignored, but otherwise the adjusted analysis is quoted. The demographic, social and reproductive data is also examined to determine whether this information can usefully identify groups at special risk of parasitic infection, poor nutritional status or adverse pregnancy outcomes.

4.5 SUMMARY

1. Baseline information on the demographic, social and reproductive characteristics of the study population comprising pregnant women in their first trimester in Western Sierra Leone was obtained by questionnaire. These characteristics are described, and the homogeneity of the four intervention groups with respect to the characteristics is assessed.
2. Both rural and peri-urban communities appeared to be well supplied with sanitary and water facilities, although access to sanitary facilities was controlled by social rules and access to water facilities was limited by restrictions on the opening times of the wells and the seasonality of the water supply.
3. Most women were of the Temne, Susu or Mende ethnic group, were married, had no formal education and were petty traders or farmers.
4. The reproductive histories of the women were characterised by high gravidity and high pregnancy wastage. Despite the dependence on abstinence as a means of contraception, deliveries appeared to be well spaced, unless a pregnancy resulted in miscarriage or stillbirth or if a child died.
5. The prevalence of smoking during pregnancy was low, and the smoking habits reported by smokers are unlikely to pose a significant threat to pregnancy outcome in the study population.
6. Sickle cell disease was under-reported; it is possible that symptoms of heterozygous sickle cell trait are not recognised amidst the plethora of other illnesses occurring in the population.
7. The four intervention groups were statistically similar at baseline with respect to almost all baseline demographic, social and reproductive characteristics. The distribution of baseline demographic, social and reproductive characteristics was also similar among the four intervention groups for the longitudinal cohort. This suggests that the drop-outs from the study were not markedly atypical with respect to these variables.

8. The clinical importance of differences that were not statistically significant is discussed.

Table 4.1 Baseline (first trimester) demographic, social and reproductive characteristics of all pregnant women enrolled at baseline.

			Intervention group†					Total (*n*=184)
			FeA (*n*=43)	FeP_A (*n*=46)	$P_{Fe}A$ (*n*=44)	$P_{Fe}P_A$ (*n*=45)	None (*n*=6)	
Age (years)		mean (SD)	25.4 (6.0)	24.5 (5.5)	24.5 (5.9)	26.2 (6.0)	20.8 (3.1)	25.0 (5.8)
Residence	rural	frequency (%)	33 (76.6)	29 (63.0)	27 (61.4)	26 (57.8)	2 (33.3)	117 (63.6)
	peri-urban	frequency (%)	10 (23.3)	17 (37.0)	17 (38.6)	19 (42.2)	4 (66.7)	67 (36.4)
Displaced persons		frequency (%)	1 (2.3)	1 (2.2)	5 (11.4)	3 (6.7)	0 (0.0)	10 (5.4)
Ethnic group	Temne	frequency (%)	29 (67.4)	27 (58.7)	26 (59.1)	22 (48.9)	5 (83.3)	109 (59.2)
	Susu	frequency (%)	7 (16.3)	9 (19.6)	7 (15.9)	10 (22.2)	0 (0.0)	33 (17.9)
	Mende	frequency (%)	4 (9.3)	4 (8.7)	5 (11.4)	4 (8.9)	1 (16.7)	18 (9.8)
	other	frequency (%)	3 (7.0)	6 (13.0)	6 (13.6)	9 (20.0)	0 (0.0)	24 (13.0)
Marital status	single	frequency (%)	2 (4.7)	5 (10.9)	4 (9.1)	3 (6.7)	1 (16.7)	15 (8.2)
	married	frequency (%)	41 (95.3)	41 (89.1)	39 (88.6)	40 (88.9)	5 (83.3)	166 (90.2)
	widowed	frequency (%)	0 (0.0)	0 (0.0)	1 (2.3)	2 (4.4)	0 (0.0)	3 (1.6)
Education	none	frequency (%)	31 (72.1)	32 (69.6)	27 (61.4)	28 (62.2)	2 (33.3)	120 (65.2)
	primary	frequency (%)	8 (18.6)	5 (10.9)	5 (11.4)	6 (13.3)	1 (16.7)	25 (13.6)
	secondary/ tertiary	frequency (%)	4 (9.3)	9 (19.6)	12 (27.3)	11 (24.4)	3 (50.0)	39 (21.2)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 4.1 Baseline (first trimester) demographic, social and reproductive characteristics of all pregnant women enrolled at baseline (continued).

			Intervention group[†]					Total (n=184)
			FeA (n=43)	FeP_A (n=46)	$P_{Fe}A$ (n=44)	$P_{Fe}P_A$ (n=45)	None (n=6)	
Sanitation	pit latrine	frequency (%)	41 (95.3)	41 (89.1)	38 (86.4)	39 (86.7)	6(100.0)	165 (89.7)
Water supply	natural	frequency (%)	3 (7.0)	1 (2.2)	3 (6.8)	1 (2.2)	1 (16.7)	9 (4.9)
	well	frequency (%)	18 (41.9)	13 (28.3)	15 (34.1)	20 (44.4)	2 (33.3)	68 (37.0)
	stand pipe	frequency (%)	22 (51.2)	32 (69.6)	26 (59.1)	24 (53.3)	3 (50.0)	107 (58.2)
Occupation	at home	frequency (%)	4 (9.3)	9 (19.6)	10 (22.7)	9 (20.0)	2 (33.3)	34 (18.5)
	trader	frequency (%)	28 (65.1)	29 (63.0)	25 (56.8)	30 (66.7)	3 (50.0)	115 (62.5)
	farmer	frequency (%)	7 (16.3)	5 (10.9)	7 (15.9)	5 (11.1)	1 (16.7)	25 (13.6)
	other	frequency (%)	4 (9.3)	3 (6.5)	2 (4.5)	1 (2.2)	0 (0.0)	10 (5.4)
Husband's occupation[‡]	farmer	frequency (%)	6 (14.6)	6 (14.6)	9 (23.1)	8 (20.0)	0 (0.0)	29 (17.5)
	fisherman	frequency (%)	16 (39.0)	13 (31.7)	7 (17.9)	8 (20.0)	3 (60.0)	47 (28.3)
	trader	frequency (%)	8 (19.5)	6 (14.6)	2 (5.1)	5 (12.5)	1 (20.0)	22 (13.3)
	other	frequency (%)	11 (26.8)	16 (39.0)	21 (53.8)	19 (47.5)	1 (20.0)	68 (41.0)
Radio ownership		frequency (%)	33 (76.7)	31 (67.4)	28 (63.6)	29 (64.4)	4 (66.7)	125 (67.9)
Socio-economic status	low	frequency (%)	22 (51.2)	25 (54.3)	21 (47.7)	19 (42.2)	2 (33.3)	89 (48.4)

[†] Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo
[‡] Among married subjects only.

Table 4.1 Baseline (first trimester) demographic, social and reproductive characteristics of all pregnant women enrolled at baseline (continued).

		Intervention group†					Total (n=184)
		FeA (n=43)	FeP_A (n=46)	$P_{Fe}A$ (n=44)	$P_{Fe}P_A$ (n=45)	None (n=6)	
Gravidae	median (QR‡)	3 (2, 5)	3 (2, 6)	3 (2, 5)	4 (2, 6)	2 (1, 3)	3 (2, 6)
Primigravidae	frequency (%)	7 (16.3)	10 (21.7)	8 (18.2)	3 (6.7)	2 (33.3)	30 (16.3)
Grandmultigravidae	frequency (%)	10 (23.3)	13 (28.3)	10 (22.7)	13 (28.9)	0 (0.0)	46 (25.0)
Months since previous delivery§	median (QR‡)	27 (24, 43)	26 (12, 36)	27 (22, 36)	36 (24, 48)	28 (22, 36)	28 (22, 36)
Number of children§	median (QR‡)	2 (1, 3)	2 (1, 3)	2 (1, 4)	2 1, 4)	1 (1, 2)	2 (1, 3)
Previous miscarriage§	frequency (%)	9 (25.0)	14 (38.9)	10 (27.8)	9 (21.4)	1 (25.0)	43 (27.9)
Previous still birth§	frequency (%)	4 (11.1)	6 (16.7)	2 (5.6)	1 (2.4)	0 (0.0)	13 (8.4)
Previous child death§	frequency (%)	18 (50.0)	16 (44.4)	15 (41.7)	17 (40.5)	0 (0.0)	66 (42.9)
Use of modern contraceptives	frequency (%)	5 (11.6)	5 (10.9)	3 (6.8)	9 (20.0)	0 (0.0)	22 (12.0)
Smokers	frequency (%)	4 (9.3)	6 (13.0)	3 (6.8)	2 (4.4)	0 (0.0)	15 (8.2)
Number of cigarettes per week††	median (range)	1 (1, 1)	2 (1, 7)	1 (1, 4)	8 (1, 14)	0	1 (1, 14)
Sickle cell disease	frequency (%)	3 (7.0)	4 (8.7)	1 (2.3)	1 (2.2)	0 (0.0)	9 (4.9)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo

‡ QR = quartile range; range is given for the 'none' intervention group.

§ Among multigravidae only.

†† Among smokers only.

Table 4.2 Season and gestational age at the first trimester assessment of all pregnant women enrolled at baseline.

			Intervention group[†]					Total (n=184)
			FeA (n=43)	FeP_A (n=46)	$P_{Fe}A$ (n=44)	$P_{Fe}P_A$ (n=45)	None (n=6)	
Season at first trimester assessment[‡]	early dry	frequency (%)	25 (58.1)	19 (41.3)	15 (34.1)	19 (42.2)	2 (33.3)	80 (43.5)
	late dry	frequency (%)	13 (30.2)	19 (41.3)	12 (27.3)	19 (42.2)	2 (33.3)	65 (35.3)
	early wet	frequency (%)	5 (11.6)	8 (17.4)	17 (38.6)	7 (15.6)	2 (33.3)	39 (21.2)
Gestation at first trimester assessment (weeks)		mean (SD)	12.5 (2.4)	12.7 (2.7)	11.4 (1.9)	12.4 (3.1)	12.8 (3.4)	12.3 (2.6)

[†] Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo
[‡] Early dry = November to January; late dry = February to April; early wet = May to July.

Table 4.3 Baseline (first trimester) demographic, social and reproductive characteristics of the longitudinal cohort of pregnant women.

			Intervention group[†]				Total (n=125)
			FeA (n=32)	FeP_A (n=35)	$P_{Fe}A$ (n=29)	$P_{Fe}P_A$ (n=29)	
Age (years)		mean (SD)	25.5 (6.0)	24.7 (5.2)	24.7 (5.6)	25.7 (5.2)	25.1 (5.5)
Residence	rural	frequency (%)	25 (78.1)	22 (62.9)	18 (62.1)	17 (58.6)	82 (65.6)
	peri-urban	frequency (%)	7 (21.9)	13 (37.1)	11 (37.9)	12 (41.4)	43 (34.4)
Displaced persons		frequency (%)	0 (0.0)	1 (2.9)	4 (13.8)	0 (0.0)	5 (4.0)
Ethnic group	Temne	frequency (%)	22 (68.8)	20 (57.1)	19 (65.5)	14 (48.3)	75 (60.0)
	Susu	frequency (%)	6 (18.8)	7 (20.0)	2 (6.9)	8 (27.6)	23 (18.4)
	Mende	frequency (%)	2 (6.2)	3 (8.6)	4 (13.8)	3 (10.3)	12 (9.6)
	other	frequency (%)	2 (6.2)	5 (14.3)	4 (13.8)	4 (13.8)	15 (12.0)
Marital status	single	frequency (%)	2 (6.3)	2 (5.7)	2 (6.9)	2 (6.9)	8 (6.4)
	married	frequency (%)	30 (93.8)	33 (94.3)	26 (89.7)	26 (89.7)	115 (92.0)
	widowed	frequency (%)	0 (0.0)	0 (0.0)	1 (3.4)	1 (3.4)	2 (1.6)
Education	none	frequency (%)	23 (71.9)	24 (68.6)	18 (62.1)	14 (48.3)	79 (63.2)
	primary	frequency (%)	6 (18.8)	3 (8.6)	5 (17.2)	6 (20.7)	20 (16.0)
	secondary/ tertiary	frequency (%)	3 (9.4)	8 (22.9)	6 (20.7)	9 (31.0)	26 (20.8)

[†] Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo

Table 4.3 Baseline (first trimester) demographic, social and reproductive characteristics of the longitudinal cohort of pregnant women (continued).

			Intervention group†				Total (n=125)
			FeA (n=32)	FeP_A (n=35)	$P_{Fe}A$ (n=29)	$P_{Fe}P_A$ (n=29)	
Sanitation	pit latrine	frequency (%)	30 (93.8)	30 (85.7)	26 (89.7)	26 (89.7)	112 (89.6)
Water supply	natural	frequency (%)	2 (6.3)	1 (2.9)	2 (6.9)	0 (0.0)	5 (4.0)
	well	frequency (%)	13 (40.6)	12 (34.3)	13 (44.8)	12 (41.4)	50 (40.0)
	stand pipe	frequency (%)	17 (53.1)	22 (62.9)	14 (48.3)	17 (58.6)	70 (56.0)
Occupation	at home	frequency (%)	2 (6.3)	7 (20.0)	6 (20.7)	5 (17.2)	20 (16.0)
	trader	frequency (%)	19 (59.4)	21 (60.0)	17 (58.6)	21 (72.4)	78 (62.4)
	farmer	frequency (%)	7 (21.9)	4 (11.4)	4 (13.8)	2 (6.9)	17 (13.6)
	other	frequency (%)	4 (12.5)	3 (8.6)	2 (6.9)	1 (3.4)	10 (8.0)
Husband's occupation‡	farmer	frequency (%)	6 (20.0)	5 (15.2)	5 (19.2)	4 (15.4)	20 (17.4)
	fisherman	frequency (%)	12 (40.0)	10 (30.3)	5 (19.2)	5 (19.2)	32 (27.8)
	trader	frequency (%)	6 (20.0)	4 (12.1)	1 (3.8)	2 (7.7)	13 (11.3)
	other	frequency (%)	6 (20.0)	14 (42.4)	15 (57.7)	15 (57.7)	50 (43.5)
Radio ownership		frequency (%)	24 (75.0)	25 (71.4)	18 (62.1)	21 (72.4)	88 (70.4)
Socio-economic status	low	frequency (%)	19 (59.4)	19 (54.3)	14 (48.3)	9 (31.0)	61 (48.8)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo
‡ Among married subjects only.

Table 4.3 Baseline (first trimester) demographic, social and reproductive characteristics of the longitudinal cohort of pregnant women (continued).

		Intervention group[†]				Total (n=125)
		FeA (n=32)	FeP_A (n=35)	$P_{Fe}A$ (n=29)	$P_{Fe}P_A$ (n=29)	
Gravidae	median (QR[‡])	4 (3, 7)	4 (3, 7)	4 (3, 6)	4 (3, 6)	4 (3, 6)
Primigravidae	frequency (%)	6 (18.8)	7 (20.0)	4 (13.8)	2 (6.9)	19 (15.2)
Grandmultigravidae	frequency (%)	8 (25.0)	10 (28.6)	8 (27.6)	8 (27.6)	34 (27.2)
Months since previous delivery[§]	median (QR[‡])	31 (18, 48)	27 (12, 37)	24 (22, 36)	36 (24, 48)	28 (22, 42)
Number of children[§]	median (QR[‡])	3 (1, 3)	2 (1, 3)	2 (1, 4)	3 (1, 4)	2 (1, 3)
Previous miscarriage[§]	frequency (%)	8 (30.8)	9 (32.1)	7 (28.0)	4 (14.8)	28 (26.4)
Previous still birth[§]	frequency (%)	4 (15.4)	6 (21.4)	2 (8.0)	0 (0.0)	12 (11.3)
Previous child death[§]	frequency (%)	12 (46.2)	12 (42.9)	10 (40.0)	10 (37.0)	44 (41.5)
Use of modern contraceptives	frequency (%)	4 (12.5)	4 (11.4)	2 (6.9)	8 (27.6)	18 (14.4)
Smokers	frequency (%)	3 (9.4)	4 (11.4)	1 (3.4)	0 (0.0)	8 (6.4)
Sickle cell disease	frequency (%)	2 (6.3)	3 (8.6)	0 (0.0)	0 (0.0)	5 (4.0)

[†] Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo
[‡] QR = quartile range.
[§] Among multigravidae only.

Table 4.4 Season at first trimester assessment and gestational age assessments of the longitudinal cohort of pregnant women.

			Intervention group[†]				Total (*n*=125)
			FeA (*n*=32)	FeP_A (*n*=35)	$P_{Fe}A$ (*n*=29)	$P_{Fe}P_A$ (*n*=29)	
Season at first trimester assessment[‡]	early dry	frequency (%)	18 (56.3)	17 (48.6)	9 (31.0)	12 (41.4)	56 (44.8)
	late dry	frequency (%)	12 (37.5)	12 (34.3)	8 (27.6)	14 (48.3)	46 (36.8)
	early wet	frequency (%)	2 (6.3)	6 (17.1)	12 (41.4)	3 (10.3)	23 (18.4)
Gestation at assessments (weeks)	first trimester	mean (SD)	12.7 (2.4)	12.9 (2.9)	11.4 (2.1)	12.7 (3.2)	12.4 (2.8)
	second trimester	mean (SD)	23.0 (3.6)	22.8 (2.8)	23.1 (2.7)	22.4 (2.2)	22.9 (2.9)
	third trimester	mean (SD)	36.5 (2.0)	36.1 (1.9)	36.3 (1.6)	36.4 (2.3)	36.3 (1.9)
Time interval between first and third assessments (weeks)		mean (SD)	23.8 (3.0)	23.3 (2.7)	24.9 (2.2)	23.8 (3.2)	23.9 (2.8)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo
‡ Early dry = November to January; late dry = February to April; early wet = May to July.

Chapter 5

Dietary iron intake

5.1 INTRODUCTION

5.1.1. Dietary intake during pregnancy

The close association between maternal nutrition, pregnancy outcome and the future health of the mother and child has been recognised for many years. During pregnancy, the requirements for energy, macronutrients and micronutrients are increased to support the expansion of the maternal and fetal tissues (Hytten & Leitch, 1971). The increased requirements of some nutrients are met in part or in whole by the pre-pregnancy nutrient stores, behavioural adaptation (for example, reduced physical activity) or physiological adaptation. These mechanisms do not meet the additional requirements of all nutrients, and there is little doubt that dietary intake should increase (FAO/WHO 1974; 1985; 1988).

The inadequate intake of absorbable dietary iron is regarded as one of the most important causes of negative iron balance during pregnancy (ACC/SCN, 1997). The nutritional adequacy of dietary iron intake depends not only on the absolute amount in the diet, but on the proportion absorbed. There are two types of dietary iron, haem iron and non-haem iron. Haem iron is present in haemoglobin and myoglobin, and is usually consumed as meat or fish. The absorption of haem iron is high (about 25%) and is little affected by the iron status of a subject or the composition of the diet (Bothwell *et al.*, 1989). Non-haem iron is predominantly of vegetable origin. The absorption of non-haem iron is determined by iron status and the interplay of dietary factors which enhance and inhibit absorption (Bothwell *et al.*, 1989). These factors must also be considered when dietary iron intake is assessed. Meat and vitamin C (ascorbic acid) are known to promote non-haem absorption, while polyphenols (including tannins), components of dietary fibre (such as phytates) and calcium inhibit non-haem absorption (Bothwell *et al.*, 1989). In developing countries, the dietary intake tends to be low in haem-iron content, low in promoters of non-haem iron absorption and high in inhibitors of non-haem iron absorption. The quantity and quality of this intake is determined not only by socio-economic status and the variation in food availability, but by cultural restrictions on the choice of acceptable food items during pregnancy (Jackson & Jackson, 1987).

5.1.2 Assessment of dietary intake during pregnancy

Information on dietary intake can help identify whether poor dietary iron intake is an underlying cause of maternal iron-deficiency and anaemia. There are several methods for quantifying dietary intake. These vary in their requirements for material and human resources, the ease of administration, labour intensivity, the degree of invasiveness and reliability of the data. The 24-hour dietary recall method is the most frequently used technique and is recommended for large scale field studies in pregnancy (Rush & Kristal, 1982). Relative to other dietary intake methods, it requires minimal resources, is quick and simple to conduct, is appropriate for illiterate subjects and can be integrated into standard antenatal care thereby minimising respondent burden (Gibson, 1990c).

5.1.3 Aims and objectives

The aim of the work described in this chapter was to examine the adequacy of the dietary intake of the women during pregnancy, and to assess the contribution of poor dietary iron intake to iron deficiency and anaemia.

The specific objectives were:

1. To present the mean dietary intake of energy, protein, carbohydrate, fat, iron, calcium vitamin A, vitamin C and folate of the women during pregnancy.
2. To compare these intakes with the daily requirements of energy and nutrients proposed by joint expert panels of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) (FAO/WHO, 1974; 1985; 1988).

5.2 METHODS

5.2.1 Limitations of the data

A single 24-h recall with adequate representation of the days of the week is most appropriate for assessing the average intake for a group (Gibson, 1990). As the day-to-day variation in dietary intake is known to be considerable (Balogh *et al.*, 1971; Sempos *et al.*, 1985; Nelson *et al.*, 1989), repeat 24-h recalls are necessary if the purpose of the assessment is to estimate the usual intake of individuals, the proportion of the population at risk of inadequate intake or for statistical analyses involving correlation or regression (Balogh *et al.*, 1971; Sempos *et al.*, 1985; Nelson *et al.*, 1989; Launer *et al.*, 1991). In the present study, repeat recalls were not feasible due to insufficient human resources and time and budgetary constraints. Therefore, the study did not seek to measure the usual intakes of individuals, but to characterise the intake of the pregnant women as a group. The sources of error in the dietary intake data will be largely eliminated in the overall group means.

Seasonal variation in food intake is common in communities with a monomodal wet season that rely on locally grown crops (Bates *et al.*, 1994). There is a marked wet and dry season in Sierra Leone, but the effect of season on dietary intake was not examined in the present study as this would have required a much more extensive investigation with multiple repeat dietary recalls. The subjects were enrolled over a six-month period, with the first trimester 24-h dietary recalls obtained between December 1995 and June 1996. This period corresponds to the interval between the early dry season and beginning of the rains. Dietary intakes at each assessment represent the mean value over a six-month period, and are not representative of the average yearly intake.

5.2.2 Data analysis

No recommended daily intake[1] (RDI) standards have been formulated for Sierra Leone. The adequacy of the dietary intakes was assessed by comparison with the internationally recognised standards set by the FAO and WHO (FAO/WHO 1974; 1985; 1988). These standards values, and the limitations associated with their use as indicators of dietary sufficiency, are given in Appendix 8.

(i) Baseline (first trimester) analysis

The mean (±SD) daily intake for energy and each macronutrient was computed. As the daily intake of micronutrients (minerals and vitamins) was highly skewed, the data was transformed using natural logarithms, and the geometric means (±SD) presented. Intake from mineral and vitamin supplements is not included in the estimates of micronutrient intake.

(ii) Longitudinal analysis

Only the longitudinal cohort (n=125) was included in the longitudinal analysis, as only these subjects provided all necessary measurements and samples at each assessment. The mean (±SD) daily intake for energy and each macronutrient and the geometric mean (±SD) of each micronutrient was computed for each trimester assessment. The micronutrient density (dietary intake per 1 MJ) was also determined. Intake from mineral and vitamin supplements is not included in the estimates of micronutrient intake.

5.3 RESULTS

5.3.1 Diet and feeding patterns

Meals tend to be communal affairs in Sierra Leone, and family members usually eat from a common dish. Men tend to eat separately from women and children. Food is prepared on open fires located within the household compound. Although meal frequency and time of eating is not fixed, most families have two main meals a day, one in the morning and one in the late afternoon or early evening. The first meal of the day may comprise food left over from the evening meal of the previous day. Food intake does not change markedly according to the

[1] Also known as safe level of intake (FAO/WHO, 1988)

day of the week, although the Creole traditionally eat *fu-fu*[2] (a thick gelatinous paste made from cassava) at the weekends.

The diet is simple and monotonous, as it is dictated by the local availability and prices of the foods. Typical meals contain either boiled rice or cassava roots with soup or *plasas*. The soup usually comprises a vegetable based gruel containing boiled or fried fish; *plasas* is a dish containing green leaves (for example, cassava leaves or sweet potato leaves) and dried fish. Bean dishes (black-eye beans, broad beans) are also popular, but are a less common component of the diet. The condiments that are most frequently added to these dishes include hot peppers, tomatoes, aubergines and groundnuts. *Yebeh* is a popular cassava porridge containing other starchy vegetables such as breadfruit, sweet potatoes or bananas and fish. Cassava is processed using fermentation and drying procedures to produce *gari* (a porridge-like dish) and *fu-fu*. *Gari* may be served as a sweet or savoury dish; the former usually contains sugar and occasionally dried milk, the latter contains savoury condiments such as hot peppers, salt, and oil. *Fu-fu* is usually eaten with *plasas* or 'slippery' soups containing okra.

All the communities studied are coastal, and marine fish is the predominant source of animal protein. Very little non-fish meat was consumed: chicken, beef, goat or mutton occasionally replace the fish in soup or *plasas*, but domestic livestock are usually reserved for religious occasions or for sale. In the communities studied, 'bush' game did not make sizable or consistent contribution to the habitual diet, and was not reported in any 24-h recall.

Palm oil is available widely and accounts for the rarity of overt vitamin A deficiency in the country. It is used in the preparation of *plasas*, although it is sometimes replaced with groundnut oil, coconut oil or palm nut oil. Soup dishes may contain any one of these oils, but imported vegetable (maize) oils have also become popular. 'White' *plasas* or soup contains very little or no oil, but groundnuts are usually added instead.

Vegetable gardens and fruit trees are found on most household compounds, even those in the peri-urban areas. Groundnuts, fruits (orange, bananas, mangoes and guavas) and raw chunks of cassava are mainly eaten as snacks between meals. Many adults chew on cola nut (*Cola nitidi*) in order to stimulate activity and reduce hunger pains (FAO, 1986). Other foods that are sold at road-side stalls and eaten as snacks include home-made cakes and biscuits. Beverages, which include tea (usually served with sugar and powdered milk), soft drinks, beer and palm-wine are not a frequent component of the maternal diet.

A sample 24-h dietary intake profile, based on the most frequent dish combinations and typical portion sizes reported by the subjects, is given in Table 5.1.

---

[2] Local terms have been transcribed using the Roman alphabet and italicised.

5.3.2 Twenty-four-hour dietary recall

(i) Baseline (first trimester) analysis

Values for the energy, macronutrient and micronutrient intake obtained by 24-h dietary recall at baseline are presented in Table 5.2. The mean daily energy intake was 7.72 MJ (1845 kcal), of which 58.6% was estimated to be provided by carbohydrates, 29.2% by fats and 12.2% by proteins. The group mean energy intake was below the FAO/WHO standard for pregnant women, assuming maintenance of the pre-pregnant activity level. The group mean dietary intake of protein was greater than the FAO/WHO standard.

The group geometric mean intake of iron was 6.2 mg. Most of this intake was provided by plant food items: fish, meat and poultry provided only 10.4% (SD 4.0%) of the total energy intake, 51.7% (SD 16.4%) of the total protein intake and 13.2 % (SD 10.9%) of the total iron intake. The FAO/WHO expert panel (FAO/WHO, 1988) only stipulate a daily iron requirement for non-pregnant women, as the requirements in the second and third trimester are not expected to be met by dietary intake, regardless of the quality of the diet. The median basal requirement for non-pregnant women is 10.5 mg (13 mg including variability), and the median amount required to prevent anaemia in non-pregnant women is 7 mg (9 mg including variability) (FAO/WHO, 1988). Relative to the non-pregnant state, iron requirements actually decrease in the first trimester, as the iron conserved by the cessation of menstruation compensates for the slow fetal and maternal growth during this period (thereafter, the high rate of fetal and maternal growth elevate the iron requirements rapidly) (Bothwell *et al.*, 1979). Nonetheless, the group geometric mean intake of iron is low relative to the standards, and it is likely that the iron intake was inadequate for a substantial proportion of the pregnant women.

The group geometric mean dietary intake of folate and calcium were also lower than the FAO/WHO standards, while the group geometric mean dietary intakes of vitamin A and vitamin C exceeded the FAO/WHO standards.

(ii) Longitudinal analysis

Dietary intake of macronutrients and micronutrients at each assessment during pregnancy are given in Table 5.3 and Table 5.4 respectively for the longitudinal cohort of subjects who completed all three assessments.

The group mean energy intake increased by 0.35 MJ (83 kcal) between the first trimester and second trimester and by 0.49 MJ (117 kcal) between the first and third trimester assessments. The group mean energy intake was lower than the FAO/WHO standards at each assessment, even if allowance is made for a decrease in physical activity during pregnancy.

The group geometric mean dietary intake and density (dietary intake per 1 MJ) of iron, calcium, vitamin A, vitamin C and folate increased between the first and third trimester.

At each assessment during pregnancy, the group geometric mean intake of iron was low compared with the FAO/WHO standards for non-pregnant women. The dietary intake of calcium and folate were also below the FAO/WHO standards, while the intake of vitamin A and vitamin C were above the FAO/WHO standards.

Due to intra-subject variation in intake, statistical comparisons between the daily dietary intake at different assessments is not recommended for single 24-h recall data (Launer *et al.*, 1991; J. McReilly, personal communication[3]). Thus, a further study with multiple repeat dietary recalls is required to confirm the trends in intake across pregnancy.

5.4 DISCUSSION

5.4.1 Dietary iron intake during pregnancy in Western Sierra Leone

The primary purpose of the 24-h dietary recall investigation was to determine whether iron deficiency and anaemia has a dietary basis among the group of pregnant women enrolled on the study. The mean daily intake of iron, estimated from a single 24-h dietary recall each trimester, increased slightly across pregnancy but did not exceed 8 mg. The FAO/WHO expert panel on human nutrient requirements did not stipulate a daily iron requirement for pregnant women, as the requirements in the second and third trimester are not expected to be met by dietary intake regardless of the quality of the diet (FAO/WHO, 1988). Nonetheless, it is clear that the estimated dietary iron intake was consistently below the basal daily iron requirements of non-pregnant women, and only the third trimester estimate exceeded the median amount required to prevent the development of anaemia in non-pregnant women. Given that iron requirements are increased during pregnancy to support the expansion of the red cell mass and maternal and fetal growth (Bothwell *et al.*, 1989; Hallberg & Rossander-Hulten, 1991), it highly likely that the dietary iron intake in this pregnant population is inadequate.

This dietary iron intake was considerably lower than values reported from dietary intake studies in developed countries. Most food consumption studies on Western diets have indicated a daily dietary iron intake of between 12 mg and 18 mg (Bothwell *et al.*, 1989). The daily intake of iron in many developing countries is between 15 mg and 30 mg (Baker & de Maeyer, 1979), although low values have also been reported, such as 7 mg among women of child-bearing age in urban South Africa (Bourne *et al.*, 1993). The low dietary iron intake among the Sierra Leonean pregnant women reflects the low intake of fish, meat and poultry, which are generally denser sources of iron than plant food items. The meat and fish component of the diet was poor, with only 10.4% of the total energy intake and 13.2% of the

[3] Doctor of Nutrition, Department of Human Nutrition, Glasgow University.

total iron intake provided by animal food items in the first trimester. Total energy intake is closely linked with total dietary iron intake (Torrance, 1992), and the low intake of energy relative to the FAO/WHO standard also contributes to the low dietary iron intake.

The low estimate of dietary iron intake in the present study may be due to inaccuracies in dietary intake estimates occurring during data collection or data analysis. Because the variety of dishes was limited, it was usually easy for the subjects to recall what they had consumed the previous day. The interviewer was trained to assist the subjects in recalling all that had been consumed, with particular attention to snacks and items that the subjects may not have perceived as food. However, the custom of eating from the same dish as other family members made it difficult for some subjects to quantify their individual intake. The Western Area occasionally benefits from food aid, and it is possible that subjects under-reported food intake so that they did not jeopardise their opportunity for food donations. Furthermore, the lack of information on the micronutrient composition of local foods and failure to account for contamination iron may have resulted in an underestimation of the true dietary iron intake. Prepared meals in developing countries usually contain a large amount of contaminant iron derived from non-food sources such as soil residues, dust, cooking utensils or the water used for cooking (Hallberg & Bjorn-Rasmussen, 1981). Studies from West Africa indicate that this contaminant iron contributes substantially to the total dietary iron intake (Galan *et al.*, 1990; Guiro *et al.*, 1991). Contaminant iron is usually regarded to have a very low bioavailability, and is thought to account for the high prevalence of iron deficiency in areas where the estimated dietary iron intake is relatively high (Hallberg *et al.*, 1983). However, there is marked variation in the bioavailability of contaminant iron depending on the source of this iron, and its role in iron nutrition cannot be neglected (Hallberg & Bjorn-Rasmussen, 1981).

The estimated dietary iron intake represents the amount of iron present in the ingested food, but does not reflect the amount that is actually absorbed and utilised by the body. The proportion absorbed depends on the chemical nature of the iron (haem or non-haem), the presence of dietary factors that inhibit or promote the absorption of iron and the iron status of the subject. Haem iron is the most important form of dietary iron because it is highly bioavailable. The low intake of animal food items suggests that the dietary iron is predominantly the non-haem form, and it is therefore important to consider the presence of dietary factors known to inhibit non-haem absorption, such as polyphenols, components of dietary fibre and calcium. It was not possible to estimate the quantity of polyphenols and dietary fibre in the diet as the necessary food composition data was unavailable, but rice-based diets with vegetable soups or sauces and little animal protein are known to contain a substantial amount of these components (Bothwell *et al.*, 1989). The low intake of calcium

(230 mg to 290 mg) is beneficial for iron nutrition as calcium inhibits iron absorption at a rate that is dose dependent (Hallberg *et al.*, 1991). Daily intakes of 200-500 mg calcium are typical in African diets, where calcium is derived principally from plant sources and where the consumption of animal milks is low (Prentice, 1991)[4]. Balanced against these inhibitors, the high dietary intake of vitamin C relative to the FAO/WHO standard is encouraging, as vitamin C is a powerful promoter of non-haem iron absorption (Bothwell *et al.*, 1989). Vitamin C prevents the dose-dependent inhibitory effects of polyphenols and phytates on non-haem iron absorption (Siegenberg *et al.*, 1991). However, vitamin C can only enhance intestinal iron absorption if it is consumed with non-haem iron, and in this community, fruits containing vitamin C were usually eaten as snacks in between meals. The practice of cooking the vegetable sauces or soups for prolonged periods is likely to destroy much of the vitamin C that is eaten together with non-haem iron.

The iron bioavailability of a diet is described as low, moderate or high, corresponding to a dietary iron absorption of approximately 5%, ±10% and 15% respectively by an individual of borderline iron status (absent iron stores but with normal iron transport) (FAO/WHO, 1988). A low bioavailability diet is defined as a cereal-, root- or tuber-based diet with negligible quantities of meat, fish or vitamin C and a preponderance of components that inhibit iron absorption. A diet of intermediate bioavailability consists mainly of cereals, roots or tubers but includes some foods which promote iron absorption (meat, fish, or vitamin C). The dietary iron absorption of the Sierra Leone diet has not been quantified, but the bioavailable iron density[5] of typical West African meals is reported to be less than half that of Western diets (Galan *et al.*, 1990; Guiro *et al.*, 1991). Iron absorption from a West African rice-based meal containing dried fish was estimated to be 11.5% for individuals of borderline iron status (Guiro *et al.*, 1991). The low content of animal foods, the high dietary fibre (phytate) and polyphenol content, balanced against the low intake of calcium and high intake of vitamin C, would suggest that the typical Sierra Leonean diet is of low-moderate iron bioavailability. If the conservative estimate of 10% absorption is assumed for the typical Sierra Leonean diet, then the average daily diet of these pregnant women supplied only 0.6 mg of absorbable iron in the first trimester, rising to 0.8 mg in the third trimester. Given that the daily iron requirement is about 5-6 mg during the second and third trimesters of pregnancy

[4] Although the low intake of calcium is favourable for iron nutrition, it is also cause for concern, as it indicates a substantial risk of calcium deficiency in this group of pregnant women. The estimated intake of calcium was consistently below the reference daily requirement. Insufficient calcium supply during pregnancy and lactation may result in hypertensive disorders of pregnancy, maternal bone loss, reduced breast-milk calcium secretion or impaired infant bone development (Prentice, 1994). The efficiency of calcium absorption may be increased in people who habitually consume low calcium diets and during pregnancy, although this adaptive mechanisms may not be adequate at very low levels of dietary calcium intake (Allen *et al.*, 1982; Prentice, 1994).

[5] The amount of iron absorbed per unit of energy intake by subjects who are borderline iron deficient.

(Bothwell *et al.*, 1989), the amount of bioavailable iron is highly inadequate, even without any additional faecal iron losses due to parasitic infection.

Iron is not the only nutrient implicated in the aetiology of iron-deficiency and iron-deficiency anaemia. Deficiencies of vitamin A in Indonesia are reported to be important in limiting the utilisation of iron (Suharno *et al.*, 1993). In populations where both iron and vitamin A are limiting, intervention studies have indicated that haemoglobin response of pregnant women to iron and vitamin A supplementation is greater than to iron supplementation alone (Panth *et al.*, 1990; Suharno *et al.*, 1993). The abundance of palm oil, mangos, maize and yellow varieties of sweet potato in the study area accounts for the high intake of vitamin A and the rarity of overt vitamin A deficiency in Sierra Leone. The intake of vitamin A was consistently above the FAO/WHO standard at each trimester assessment. There is a very small risk of vitamin A deficiency among the group of pregnant women, and it is not likely that iron-deficiency anaemia is caused by a deficiency of vitamin A deficiency in this area. Riboflavin deficiency has also been linked with iron deficiency in the Gambia. Daily riboflavin supplements in addition to iron supplements significantly improved iron stores in lactating women in the Gambia relative to the placebo and either riboflavin or iron given alone, although these findings were not observed in pregnant women (Powers *et al.*, 1985).

Other nutrients implicated in the aetiology of anaemia include folate and vitamin B_{12} deficiency, which both cause megaloblastic anaemia, and prolonged protein deficiency, which may lead to normoblastic normochronic anaemia (Masawe, 1981). In tropical Africa, folate deficiency is the usual cause of megaloblastic anaemia in pregnancy, as women with vitamin B_{12} deficiency are usually infertile (Masawe, 1981). Most of the dietary folate in the typical Sierra Leonean diet is present in the green leafy sauces that accompany rice, although sweet potatoes, bananas, plantain, mangoes and fish are other good sources. The intake of folate increased across pregnancy but was constantly below the FAO/WHO standard. The discrepancy between estimated group intake and this standard indicates a risk of deficiency within the group, which could contribute to anaemia in some subjects. This risk cannot be quantified on the basis of this information alone as RDI values represent an average requirement augmented by a factor that allows for inter-individual variation in requirements. A mean group intake equal to the RDI standard can be taken to represent a small risk of deficiency in the population, while increasing disparity between the two values indicates an increasing risk of deficiency (FAO/WHO, 1985). The bioavailability of folates from food is a less important consideration than is the case for iron in the aetiology of anaemia. However, folates are heat labile, and the common practice in Sierra Leone of cooking sauces and soups for prolonged periods could be an important factor in the development of folate deficiency (WHO, 1993). Megaloblastic anaemia attributed to folate deficiency has been reported in pregnant women in the Gambia, but the high intake of green leafy vegetables in Liberia is thought to account for the absence of folic acid deficiency in pregnant women (Jackson & Latham, 1982). Liberia borders Sierra Leone and shares a similar climate, agricultural environment and dietary patterns (Jackson & Latham, 1982; Jackson & Jackson, 1987).

Protein is required to provide amino acids for the synthesis of red cell proteins such as globin. The protein intake of the subjects exceeded the FAO/WHO standard at each assessment during pregnancy. It should be noted that the energy requirements were not met by all the pregnant women, and in some individuals, dietary protein may be used to provide energy, thus invalidating the standard. However, the pool of protein in the body is very large and given that haematopoiesis is only affected after prolonged severe protein deficiency (Masawe, 1981), the risk of depressed haematological values due to protein deficiency is probably negligible in this population.

The dietary energy intake of the women was in the range reported for women in other developing countries (5.6 to 8.6 MJ/d: Prentice, 1980), but was less than intakes recommended by FAO/WHO (1985). Deficiency of energy intake is of relevance in the present study as it is known to adversely affect maternal weight gain and birth-weight (Paul *et al.*, 1979). Inadequate energy intake among some subjects could confound the relationship between intervention and anthropometrical outcomes. The FAO/WHO (1985) recommended increase in energy intake during pregnancy is 1.2 MJ/d for women who maintain their pre-pregnant level activity or 0.84 MJ/d for those who reduce their activity level. It is not known whether the energy intake of the subjects was altered during pregnancy relative to the pre-pregnant state, as pre-pregnancy dietary intake data was not obtained. However, mean group energy intake did increase between the first trimester and third trimester. In addition, the density of all micronutrients except vitamin A also increased across pregnancy, which suggests an increase in the quality as well as quantity of dietary intake. Nonetheless, the group mean intake of energy at each trimester assessment was markedly below the FAO/WHO standard. The standard value represents the recommended population mean requirement, and therefore the discrepancy between the mean intake of the group and this standard indicates an inadequate intake for the group and perhaps for most individual within the group (FAO/WHO, 1985). The significance of the deficit in energy intake is difficult to assess, as studies in the Gambia indicate that nutritional adaptation to low energy intake may occur during pregnancy. These studies found that the extra energy requirement may be met by a depression of the basal metabolic rate, mobilisation of fat stores and a progressive reduction in activity during pregnancy (Roberts *et al.*, 1982; Lawrence *et al.*, 1984; Lawrence *et al.*, 1987b; Lawrence & Whitehead, 1988; Poppitt *et al.*, 1993). Such mechanisms are believed to account for findings from both developed and developing countries, which indicate that normal pregnancy outcomes can occur even if energy intake is not substantially altered during pregnancy (Whitehead & Paul, 1982; Durnin *et al.*, 1985; Durnin *et al.*, 1987; Pelto, 1987; Durnin, 1993).

In the Gambia (West Africa), where there is a marked seasonal pattern in rainfall and large seasonal differences in the intake of several nutrients, seasonality is a major determinant of micronutrient status in pregnant women, including Vitamin C (Bates *et al.*, 1982; Bates *et al.*, 1994). The effect of seasonality on iron intake and on the bio-availability of this intake was not quantified in the present study, although observations were made on the seasonal availability of food items. The climate in Sierra Leone is monomodal, with the rainfall peaking in August and no or little rain between November and April. During the wet season, the depletion of indigenous rice stores rice coincides with the period of highest work activity in the fields. Despite being known as the 'hungry season', the wet season is not associated with the acute seasonal shortages in energy intake that occur in the savannah zone of West Africa, which includes the Gambia (Annegers, 1973). In Sierra Leone and other parts of the guinean zone, cassava and yam crops supplement or replace rice during the wet season. Imported rice is also available throughout the year in Sierra Leone, particularly in the Western Area. The explanation for the lay term is that people will claim that they have not eaten unless they have eaten rice. There is, however, marked variation in the availability of several plant food items that may affect the bioavailability of non-haem iron. Citrus fruits and mangos containing vitamin C are most abundant during the late dry season. Mangoes are also rich in vitamin A and the intake of vitamin A may also be seasonal, although hepatic stores accumulated during periods of high intake are probably sufficient to maintain plasma retinol levels at other times. During the dry season, the low demand for agricultural activity and ideal weather conditions facilitate fishing activities and may increase the intake of fish, which is the predominant source of haem iron in these communities.

It should be noted that seasonality affects the adequacy of the dietary intake in ways other than the availability of food items. Dietary requirements are likely to increase in the wet season, when there are increases in physical activity associated with agriculture (Roberts *et al.*, 1982; Lawrence & Whitehead, 1988) and in the transmission of infections such as malaria (Blacklock & Gordon, 1925; Powers *et al.*, 1985; Aitken, 1990; Bouvier *et al.*, 1997; Greenwood *et al.*, 1987c) and hookworm infection (Chandiwanna, 1990; Smith, 1990; Pawlowski *et al.*, 1991; see Chapter 6). During the wet season, pregnant women in Sierra Leone are likely to participate in farming activities, even in their third trimester of pregnancy, although they are usually given lighter jobs (Aitken, 1990; personal observations). The FAO/WHO estimates of energy and nutritional requirements are based on healthy women who are only moderately active and do not bear the additional stress of parasitic infections.

5.4.2 Implications for the control of iron deficiency and anaemia

The dietary intake data indicates that the iron nutrition of pregnant women in this community may be compromised by a low intake of absorbable dietary iron. Given that many of these women also experience intestinal blood loss due to infection with *N. americanus* and *T. trichiura* (see Chapter 6), the significance of this inadequate intake is particularly great. Poor dietary iron intake has been identified as the underlying cause of iron-deficiency anaemia among pregnant women in Liberia (Jackson & Latham, 1982; Jackson & Jackson, 1987). In both countries most of the dietary iron intake is of plant origin and thus predominantly non-haem iron. Fruits containing vitamin C tend to be eaten as snacks in between meals when they are unlikely to enhance non-haem absorption. Haem-iron containing foods of animal origin are consumed only in small amounts. Dietary iron deficiency is also reported to be the main cause of iron deficiency among pregnant women in other West African countries, including the Gambia (Powers *et al.*, 1985) and Nigeria (Ogunbode *et al.*, 1979).

Iron requirements during pregnancy are unlikely to be met by dietary iron intake, regardless of the quality and quantity of the diet (FAO/WHO, 1988). Nonetheless, there is a need to address the dietary practices among these pregnant women so that the iron intake is optimised. Dietary behaviour is dynamic (Sanjur, 1982), and the incidence of nutritional deficiencies can be reduced through dietary modifications (ACC/SCN, 1991; WHO, 1993). The majority of the iron-relevant nutrients in the typical Sierra Leonean diet are found in the sauces or soups that accompanies rice. As economic constraints limit the intake of animal foods, the most appropriate means to improve the quantity of iron in the diet would be to increase the intake and bioavailability of non-haem iron. Green leafy vegetables and pulses, which are good sources of both non-haem iron and folate, form the basis of the sauces and soups. The absorption of non-haem iron can be enhanced by adding vitamin C-containing fruits to the meal, rather than eating them as snacks between meals. As little as 50 mg of vitamin C, which could be provided by an orange or 120 g of mango (WHO, 1993), can double iron absorption (Monsen *et al.*, 1978). In addition, the common practice of cooking the sauces and soups for prolonged periods should be discouraged, as this destroys much of the vitamin C and folate content (ACC/SCN, 1991).

Although these recommendations appear relatively simple, they are unlikely to be effective if they conflict with long-established dietary habits in the community (WHO, 1993). Any campaign to promote positive dietary practices must consider the cultural as well as economic constraints on dietary intake in the community. Traditional food beliefs and customs play a significant role in the dietary behaviour of non-industrialised societies (Sanjur, 1982), and the effectiveness of nutrition intervention programmes is greater when it is possible to reinforce or modify concepts which reflect rather that conflict with these beliefs (McKay, 1971; Pratt, 1983; Thompson-Clewry, 1972; Sanjur, 1982). Rapid ethnographic techniques can help in the design of culturally appropriate nutrition intervention programmes by uncovering the beliefs that create barriers to nutritional adequacy, and by identifying culturally appropriate methods by which to improve dietary practices (Jerome, 1997). In Chapter 11, ethnographic data on the dietary habits of pregnant women belonging to the Temne ethnic group in Western Sierra Leone are examined.

Dietary intake data are a measure of what an individual eats, but not of what is actually metabolised (Dwyer, 1991). Therefore, they give an indication of the risk for nutrient inadequacy in a population but do not identify the presence or severity of nutrient deficiencies. Objective measures of nutritional status, including biochemical and anthropometrical assessments, are necessary to confirm the presence of undernutrition (FAO/WHO, 1974; Shetty *et al.*, 1994), and are the focus of Chapters 7 and 8.

5.5 SUMMARY

1. The group mean dietary intake of energy, protein, carbohydrate, fat, iron, calcium, vitamin A, vitamin C and folate of the pregnant women in Western Sierra Leone was estimated using data from a single 24-h dietary recall in each trimester of pregnancy.
2. Relative to the FAO/WHO recommended daily intake standards, the dietary intake of the women during pregnancy was characterised by low intake of energy, iron, calcium and folate and high intake of vitamin A and vitamin C.
3. The group mean dietary intake of energy, protein, iron, calcium, vitamin A, vitamin C and folate increased across pregnancy. The density of micronutrients also increased across pregnancy, suggesting an increase in the quality as well as quantity of the dietary intake.
4. The dietary intake data indicates that iron deficiency during pregnancy may have a dietary basis in this group of women. The dietary iron intake was predominantly non-haem, and supplied only an estimated 0.6 mg of absorbable iron in the first trimester, rising to 0.8 mg in the third trimester.

Table 5.1 Sample meal pattern reported by pregnant women in Western Sierra Leone.

Meal	Quantity[†]
Morning meal	
Boiled white rice (imported)	½ 'butter cup' uncooked rice
Cassava leaf *plasas*	1 spoon
Mid-afternoon snack	
Oranges, juice only	4 medium
Evening meal	
Boiled white rice (imported)	½ 'butter cup' uncooked rice
Fried fish soup	1½ spoon

[†] Local measures and monetary values used to quantify intake; sample meal contains 7.63 MJ

Table 5.2 Energy and nutrient composition of the daily dietary intake of women in the first trimester of pregnancy in Western Sierra Leone.

Nutrient		Mean	(±SD)[†,‡]
Energy	MJ	7.72	(1.44)
Carbohydrate	g	284	(70)
	%[§]	58.6	(9.3)
Fat	g	61	(20)
	%[§]	29.2	(8.8)
Protein	g	55	(13)
	%[§]	12.2	(2.3)
Iron	mg	6.2	(-3.1, +6.2)
Calcium	mg	235	(-125, +266)
Vitamin A (RE[††])	mg	1318	(-966, +3634)
Vitamin C	mg	131	(-99, +415)
Folate	µg	183	(-73, +122)

[†] Estimated from a single 24-h dietary recall, *n*=184.
[‡] Geometric means are given for micronutrients.
[§] Percentage of total energy intake provided by macronutrient.
[††] RE = retinol equivalents

Table 5.3 Energy and macronutrient composition of the daily dietary intake of women during pregnancy in Western Sierra Leone.

Macronutrient		Trimester	Mean	(SD)†
Energy	MJ	first	7.72	(1.38)
		second	8.07	(1.39)
		third	8.21	(1.40)
Carbohydrate	g	first	281	(68)
		second	300	(67)
		third	283	(59)
	%‡	first	58.0	(8.9)
		second	59.6	(9.3)
		third	56.0	(8.7)
Fat	g	first	62	(20)
		second	62	(23)
		third	70	(26)
	%‡	first	29.8	(8.3)
		second	28.1	(9.0)
		third	31.5	(8.6)
Protein	g	first	55	(12)
		second	58	(14)
		third	60	(15)
	%‡	first	12.3	(2.2)
		second	12.2	(2.1)
		third	12.4	(2.3)

† Estimated from a single 24-h dietary recall, *n*=125

‡ Percentage of total energy intake provided by macronutrient

Table 5.4 Micronutrient composition of the daily dietary intake of women during pregnancy in Western Sierra Leone.

Micronutrient		Trimester	Geometric mean (SD)†			
			Daily micronutrient intake		Micronutrient density	
Iron	mg	first	6.2	(-3.1, +6.3)	0.81	(-0.38, +0.72)
		second	6.7	(-3.4, +6.7)	0.85	(-0.39, +0.73)
		third	8.0	(-4.1, +8.5)	0.98	(-0.48, +0.94)
Calcium	mg	first	232	(-127, +282)	31.2	(-16.2, +33.6)
		second	251	(-129, +264)	31.5	(-15.0, +28.6)
		third	294	(-151, +343)	36.4	(-17.8, +38.2)
Vitamin A (RE)	mg	first	1300	(-965, +3474)	171.4	(-124.5, +455.6)
		second	1246	(-907, +3332)	156.8	(-111.5, +385.6)
		third	1647	(-1120, +3499)	203.6	(-133.3, +385.6)
Vitamin C	mg	first	123	(-98, +477)	16.2	(-12.7, +59.3)
		second	126	(-85, +266)	15.8	(-10.5, +31.5)
		third	152	(-105, +342)	18.8	(-12.9, +41.3)
Folate	mg	first	180	(-72, +119)	23.8	(-8.1, +12.3)
		second	196	(-75, +121)	24.7	(-7,6, +12.5)
		third	200	(-79, +129)	24.8	(-8.5, +12.8)

† Estimated from a single 24-h dietary recall, n=125.

Chapter 6

Intestinal nematode infections

6.1 INTRODUCTION

6.1.1 Assessment of intestinal nematode infections

Little is known about the distribution of intestinal nematode infections in pregnant women, or indeed whether pregnancy affects the susceptibility to infection (Brabin, 1985; Stoltzfus *et al.*, 1997). Knowledge of the prevalence, intensity, incidence and epidemiology of these infections in pregnant women contributes to risk assessment and the formulation of appropriate intervention strategies.

The public health significance of intestinal nematode infections during pregnancy will depend on number of cases of infection and clinical illness. The clinical severity of these infections is closely related to the intensity of infection (Stephenson & Holland, 1987; Bundy & Cooper, 1989; Lwambo *et al.*, 1992). Therefore, research on the nutritional impact of intestinal nematode infections logically begins with an assessment of the prevalence and intensity of infection in the study population. This information can then be related to indicators of maternal nutritional status and neonatal outcomes, so that the impact of these infections can be ascertained. Information on the incidence of infection in pregnant women is also useful, as the appropriate frequency of anthelminthic treatment will depend on the rate of reinfection following intervention (WHO, 1996a). In addition, an understanding of the epidemiology of infection can help identify pregnant women who are at increased risk of infection and morbidity. Studies among non-pregnant groups indicate that specific sectors of a population are more susceptible to infection, and among those infected, the parasites are highly aggregated (overdispersed) with most subjects lightly infected and a small number carrying the majority of the total parasite load (Anderson & May, 1982).

6.1.2 Aims and objectives

The aim of the work described in this chapter was to examine the prevalence, intensity and distribution of intestinal nematode infections in the women in the first trimester of pregnancy and to examine the efficacy of albendazole administered as a single dose at the beginning of the second trimester.

The specific objectives were:

1. To identify the presence of intestinal nematode infections in the women in the first trimester of pregnancy.
2. To relate the prevalence and intensity of infection in the women in the first trimester of pregnancy to socio-demographic factors, socio-economic status (SES) and season at assessment.

3. To compare the prevalence and intensity of infection in the women in the first trimester of pregnancy with non-pregnant women of child-bearing age residing in the same household.
4. To evaluate the therapeutic efficacy of albendazole (400 mg) in terms of the cure rate and egg reduction rate for each intestinal nematode.
5. To determine the appropriate frequency of albendazole treatment during pregnancy by examining infection and reinfection with intestinal nematodes following intervention.
6. To report on side-effects experienced after intervention with albendazole or the albendazole placebo.

6.2 METHODS

6.2.1 Limitations of the data

The prevalence and intensity of intestinal nematode infections can be measured directly by counting the number of worms expelled after anthelminthic treatment. This method requires that all subjects receive anthelminthic treatment each time the intensity of infection is assessed and is therefore not appropriate for all studies. As an alternative, faecal egg counts are often used to diagnose infection and indirectly assess the intensity of infection. The use of egg counts is based on the assumption that an increase in worm burden is reflected by an increase in the number of eggs excreted per unit mass of faeces (typically 1 g).

There are a number of limitations associated with the use of faecal egg counts. Failure to detect eggs does not preclude the presence of nematode parasites in the intestinal tract; infections may be inapparent if (1) the worm load is low (2) the infection comprises single sexed nematodes, or in the case of *Ascaris*, male only nematodes[1] (3) the nematodes have not reached sexual maturity. Egg counts are affected by numerous factors including daily fluctuations in egg production and excretion, density-dependent constraints on parasite fecundity and the age of the mature worms (Hall, 1981; Croll *et al.*, 1982; Hall, 1982; Bundy *et al.*, 1985a; Schad & Anderson, 1985). Host biology is also of relevance, particularly the extent of faecal dilution of eggs and non-homogenous mixing of eggs in the stool (Hall, 1981; Hall, 1982). Other sources of error include sampling error, poor standardisation of laboratory techniques and intra- or inter-observer error by examining technicians (undercounting or overcounting) (Davis, 1994).

In describing the prevalence of infection and in assessing the therapeutic efficacy of albendazole, the adjective 'uninfected' is used to describe subjects in whose stool samples neither nematode eggs or larvae were observed. As the Kato-Katz technique of faecal

[1] Ascaris is known to release unfertilised eggs (Sawitz, 1942).

examination is insensitive to low intensity infections, it is likely that some false-negative results were obtained. Epidemiological estimates of cure rates based on single post-treatment samples are therefore likely to be over-optimistic.

6.2.2 Data analysis

(i) Baseline (first trimester) analysis

The association between maternal factors and the baseline (first trimester) prevalence of infection, prevalence of polyparasitism and intensity of infection was examined. The maternal factors included age, place of residence, marital status, maternal education, sanitation facilities, source of drinking water, maternal and partner's occupation, SES and season at assessment.

The univariate analyses for the prevalence of infection and polyparasitism included Chi-square test (χ^2) with Yate's correction for nominal data, or Fisher's exact test where expected cell sizes were less than 5 (2 x 2 contingency tables only), and the Chi-square test for trends (χ^2_{trend}) for ordinal data. Multiple logistic regression was employed to relate several maternal variables to the prevalence of infection or polyparasitism. Variables were analysed using the forward stepwise method with an entry *p*-value of 0.05 and a removal *p*-value of 0.06. Odds ratios with 95% confidence intervals (CI) were calculated. The referent category for each categorical variable was the category with the lowest prevalence, unless otherwise stated. The multivariate results are shown only for significant factors as non-significant factors were not retained in the final multiple logistic regression model.

Egg counts per gram (epg) were highly skewed and therefore transformed using natural logarithms, $\log_e$(epg). If uninfected subjects were included in an analysis of intensities, the transformation $\log_e$(epg+1) was used, as the logarithm of zero is undefined. Geometric mean egg counts were estimated as the antilog of the mean of the transformed data. Levene's test (univariate analysis) and Bartlett Box Test (multivariate analysis) were used to assess the homogeneity of variances between groups of samples. For homogenous samples, baseline transformed egg counts data were analysed using t-tests, one-way analysis of variance (ANOVA) and two-way ANOVA. Following a significant one-way ANOVA analysis, pair-wise comparisons between the groups were used to determine which groups were significantly different, with the Bonferroni correction to allow for multiple comparisons. For heterogeneous samples, t-tests corrected for unequal variances and Kruskal-Wallis tests were employed.

The difference in the prevalence of intestinal nematode infections between pregnant women and non-pregnant women matched for the same household was examined using McNemar's Test. The intensity of infection was compared using paired t-tests.

(ii) Longitudinal analysis

The efficacy of treatment was estimated in terms of cure rate (CR) and egg reduction rate (ERR) at the post-treatment stool assessment. Only those subjects who were infected for a particular infection at baseline were considered in this analysis. The CR is defined as the proportion of individuals excreting eggs at baseline with no evidence of infection at the post-treatment stool assessment (Albonico *et al.*, 1994). The ERR is the percentage reduction in baseline geometric egg counts at a subsequent stool assessment for individuals who were infected at baseline, and was estimated as $100(1\text{-}e^{-D})$, where D is the mean difference in transformed egg counts between the baseline and post-treatment assessments (Albonico *et al.*, 1994).

The effect of intervention with albendazole on the prevalence and intensity of infection was the primary factor of interest. However subjects also received iron-folate supplements or the iron-folate placebo. It was therefore necessary to determine whether the iron-folate supplements were associated with a significant change in infection status or egg counts. This preliminary analysis employed multiple logistic regression for CRs and the Scheirer-Ray-Hare extension of the Kruskal-Wallis Test for ERRs. The main intervention effects (iron-folate supplements and albendazole treatment) and the interaction effect for these interventions were entered into the models simultaneously. These analyses indicated that iron-folate supplements were not associated with any change in infection status or egg counts, either as a main effect or as an interaction effect. Therefore, only albendazole treatment was considered in further analysis. The CRs and ERRs associated with albendazole and the albendazole placebo were compared using the Chi-square test and t-test respectively.

Only the longitudinal cohort (n=125) was included in the analysis of infection and reinfection following albendazole treatment, as only these subjects provided all necessary measurements and samples at each assessment during pregnancy. Preliminary analyses indicated that supplementation with iron and folate was not associated with the incidence or ERR, either as a main effect of interaction effect.

The incidence of infection between the post-treatment assessment and third trimester assessment was determined for subjects who were negative at the post-treatment assessment for a particular infection. The incidence of infection associated with albendazole and albendazole placebo were compared using Fisher's Exact Test. The ERRs were calculated at the post-treatment, second trimester and third trimester assessments. The ERRs associated with albendazole and albendazole placebo were compared using t-tests.

Kendall's rank correlation was employed to examine the correlation between baseline egg counts and egg counts at the third trimester assessment. Tests were performed only for *T. trichiura* data as there were insufficient new *A. lumbricoides* and *N. americanus* infections during this period. A standardisation procedure was used to correct for age-related differences in the intensity of *T. trichiura* infection. The subjects were stratified by age group (15-19, 20-24, 25-29, 30-40 years) and the standardised count $Y_{i,j}$ was calculated as

$$Y_{i,j} = (X_{i,j} - X_j)/s_j$$

where $X_{i,j}$ is the egg count in patient i who was in age class j, X_j is the mean count in patients of age class j, and s_j is the standard deviation for patients of age class j.

The intervention groups are defined as follows: FeA = iron-folate supplements and albendazole; FeP_A = iron-folate supplements and albendazole placebo; $P_{Fe}A$ = iron-folate placebo and albendazole; $P_{Fe}P_A$ = iron-folate placebo and albendazole placebo.

6.3 RESULTS

6.3.1 Baseline (first trimester) analysis

(i) Infections identified

Between December 1995 and June 1996, stool samples were obtained from 184 women in their first trimester of pregnancy. Infections with *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* were identified by light microscopial examination of nematode eggs in stool samples. Eggs of the trematode *Schistosoma mansoni* were also observed. Using the Harada-Mori method of faecal culture, hookworm larvae were isolated from 16 of 50 unfixed stool samples (32%): examination of the larvae with a light microscope indicated the species to be *Necator americanus*.

None of the urine samples taken from women in the first trimester were found to contain *S. haematobium* eggs.

(ii) Prevalence and intensity of intestinal nematodes

The baseline prevalence of the intestinal nematode infection was as follows: *A. lumbricoides* 21.2%; *N. americanus* 66.9%; and *T. trichiura* 71.7% (Table 6.1). For reasons outlined in Section 6.2.1, it is likely that these prevalence levels underestimate the actual values. The geometric mean intensities were: *A. lumbricoides* 267 epg; *N. americanus* 191 epg; and *T. trichiura* 93 epg (Table 6.1). The majority of intestinal nematode infections were light (Table 6.2). However, extreme variations in egg counts were observed. Egg counts were highly overdispersed (aggregated), with 75% of the total egg count of *A. lumbricoides*, *N. americanus* and *T. trichiura* excreted by 12.8%, 22.8% and 25.0% of subjects respectively.

Schistosoma mansoni eggs were observed in 9.8% of subjects. The geometric mean intensity of infection was 70 epg. Infection with *S. mansoni* is not considered hereafter as it is not an intestinal nematode infection. This parasite is unlikely to be of major public health significance in the study population, as the prevalence and intensity of infection were both low.

(iii) Socio-demographic and socio-economic factors associated with intestinal nematode infection.

Using univariate analysis, the prevalence of *N. americanus* infection was significantly higher among subjects with a lower level of maternal education, women who were farmers, women whose partners were farmers, rural residents, women with low SES, women without access to a stand-pipe and those who were assessed in the early dry season (November to January) (Table 6.3). Of these factors, rural residence (p=0.0058), low SES (p=0.0003) and the season at assessment (late dry p=0.0027; early wet p=0.011) remained independently significant when entered into the multiple logistic model. Thus, the multivariate analysis indicated that the initial univariate analysis failed to identify level of education, occupation and standard of water supply as coparameters. In the multivariate model, the odds on *N. americanus* infection were over three times higher for women who were assessed in the early dry season than the late dry season (February to April) or early wet season (May to July), almost three times greater for rural residents than peri-urban residents and over four times greater for subjects with low SES as compared to those of high SES. None of the socio-demographic or socio-economic factors was significantly associated with *A. lumbricoides* infection or *T. trichiura* infection.

The geometric mean intensity of *A. lumbricoides* and *T. trichiura* infection decreased with age group (One-way ANOVA: *A. lumbricoides* $F_{3,38}$=3.17, p=0.036; *T. trichiura* $F_{3,131}$=4.00, p=0.0092). Post-hoc pairwise comparison of age groups indicated that the main explanation for the difference in intensities of *A. lumbricoides* and *T. trichiura* with age was the difference between the age groups 15-19 years and 30-40 years (Figure 6.1). The geometric mean intensity of *N. americanus* also varied with age group (One-way ANOVA: $F_{3,122}$=3.23, p=0.025) but differences between age groups 15-19 years and 20-24 years appeared to account for most of this variation (Figure 6.1). For each age group, the mean intensity of *T. trichiura* infection was independently higher among subjects of low SES (Two-way ANOVA: SES, $F_{1,131}$=6.32, p=0.013; age group, $F_{3,131}$=4.27, p=0.007; interaction, $F_{3,131}$=0.08, p=0.97) (Figure 6.2). The distribution of egg counts did not vary with any of the other socio-demographic factors examined.

(iv) Polyparasitism with intestinal nematodes

The distribution of single, double and triple intestinal nematode infections among the pregnant women is shown in Table 6.4. Of the 184 stool samples examined, 156 (84.8%) showed evidence of at least one intestinal nematode. Double infections accounted for the largest proportion of samples (41.3%), followed by single infections (26.6%) and triple infections (16.8%).

The prevalence of intestinal nematode infections was investigated to determine if combinations of infections were more or less likely to occur together than would be expected by random events. Of those infected, 102 (65.4%) were infected with both *N. americanus* and *T. trichiura*; 31 (30.4%) of these subjects were also infected with *A. lumbricoides*. Significant associations were identified between infection with *A. lumbricoides* and *N. americanus* (Chi-square test: χ^2=8.10, p=0.0044), and between infection with *N. americanus* and *T. trichiura* (Chi-square test: χ^2=21.30, p<0.0001). *Ascaris lumbricoides* and *T. trichiura* were not significantly associated (Chi-square test: χ^2=3.28, p=0.070). Multiple logistic regression was employed to determine whether *A. lumbricoides* and *T. trichiura* were independently or dependently associated with *N. americanus*. An interaction between *A. lumbricoides* and *T. trichiura* indicated a dependent association (Multiple logistic regression: interaction term Wald=5.63, p=0.018). This relationship was further investigated by regressing *Ascaris lumbricoides* on *N. americanus* separately for subjects with or without *T. trichiura* and regressing *T. trichiura* on *N. americanus* separately for those with or without *A. lumbricoides*. *A. lumbricoides* infection was associated with an increased odds on *N. americanus* infection only among subjects with *T. trichiura* infection (p=0.018), while *T. trichiura* infection was associated with increased odds on *N. americanus* infection regardless of whether the subjects were infected (p=0.012) or uninfected (p=0.0003) with *A. lumbricoides* (Table 6.5). The odds on *N. americanus* infection among those with both *T. trichiura* and *A. lumbricoides* was over four times greater than among those with *T. trichiura* only, albeit with overlapping 95% confidence intervals.

An increased odds on poly-parasitism was associated with assessment in the early dry season, a lower level of education, farming as the maternal occupation, farming as the partner's occupation, rural residence and low SES (Table 6.6). When these variables were entered into a multiple logistic regression, rural residence (p=0.0002) and low SES (p=0.0035) accounted for most of the explainable variation and adding further risk factors to the model did not improve the fit significantly. In the multivariate model, the odds on polyparasitism were over three times greater for rural residents that peri-urban residents and more than twice as great for subjects of low SES as compared to those of high SES.

There were positive bivariate correlations between the log transformed egg counts of infections but none of these correlations was significant. There was no significant difference in the intensity of *A. lumbricoides* infection or *N. americanus* infection for subjects with one, two or three intestinal nematode infections (One-way ANOVA: *A. lumbricoides* $F_{2,38}$=1.19, p=0.32; *N. americanus* $F_{2,122}$=1.27, p=0.28). However, the intensity of *T. trichiura* infection varied significantly between these groups (One-way ANOVA: $F_{2,131}=5.74$, $p=0.0041$). Pair-wise comparisons between the groups indicated that the significant differences were between subjects with one and two infections and those with one and three infections (Figure 6.3).

(v) Comparison of the prevalence and intensity of infection between first trimester pregnant women and non-pregnant women

In order to compare the prevalence and intensity of intestinal nematodes in pregnant women with non-pregnant women, 145 of the pregnant women participating in the study were matched to a non-pregnant woman residing in the same household (39 pregnant women were unable to provide a non-pregnant partner). The non-pregnant women comprised blood-relatives, in-laws and friends of the pregnant women. The need to control for household was based on studies that have demonstrated familial aggregation of *A. lumbricoides* and *T. trichiura* (Forrester *et al.*, 1988) and predisposition at a family level (Forrester *et al.*, 1990; Chan *et al.*, 1994a), probably as a consequence of environmental or behaviour similarities rather than genetic relatedness (Chan *et al.*, 1994b).

The mean age of the pregnant and non-pregnant women was 24.6 (SD 5.6) years and 25.8 (SD 6.9) years respectively: there was no significant difference between the ages of the matched pairs (Paired t-test $t=1.76$, $p=0.080$). There was no significant difference in the prevalence of *A. lumbricoides*, *N. americanus* or *T. trichiura* between pregnant and non-pregnant subjects (Table 6.7). The intensity of infection in pregnant women and non-pregnant women was compared for all pairs of women regardless of whether either of the pair were infected or uninfected (Table 6.8) and for pairs where both the non-pregnant and pregnant woman were infected (Table 6.9). If all pairs are examined, the intensity of *T. trichiura* was significantly greater for pregnant subjects ($p=0.042$), but this may reflect the higher prevalence of infection among this group (albeit insignificant) and there was no difference in the intensity of infection if only infected pairs were compared ($p=0.28$). Conversely, there was no significant difference in the intensity of *N. americanus* between non-pregnant and pregnant women if all pairs were included in the analysis ($p=0.076$), but if only infected pairs were compared, the intensity of infection was significantly greater among pregnant women ($p=0.002$).

6.3.2 Longitudinal analysis

(i) Efficacy of anthelminthic treatment

All post-treatment stool samples were collected within 35 d of intervention with albendazole or the albendazole placebo and 88% between 14 and 28 d of treatment. A post-treatment stool sample was not obtained from 6 (6.9%) women who received albendazole and 2 (2.2%) women who received the placebo (Fisher's Exact Test, $n=178$, $p=0.16$). The baseline prevalence and intensity of intestinal nematode infections was statistically similar in the groups of women treated with albendazole and the placebo (Table 6.10).

The CR induced by albendazole treatment was greatest for *N. americanus* (94.5%) followed by *A. lumbricoides* (93.3%) and *T. trichiura* (63.8%) (Table 6.11). These CRs were significantly greater than those induced by the albendazole placebo ($p<0.0001$) (Table 6.11).

Compared to the albendazole placebo, albendazole treatment was associated with a significantly greater reduction in the baseline geometric mean egg counts of *A. lumbricoides* ($p<0.001$), *N. americanus* ($p<0.001$) and *T. trichiura* ($p<0.001$) (Table 6.12). The ERRs in those treated with albendazole were marginally higher for *A. lumbricoides* (99.7%) than *N. americanus* (99.4%) and *T. trichiura* (96.0%). However, these differences may partly reflect the higher baseline geometric mean egg count of *A. lumbricoides* in comparison with *N. americanus* and *T. trichiura*. The distribution of egg counts at baseline and 2-4 weeks after treatment is shown in Figure 6.4.

(ii) Reinfection and infection following treatment

The baseline prevalence and intensity of intestinal nematode infections for the longitudinal cohort by albendazole treatment is given in Table 6.13. The intensity of *A. lumbricoides* was significantly higher at baseline among subjects who received albendazole treatment (t-test: n=37, t-value =2.29, p=0.031), but otherwise the treatment groups were similar. The baseline prevalence and intensity of intestinal nematode infections for the longitudinal cohort by all intervention groups (FeA, FeP_A, $P_{Fe}A$ and $P_{Fe}P_A$) is given in Table 6.14. Although this comparison is not strictly relevant here, it is included for future reference: when the effects of intervention on maternal nutritional status and neonatal outcomes are examined, it is important to know whether the intervention groups were similar at baseline with respect to the prevalence and intensity of intestinal nematode infection. There were no differences in the prevalence and intensity of intestinal nematode infections by intervention with iron-folate supplements.

The incidence of new infections during pregnancy following intervention with albendazole/albendazole placebo was examined by determining the proportion of subjects uninfected at the post-treatment assessment who became infected at the second or third trimester assessment. The incidence of each intestinal nematode infection by albendazole treatment group is given in Table 6.15. There was no significant difference in the incidence of *A. lumbricoides* infection (p=0.18) or *N. americanus* infection (p=1.00) by treatment group. However, albendazole treatment was associated with an increased incidence of *T. trichiura* infection (p=0.024).

The prevalence of infection at each assessment for the cohort of 125 subjects who provided a stool sample at every assessment during pregnancy is shown in Figure 6.5. Among subjects who received albendazole, the increase in the prevalence of *T. trichiura* subsequent to the post-treatment assessment clearly exceeded that of both *A. lumbricoides* and *N. americanus*. At the third trimester assessment, the prevalence of *A. lumbricoides* and *N. americanus* remained below 10%, while the prevalence of *T.trichuria* increased to almost 50%. This difference was partly due to the lower CR of albendazole for *T. trichiura* infection, but also reflects the higher incidence of *T. trichiura* infection subsequent to the post-treatment assessment.

At each assessment subsequent to treatment, albendazole treatment was associated with a significantly greater decrease in the baseline geometric egg counts of *A. lumbricoides* ($p<0.001$), *N. americanus* ($p<0.001$) and *T. trichiura* ($p<0.001$) as compared to the placebo (Table 6.16). The percentage reduction in baseline geometric mean egg counts for *A. lumbricoides*, *N. americanus* and *T. trichiura* is shown in Figure 6.6 for the longitudinal cohort. Among those treated with albendazole, the geometric mean egg counts of *A. lumbricoides* and *N. americanus* remained below 1% of the baseline values at the third trimester assessment. A greater change in geometric mean egg count was observed for *T. trichiura* and by the third trimester assessment the geometric mean egg count was 12.4% of the baseline value.

The *T. trichiura* egg counts of subjects treated with albendazole were examined to determine whether those subjects with heavy (or light) baseline egg counts tended, on average, to re-acquire heavy (or light) infections during pregnancy. There was a positive significant positive correlation between baseline and third trimester egg counts (Kendall's coefficient of rank correlation, $\tau=0.45$, $n=61$, $p<0.001$). Standardization of the egg counts to remove host age-related trends in intensity reduced the value of the correlation coefficient, but the positive correlation remained significant (Kendall's coefficient of rank correlation, $\tau=0.38$, $n=61$, $p<0.001$). Kendall's coefficient of rank correlation can be interpreted approximately as the proportion of pairs of cases (here, 38%) in which the rank order is preserved from the baseline to the third trimester surveys.

(iii) Reported side-effects after treatment with albendazole

The pregnant women were asked to recall any adverse clinical symptoms experienced following treatment with albendazole. Symptoms reported during the 14 d following treatment are given in Table 6.17. The low frequencies of these symptoms precluded statistical evaluation, but inspection of the data indicates that the number of reported symptoms did not differ greatly between the intervention groups. The quality of recall could not be assessed, and the exact timing of these symptoms relative to the administration of albendazole treatment is unknown.

Two subjects miscarried within 14 d of albendazole treatment, one in intervention group FeA and the other in intervention group $P_{Fe}P_A$. A further six women miscarried subsequently: three of these women received albendazole and the other three the albendazole placebo. One mother who received albendazole gave birth to an infant with polydactylism, a relatively common congenital abnormality in West Africa (Onadeko *et al.*, 1992).

6.4 DISCUSSION

6.4.1 Intestinal nematode infections among pregnant women in Western Sierra Leone

One hundred and eighty-four women in their first trimester of pregnancy presented at the study clinics. Among these subjects, *T. trichiura* (71.9%) was most highly prevalent followed by hookworm (66.5%), *A. lumbricoides* (21.1%) and the trematode *S. mansoni* (9.5%). *Necator americanus* was identified as the species of hookworm, in agreement with previous studies in Sierra Leone that have examined larvae after faecal culture (Whitworth *et al.*, 1991) or adult worms expelled after anthelminthic treatment (Bayoh *et al.*, 1992). No study in Sierra Leone has reported the presence of *A. duodenale*, but this does not preclude its existence. Larvae of *Ternidens deminutus* and *Trichostrongyle* species, whose eggs resemble those of hookworm, were not observed in the faecal cultures. The intensities of *A. lumbricoides*, *N. americanus* and *T. trichiura* infections were overdispersed, with the majority of infections light. Overdispersion is typical of these nematodes (Croll *et al.*, 1982; Bundy *et al.*, 1985b; Schad & Anderson, 1985), and may reflect the spatial aggregation of infective stages in the environment (Hominick *et al.*, 1987) or result from host-dependent factors, which acting alone or concomitantly, predispose subjects to infection (Schad & Anderson, 1985; Bundy *et al.*, 1987a; Haswell-Elkins *et al.*, 1987).

There is scarce previous data on pregnant women with which to compare these results. In 1996, a cross-sectional survey was conducted among 230 pregnant women residing at displaced camps in Freetown and Bo (Yarjah, 1996). The reported prevalences (and geometric mean egg counts) of *A. lumbricoides*, hookworms and *T. trichiura* respectively were 28.7% (283 epg), 52.2% (255 epg) and 11.3% (74 epg) respectively. Elsewhere, most previous studies in pregnant women tend to focus on hookworm infection, which is usually considered to be of greatest medical significance during pregnancy. Prevalence values ranging from 12.5% to 30.8% have been reported from studies in pregnant women in Liberia, South Africa and Northeast Thailand (Jackson & Jackson, 1987; Saowakontha *et al.*, 1992; Thomson, 1997). Intensity data was given only for the study in Liberia, where the prevalence of hookworm infection was 30.0% (Jackson & Jackson, 1987). The majority of infections among the Liberian women were light: the median intensity was 26 epg, and only two women had egg counts exceeding 1,000 epg (Jackson & Jackson, 1987). Both the prevalence and intensity of hookworm infection in the present study population were high in comparison with these studies. It is possible that the *N.americanus* intensity was underestimated in the present study as hookworm eggs are fragile and may collapse as a consequence of fixing the stool samples in formalin (A.Hall[2], personal communication). In addition, clearance of the faecal slides for more than 60 min. may lead to disappearance of some hookworm eggs due to the use of glycerol (Pawlowski *et al.*, 1991).

The intensity of *T. trichiura* (among all matched pairs of pregnant and non-pregnant women) and intensity of *N. americanus* infection (among infected women only) were significantly higher among pregnant women than non-pregnant women residing in the same household. The lack of difference in the intensity of *A. lumbricoides* may be attributed to the small number of subjects infected with this nematode. In southern Nigeria, Nwosu (1981) also reported a higher prevalence and intensity of hookworm infection among women in their third trimester of pregnancy than in non-pregnant non-lactating women in the same community.

If it is assumed that pregnant and non-pregnant woman co-residing in the same household are subject to similar environmental determinants of intensity (such as the distribution of parasites in the vacinity of the household), these differences may be attributed to some aspect of pregnancy that increases susceptibility or exposure to infection. Possibilities include alterations in immunity, nutritional status, gastrointestinal function and behaviour.

During pregnancy, antibody responses are normal, but cell-mediated immunity (CMI) is down-regulated to avoid immunological rejection of the fetus (Weinberg, 1987). Pregnant women may therefore be at greater risk of acquiring nematode infections for which CMI is essential in defence. Cell-mediated immune responses have been described in animal models of intestinal nematodes (Wakelin, 1985; Carroll, 1990), but as yet, the nature and protective effect of similar responses in humans are not well characterised (Bundy & Cooper, 1989; Ottesen, 1990).

The nutritional constraints on the survival or fecundity of blood feeding nematodes, such as *N. americanus* and *T. trichiura*, have not been quantified. However, it is reasonable to suppose that a increase in the nutrient content of blood would promote both survival and fecundity. During pregnancy, the blood concentration of haemoglobin and total serum proteins decrease (Stirrat, 1981; Hytten, 1985), and so this mechanism does not adequately explain the elevated intensities and densities of these nematodes. One physiological characteristic of pregnancy that may account for these findings is constipation, a frequent gastrointestinal complaint among the pregnant women in the study. The reduction in stool volume associated

[2] Dr. A. Hall, Wellcome Centre for Epidemiology, Oxford.

with this condition may lead to a denser distribution of parasite eggs per unit volume of stool that is unrelated to increased susceptibility to infection. The association between egg density and stool consistency has been demonstrated (Nawalinski *et al.*, 1978).

Behaviour is also modified during pregnancy, and a notable example is geophagia, a form of pica that involves deliberate consumption of clay or earth (Walker *et al.*, 1985). The extent of geophagia among pregnant women varies ethnically and geographically, but tends to be more common among tropical populations (Key *et al.*, 1981; Walker *et al.*, 1985). In the present study, one pregnant woman recalled consuming a small ball of clay as part of her dietary intake. A reported history of geophagic behaviour is associated with severe *T. trichiura* infections in children (Gilman *et al.*, 1983). Deliberate soil eating may increase exposure to *T. trichiura* as this parasite is transmitted via the faeco-oral route. Furthermore, geophagia interferes with the intestinal absorption of zinc, an essential element for thymus-mediated immune responses (Wong *et al.*, 1988). Low plasma zinc concentrations are associated with intense *T. trichiura* infection (Bundy & Golden, 1987). Geophagic behaviour would need to be initiated very early in pregnancy in order to effect the elevated intensities of *T. trichiura* at the end of the first trimester, and geophagia does not explain the higher intensities of *N. americanus* infection, which is not acquired orally. Nonetheless, the possible contribution of geophagia to the faeco-oral transmission of nematodes in pregnant women merits further investigation.

While the findings of the present study indicate that pregnant women may be at greater risk of intestinal nematode infections, these horizontal findings cannot be regarded as evidence for a causal relationship between pregnancy and predisposition to infection. Further investigations should employ a longitudinal study design, with follow-up of pregnant women who were screened prior to pregnancy. Alternatively, the incidence of infection in pregnant women following anthelminthic intervention could be compared to that of non-pregnant women.

6.4.2 Epidemiological patterning of intestinal nematode infection

Analysis of the prevalence data indicated that the likelihood of intestinal nematode infection was not equivalent for all pregnant women. Subjects infected with *N. americanus* were more likely to have *T. trichiura* infection, and those with both infections were more likely to be infected with *A. lumbricoides*. The odds on having *N. americanus* infection were independently higher among rural residents, subjects of low SES and women who were assessed in the early dry season. In addition, rural residents were more likely to be polyparasitised, and those with low SES were more likely to be infected with *T. trichiura*.

Polyparasitism with intestinal nematodes is the norm in developing counties, and may reflect similarities in transmission or host susceptibility to infection. These infections are collectively known as soil-transmitted nematodes as they are all acquired following contact with faecally polluted soils. A study of the comparative prevalences of *A. lumbricoides*, *T. trichiura* and *N. americanus* infection from published literature indicated a consistent positive correlation between the prevalence of *A. lumbricoides* and *T. trichiura* in different geographical areas (Booth & Bundy, 1992). Simultaneous exposure to *A. lumbricoides* and *T. trichiura* is likely as they are both transmitted via faeco-oral contact. The prevalence of *N. americanus*, meanwhile, appeared to be independent of infection with the other species (Booth & Bundy, 1992). These findings were not observed in the present study: despite its transdermal route of transmission, *N. americanus* infection was associated with both *A. lumbricoides* and *T. trichiura* infection. It is unlikely, therefore, that a specific behaviour can determine the presence of all three nematodes (Haswell-Elkins *et al.*, 1987). Laboratory studies with animal models indicate that intestinal nematode infections induce non-specific immunosuppresssion, and thereby promote the survival and establishment of a different nematode species (Jenkins & Behnke, 1977; Crandall *et al.*, 1978; Pritchard *et al.*, 1984; Behnke *et al.*, 1994b). Pregnant women with high or low intensities of infection for one nematode did not necessarily have high or low intensities for another nematode. The lack of correlation between intensities of these three infections would appear to contradict the prevalence data but applies only to infected subjects in this case.

Rural-urban differences in *N. americanus* prevalence are frequently observed in Sierra Leone (Whitworth *et al.*, 1990; Ewald, 1994; Williams, 1996). The availability of suitable sites for hookworm larval development and agriculture-based employment may encourage greater transmission in rural areas. Agriculture is widely regarded as an occupational risk factor for *N. americanus*, as the work draws subjects away from sanitary facilities (thereby encouraging indiscriminate defaecation) and involves prolonged contact with soil (Schad *et al.*, 1983). After controlling for rural residence, farming as a maternal or paternal occupation was not associated with *N. americanus* infection status in the multivariate model. Rural residency may therefore account for the variation in infection due to farming. It is possible that women in rural areas were more likely to engage in farming as a secondary means of occupation or on a subsistence level to provide food for the family. Rural residents were more likely to have farming relatives, whom they may have been expected to assist after working hours. For example, of the 29 husbands who farmed, 28 resided in the rural areas. Non-cultivated land in rural areas in Sierra Leone tends to be vegetation-rich and is likely to promote the survival and development of the free-living stages (Smith, 1990). In the crowded environments of peri-urban Freetown, land is less readily available for household gardens and farming allotments, which are situated between residential areas, are usually closer to sanitary facilities. Peri-urban sites are often cleared of vegetation, and the exposed areas of compacted soil, gravel or tarmac are less conducive to parasite survival. Rural residency may also be a correlate of some undefined risk factor for *N. americanus* infection, such as depressed immunity to infection due to increased nutritional stress.

The relationship between SES and intestinal nematodes has not been adequately studied in Sierra Leone. Two previous studies only examined one or two indicators of SES. An assessment among children residing in Freetown did not observe any association between infection status and maternal education or the number of subjects per room per household (Webster *et al.*, 1990). However, a community-based study in Foria, a rural area in the Northern Province, reported an association between intense infection with *A. lumbricoides* and small household size (Ewald, 1994). The SES index employed in the present study represented the additive effects of maternal education, household sanitation facilities, source of drinking water supply, radio possession and maternal and paternal occupation. The underlying mechanism for the association of both *N. americanus* and *T. trichiura* with low SES may involve one or more of these factors or their correlates. For example, exposure may be increased in the absence of sanitary facilities and ignorance of health issues, or immunity to infection compromised by poverty-induced nutritional stress. This study does not resolve the actual mechanism, but clearly indicates that these infections are associated with poorer living conditions. Elsewhere in the developing world, most detailed studies have reported that intestinal nematodes are more common among subjects of lower SES (St. George, 1976; Sanjur, 1989; Holland *et al.*, 1988). These studies are supported by evidence which indicates that the risk of acquiring infection is enhanced by patterns of human behaviour, such as inadequate use of shoes or latrines (Dunn, 1972; Kloos *et al.*, 1981; Kochar *et al.*, 1983; Schad *et al.*, 1983).

The higher prevalence of *N. americanus* among pregnant women whose first trimester stool sample was obtained in the early dry season is explained by the seasonality of hookworm transmission (Pawlowski *et al.*, 1991). The early dry season immediately follows the previous wet season. The relative high humidity and lower temperatures in the wet season are most conducive to the survival and development of *N. americanus* larvae (Smith, 1990). Thus the population density of infective larvae in the soil is likely to peak during the rains (Chandiwana, 1990). Women assessed in the early wet season did not exhibit a higher prevalence of infection: the pre-patent period for *N. americanus* is about 6-8 weeks (Pawlowski *et al.*, 1991), and infections acquired during this season would not have been detected if the parasites had not reached sexual maturity.

The intensities of *A. lumbricoides* and *T. trichiura* decreased with maternal age, indicating that that younger mothers may be at greater risk of high intensity infections. As the degree of morbidity is generally related to intensity (Stephenson & Holland, 1987; Bundy & Cooper, 1989; Lwambo *et al.*, 1992; Chan *et al.*, 1994c), the latter group of subjects are at increased risk of developing disease manifestations. Irrespective of geographical location, these age-intensity profiles are usual among adults (Bundy, 1988; Bundy, 1990). There was also a trend of decreasing intensity of *N. americanus* with age, but the low intensity in the 20-24 year age groups appeared to account for most of the difference. Previous studies have suggested a wide variation in the age-intensity profiles for hookworm (Behnke, 1987), although more recently, studies using worm burdens as a direct measure of intensity have indicated that intensity reaches a stable asymptote or increases slightly in adulthood (Pritchard *et al.*, 1990b; Bradley *et al.*, 1992). These recent studies are contrary to the present findings of a marked decrease in intensity with age, perhaps due to different measures of intensity employed.

The reduction in infection intensities with age indicates that the rate of acquisition of these infections declines across the child-bearing years. These age-dependent intensity patterns suggest that there may be a reduction in exposure to infection and/or the development of acquired immunity with age (Anderson, 1986). Due to difficulties in quantifying exposure to infection and an incomplete understanding of the mechanisms which control host immunity, the relative contribution of exposure and acquired immunity in regulating intensity is unknown. Both must be important as exposure is essential for the development of immunity (Bundy & Medley, 1992). In recent years, immunoepidemiological investigations have demonstrated that age-dependent trends in antibody responses to *A. lumbricoides*, *N. americanus* and *T. trichiura* mirror those of infection intensity at the community level, and probably reflect the degree of exposure to infection with age (Haswell-Elkins *et al.*, 1989; Pritchard *et al.*, 1990b; Haswell-Elkins *et al.*, 1992; Needham *et al.*, 1996). The development of better hygienic practices with age is likely to reduce exposure and thus the intensities of infection in adulthood, as infection is preventable by avoiding contact with infectious stages of the life-cycles. Antibody responses have also been shown to negatively correlate with parasite burdens in adults, and are suggestive of an acquired immune response (Bundy *et al.*, 1991b; Needham & Lillywhite, 1994; Quinnell *et al.*, 1995). The protective effects of immune responses to intestinal nematodes have been demonstrated in non-human mammalian hosts (Carroll, 1990; Wakelin, 1996), and would appear to indicate that acquired immunity is of some importance in humans (Anderson & May, 1991b). Direct evidence for the protective effect of the human humoral immune response is lacking, although a protective role of IgE against *N. americanus* has been implicated by studies that have shown a negative correlation between total (non-specific) IgE levels and worm fecundity (Pritchard *et al.*, 1995).

The relationship between the prevalence and mean intensity of highly aggregated intestinal nematodes is non-linear, such that a large decrease in intensity will result in a modest decline in prevalence (Anderson & May, 1985; Anderson, 1986). It is not surprising therefore, that the decline in intensity of the intestinal nematodes with age was not associated with a similar decrease in prevalence.

Predisposition to infection with *T. trichiura* is indicated by the higher intensities of infection among subjects infected with all three intestinal nematodes and those with low SES. Direct causative evidence for predisposition requires the investigation of reinfection after anthelminthic intervention (Anderson, 1986). A sensitive assessment of predisposition is only possible following the re-establishment of mean pre-treatment intensities of infection (Keymer & Pagel, 1990). The use of worm burden as a direct measure of intensity is preferred, as density-dependent constraints on fecundity and sampling error are likely to reduce the sensitivity of analyses based on egg counts (Keymer & Pagel, 1990). During the five months following treatment, the egg counts of all three infections remained significantly lower than baseline values, and therefore neither of these conditions were met. Nonetheless, subjects with heavy *T. trichiura* infections at baseline tended to reacquire heavier than average infections six months after treatment. Significant predisposition remained after standardization of the egg counts to remove host-age related trends in intensity. Following anthelminthic intervention, 38% of pregnant women retained their relative position in the rank of *T. trichiura* intensities at baseline, which is typical of intestinal nematodes (Bundy & Medley, 1992). It should be noted that albendazole did not completely eradicate all *T. trichiura* infections, and this result should be interpreted in terms of continuing infection and reinfection.

Epidemiological investigations have demonstrated predisposition to infection with *A. lumbricoides* (Croll *et al.*, 1982; Elkins *et al.*, 1986; Thein Hlaing *et al.*, 1987; Holland *et al.*, 1989; Hall *et al.*, 1992), hookworms (Schad & Anderson, 1985; Haswell-Elkins *et al.*, 1988; Bradley & Chandiwana, 1990; Quinnell *et al.*, 1993) and *T. trichiura* (Bundy, 1986; Bundy *et al.*, 1987a; Haswell-Elkins *et al.*, 1987). The nature of the mechanisms underlying predisposition to intestinal nematodes, including *T. trichiura*, is a subject of controversy. These mechanisms are probably distinct from those which generate the age-dependent trends in intensity, as most studies where data has been standardised by host age have demonstrated that predisposition is independent of age (Bundy *et al.*, 1987a; Haswell-Elkins *et al.*, 1987; Haswell-Elkins *et al.*, 1988; present study). There is some evidence to indicate that predisposition relates to heterogeneity in immunological susceptibility to *T. trichiura*. Immunocompetence may be determined by variation in phenotypic factors such as nutrition, the presence of other infections, or reproductive state, or by variation in genotype (Keymer & Pagel, 1990). Nutritionally-mediated mechanisms are indicated by studies which have shown that low plasma concentrations of zinc, an essential trace element for thymus-mediated immune responses, are correlated with high intensities of *T. trichiura* (Bundy & Golden, 1987). The association of polyparasitism with intense *T. trichiura* infections may arise as a consequence of non-specific immunosuppression by an existing nematode infection. In the present study, pregnant women harboured a higher intensity of *T. trichiura* than their non-pregnant counterparts. The contribution of suppressed cell-mediated immunity to these findings, and the extent of heterogeneity in these responses, is unknown. However, defences against intestinal nematodes in non-human mammals appear to be sensitive to hormonal changes associated with pregnancy (Wakelin, 1986). Genetic restriction of the immune repertoire appears to determine susceptibility to intestinal nematode infections in non-human mammals (Wakelin, 1985; Wakelin, 1996; Kennedy, 1989), and in humans, HLA (human-leucocyte-associated antigens) correlates of intense *T. trichiura* infection have been described (Bundy, 1988). Familial clustering of heavy (or light) *T. trichiura* infections lends support to genetically determined predisposition (Forrester *et al.*, 1988), although recent analysis implicates shared environmental or behavioural characteristics as the significant determinants of these observations (Chan *et al.*, 1994b). The association between *T. trichiura* infection and low SES observed in the present study does imply that individuals living under deprived conditions are at increased exposure to infection. Despite the lack of objective evidence for the role of exposure, it is highly likely that heterogeneity in subject behaviours such as geophagia are also involved in the generation of predisposition (Bundy, 1988; Wong *et al.*, 1988). Geophagia is of particular relevance in pregnant women.

6.4.3 Therapeutic efficacy of albendazole treatment

Albendazole was imported into Sierra Leone for human use in the 1980s (M.Hodges[3], personal communication), and has been the drug of choice for school-based anthelminthic control programmes in Freetown and the provinces. It is recommended by local health care professionals but is rarely purchased by the general population as less expensive generic forms of mebendazole, levamisole and pyrantel are available. Traditional forms of medication are also common and are more widely used in rural areas where they are more familiar to the local population, readily available and less costly (see Chapter 11). The pregnant women who participated in the present study were not prevented from seeking any form of medication outside the study clinics, but were asked to report to a member of the study team if they did. No subjects reported taking any anthelminthic drug or traditional treatment other than that

[3] Paediatrician, MB BS, MRCP.

provided by the clinic. Although there may have been some under-reporting, the proportion of subjects of high SES was similar in each intervention group, and therefore the ability to purchase treatment was probably comparable.

The trial has shown that albendazole (400 mg), administered as a single dose at the beginning of the second trimester of pregnancy, was highly effective in eliminating infection and reducing the baseline egg counts of *A. lumbricoides* and *N. americanus*. Within one month of treatment, the CRs exceeded 90% and baseline mean geometric egg counts were reduced by over 99%. Albendazole was not effective in clearing over 35% of *T. trichiura* infections but reduced the geometric mean egg counts by 96%. The CRs for the albendazole placebo were inseparable from the technical errors associated with the parasitological techniques (WHO, 1996a). Iron-folate supplements did not appear to affect the prevalence or intensity of infection.

The CRs and ERRs for all intestinal nematodes fell within the upper range of values recently summarised for 80 published human albendazole trials, except the ERR of *T. trichiura* which exceeded the range of values (Table 6.17; WHO, 1996a). Several factors may have contributed to the relatively high CRs and ERRs in the present trial. Firstly, the susceptibility of nematodes to albendazole treatment may differ among geographical variants of the same nematode species. There have been no confirmed reports of anthelminthic drug resistance to intestinal nematodes in humans (de Silva *et al.*, 1997), but resistance is documented among nematode parasites of domestic livestock (Waller, 1990; Conder, 1995) and suggests that repeated anthelminthic intervention in humans could give rise to similar increases in resistant genotypes among anthrophilic species. Differential susceptibility to anthelminthic treatment has been documented (Pritchard *et al.*, 1990a; Campbell & Benz, 1984), yet published intervention studies rarely distinguish between the two species of hookworm. Secondly, the high indices may relate to the large proportion of light or moderate intensity infections at baseline. Albonico *et al.* (1994) reported that CRs among subjects who had baseline *T. trichiura* egg counts less than 1,000 epg were higher than those with egg counts greater or equal to 1,000 epg. Thirdly, differences in study design and use of parasitological methods may give rise to the disparity in CRs and ERRs values between trials. Indices based on the collection of several consecutive stool samples and use of intensive techniques including concentration procedures are likely to produce lower estimates of CR than those based on single post-treatment samples (WHO, 1996a). Fourthly, these high indices may be peculiar to anthelminthic intervention in pregnant women, although an underlying mechanism is unclear.

The central aim of an intestinal nematode control programme is not only to reduce the intensities of infection among the target population, but to maintain the intensities below the levels associated with morbidity (WHO, 1996a). In order to determine the rate at which pregnant women reacquired infections and thus the appropriate frequency of anthelminthic treatment during pregnancy, repeat stool surveys were conducted in the second and third trimesters. Infection and reinfection with *A. lumbricoides* and *N. americanus* following albendazole treatment was very low during this follow-up period and the geometric egg counts remained below 1% of the baseline values. In contrast, reinfection with *T. trichiura* was rapid, and by the third trimester assessment, the prevalence of infection had increased to 50%. However, the ERR remained above 85% and would probably have been sufficient to prevent symptomatic infection during the course of pregnancy. The more rapid return of *T. trichiura* populations to pre-control levels relative to *A. lumbricoides* and *N. americanus* is due in part to the higher basic reproductive rate, lower life expectancy and higher initial prevalence of this species in this population (Anderson & Medley, 1985; Bundy *et al.*, 1985a; Bundy, 1986).

Previous intervention studies have shown that the recovery of *T. trichiura, A. lumbricoides* and *N. americanus* populations following chemotherapy takes approximately six months (Bundy *et al.*, 1985a), one year (Haswell-Elkins *et al.*, 1987; Holland *et al.*, 1989) and several years (Schad & Anderson, 1985; Quinnell *et al.*, 1993), respectively. While these studies were carried out over a longer period than the present study, it was clear that the rates of reinfection observed in the pregnant women were low in comparison to these studies. The use of faecal egg counts in the present study may have even exaggerated the reinfection rates, as parasite fecundity appears to increase after treatment (Haswell-Elkins *et al.*, 1988).

Several factors may have contributed to the low rate of reinfection among this group of pregnant women. The absence of children in the study sample is likely to explain part of the difference, as children tend to reacquire *A. lumbricoides* and *T. trichiura* infections more rapidly than adults (Thein Hlaing *et al.*, 1987; Bundy *et al.*, 1988), although reinfection with hookworm is reported to be independent of age (Quinnell *et al.*, 1993). Previous anthelminthic intervention studies in Sierra Leone have only examined school children, among whom reinfection was also rapid (Williams & Hodges, 1995; Koroma *et al.*, 1996). The effect of host characteristics on the degree of parasite transmission is also of relevance. The contribution of pregnancy-associated alternations in exposure or physiology to the low rates of reinfection is unknown. Public health education messages obtained at the antenatal clinics may have promoted improved hygienic behaviour and self-treatment (although the latter was not reported). Furthermore, women become less mobile as pregnancy proceeds and reduced involvement in high risk activities such as gardening or farming may have decreased exposure. It is also possible that the changes in blood composition may have reduced the fecundity or survival of haemophagus nematodes, although iron-folate supplementation did not appear to influence the intensities of infection.

Published reports on the experience of albendazole treatment during pregnancy, particularly the first trimester, are scarce (Cook, 1993). Trials provide the opportunity to assess the safety of albendazole treatment during pregnancy, and it is of importance to report on clinical symptoms that are experienced following treatment. This is of particular relevance in developing countries, where pregnant women may be inadvertently treated as part of mass control programmes (Horton, 1993). Previous controlled trials among non-pregnant populations indicate that adverse effects affect only a small proportion of patients, are usually mild and transient, and are similar to those observed in placebo groups (Rossignol & Maisonneuve, 1983; Albonico *et al.*, 1994). These adverse effects include epigastric pain, diarrhea, headache, nausea, vomiting, dizziness, constipation, dry mouth, lassitude and insomnia (Cook, 1990; Dollery, 1992; de Silva *et al.*, 1997). In the present study, the range and frequency of symptoms experienced by pregnant women receiving albendazole was similar to the placebo group. The frequency of miscarriage was identical in albendazole and placebo groups. Unfortunately, the exact timing of these adverse symptoms (apart from miscarriage) relative to the administration of treatment is unknown and therefore none of the adverse clinical signs could be directly attributed to the effects of treatment.

6.4.4 Implications for the control of intestinal nematode infections during pregnancy

Chemotherapy is regarded as the most effective means of controlling the mortality and morbidity associated with intestinal nematode infections (WHO, 1996a). The present results indicate that in this community, a single dose of albendazole (400 mg) administered after the first trimester of pregnancy, was highly effective in reducing the prevalence and intensity of intestinal nematode infection. While the duration of protection afforded by a single dose of albendazole could not be assessed within the time frame of the follow-up period, it appeared to be adequate for the remaining duration of pregnancy. However, it should be noted that the full yearly cycle of seasons was not covered in the present study, as women were recruited over a six month period from December to June. Therefore, the extent of reinfection following the administration of albendazole treatment in the second trimester of pregnancy between July and November is unknown.

The implication of these results for pregnant women in other regions is difficult to ascertain, as the efficacy of treatment and reinfection after intervention is strongly dependent on local ecology, epidemiology and the species of parasites. The appropriate frequency of anthelminthic treatment during pregnancy is likely to vary and should be determined for each community by assessing the rate of reinfection following anthelminthic treatment (Chandiwana, 1990) and possible effects of seasonality on the transmission of infective stages (WHO, 1996a). In areas where the transmission of infection is more intense, the impact of chemotherapy may be short-lived, and therefore the optimal frequency of chemotherapy during pregnancy will be greater. Ideally, each antenatal control program should be designed to reflect the local epidemiological situation (WHO, 1996a).

Intestinal nematode control programs are described in terms of their degree of coverage: selective treatment, in which anthelminthics are administered to subjects with high intensity infections; targeted treatment, in which subjects in predetermined high risk groups are treated; and mass treatment, in which all or a proportion of subjects in a community are treated (Anderson & May, 1982). The highly aggregated distribution of nematode infections among the pregnant women and the identification of predisposition to *T. trichiura* may facilitate the control of morbidity by selective or targeted approaches (Anderson & May, 1982; Schad & Anderson, 1985; Elkins *et al.*, 1986). However, both these approaches have serious draw-backs. In developing countries the costs of a selective approach, that is, the identification, follow-up and treatment of heavily infected pregnant subjects, is prohibitive on a national basis. A targeted approach will only be realised if predisposition is fully characterised and is persistent, and only then if predisposed subjects can be identified by cost-effective means (Bundy *et al.*, 1987b). The concept of high risk groups is not particularly useful among the present pregnant community in Sierra Leone as easily assessed socio-demographic or socio-economic factors did not correlate with intensity (and therefore morbidity) of infection. Young mothers may constitute a high risk category, although undefined factors other than maternal age appeared to predispose pregnant women to *T. trichiura*. Furthermore, selective or targeted treatment may not be socially acceptable, particularly in African communities (Chandiwana, 1990). In this context, all pregnant women should be regarded as high risks subjects and resources for control directed at this entire group.

In the developing world, the use of chemotherapy for the control of intestinal nematode is restricted by financial constraints (Bundy *et al.*, 1991a), and these infections must compete with a multitude of other life-threatening diseases for limited financial and health resources (Medley, 1994). Information on the prevalence and intensity of these infections and the efficacy of treatment is not sufficient to justify the implementation of an antenatal intestinal nematode programme. Anthelminthic treatment must also be shown to effect a substantial health benefit. Morbidity indicators besides intensity are therefore necessary to determine the impact of albendazole treatment on the course and outcomes of pregnancy. In the following three chapters, the impact of intestinal nematode infections and albendazole treatment on maternal nutritional status and neonatal outcomes is examined.

6.5 SUMMARY

1. The distribution of intestinal nematode infections in women in the first trimester of pregnancy and the efficacy of albendazole (400 mg) administered as a single dose at the beginning of the second trimester was investigated in Western Sierra Leone.
2. The prevalence (and geometric mean intensity) of intestinal nematodes was as follows: *A. lumbricoides* 21.2% (267 epg); *N. americanus* 66.9% (191 epg); *T. trichiura* 71.7% (93 epg). In addition 9.8% (70 epg) of pregnant women were found to be infected with *S. mansoni*.
3. The presence of *N. americanus* was associated with *T. trichiura*, and subjects with both these infections were more likely to be infected with *A. lumbricoides*. The odds on *N. americanus* infection were independently higher among rural residents, subjects of low SES and women who were examined in the early dry season. Rural residents and women of low SES were also more likely to be polyparasitised.
4. The intensities of infection, as measured by faecal egg counts, were found to be overdispersed. Most infections were light. The intensity of infection appeared to decrease across the child-bearing years, indicating that younger mothers may be at greater risk of high intensity infections and associated morbidity. Age-dependent exposure to infection or acquired immunity may explain these findings.
5. The intensity of *T. trichiura* (among all matched pairs of pregnant and non-pregnant women) and intensity of *N. americanus* infection (among infected women only) were significantly higher among pregnant women than non-pregnant women residing in the same household.
6. Albendazole was highly effective in reducing the prevalence and intensity of *A. lumbricoides* and *N. americanus*. Albendazole was not effective in clearing *T. trichiura* infections but reduced the geometric mean egg counts considerably. Iron-folate supplements did not appear to induce any effect on the prevalence or intensity of infection.
7. The protection afforded by a single dose of albendazole, in terms of reducing the intensities below the level associated with morbidity, extended for the duration of pregnancy for all infections.
8. The reinfection patterns of *T. trichiura* after treatment, standardised for age, suggest that some subjects were predisposed to high or low intensity infections.
9. The concept of high risk groups is not particularly useful for pregnant women in these communities, as easily assessed socio-demographic or socio-economic factors did not correlate with intensity (and therefore morbidity) of infection. In this context, all pregnant

women should be regarded as high risks subjects and resources for control directed at this entire group.

10. The implication of these results for pregnant women in other regions is difficult to ascertain, as the efficacy of treatment and reinfection after intervention is strongly dependent on local ecology, epidemiology and the species or strain of parasite. Ideally, each antenatal control program should be designed to reflect the local epidemiological situation.

Table 6.1 Prevalence and intensity of intestinal nematode infections and *Schistosoma mansoni* in women in the first trimester of pregnancy in Western Sierra Leone (*n*=184).

Infection	Prevalence (95% CI)	Geometric mean intensity (±SD) (eggs per gram faeces)
Ascaris lumbricoides	21.2 (15.2, 27.2)	267 (-229, +1618)
Necator americanus	66.9 (60.0, 73.7)	191 (-152, +736)
Trichuris trichiura	71.7 (65.2, 78.3)	93 (-67, +241)
Schistosoma mansoni	9.8 (5.5, 14.1)	70 (-51, +191)

Table 6.2 Distribution of subjects[†] infected with intestinal nematodes among the intensity classes.

Infection	Intensity class[‡] (eggs per gram faeces)		Frequency (%)
Ascaris lumbricoides	light	1 - 4,999	35 (89.7)
	moderate	5,000 - 49,999	4 (10.3)
	heavy	50,000+	0 (0.0)
Necator americanus	light	1 - 1,999	115 (93.5)
	moderate	2,000 - 3,999	5 (4.1)
	heavy	4,000+	3 (2.4)
Trichuris trichiura	light	1 - 999	127 (96.2)
	moderate	1,000 - 9,999	5 (3.8)
	heavy	10,000+	0 (0.0)

† Subjects comprise 184 women in the first trimester of pregnancy in Western Sierra Leone.
‡ As suggested by WHO (1987) and Stoltzfus *et al.* (1996).

Table 6.3 Risk factors for infection with *Necator americanus* in women in the first trimester of pregnancy in Western Sierra Leone.

Risk factor		*n*	Frequency of infection (%)	Univariate analysis[†] Odds ratio[§] (95% CI)	χ^2	*p*	Multivariate analysis[‡] Odds ratio[§] (95% CI)	Wald	*p*
Education	none	120	87 (72.5)	2.78 (1.32, 5.84)	7.53	0.023	-	-	NS[††]
	primary	25	17 (68.0)	2.24 (0.78, 6.39)					
	secondary/tertiary	39	19 (48.7)						
Occupation	farmer	25	24 (96.0)	14.55 (1.92, 110.25)	9.62	0.0019	-	-	NS[††]
	other	159	99 (62.3)						
Partner's occupation	farmer	29	26 (89.7)	5.18 (1.50, 17.88)	6.90	0.0086	-	-	NS[††]
	other	155	97 (62.6)						
Residence	rural	117	92 (78.6)	4.27 (2.22, 8.21)	18.70	<0.0001	2.81 (1.35, 5.86)	7.60	0.0058
	peri-urban	67	31 (46.3)						
Socio-economic status	low	89	73 (82.0)	4.11 (2.09, 8.06)	16.61	<0.0001	4.06 (1.90, 8.64)	13.17	0.0003
	high	95	50 (52.6)						
Source of water	stream	9	7 (77.8)	2.44 (0.48, 12.33)	7.73	0.026	-	-	NS[††]
	communal well	68	53 (77.9)	2.46 (1.24, 4.92)					
	stand-pipe	107	63 (58.9)						
Season at first trimester assessment[‡‡]	early dry	80	66 (82.4)		15.67	0.0003			
	late dry	65	36 (55.4)	0.26 (0.12, 0.56)			0.29 (0.13, 0.65)	9.00	0.0027
	early wet	39	21 (53.8)	0.25 (0.11, 0.58)			0.27 (0.10, 0.74)	6.54	0.011

[†] Chi-square tests of independence.
[‡] Multiple logistic regression.
[§] Reference category is that with the lowest proportion, except for season at first trimester assessment, for which the reference category is that with the highest proportion.
[††] Non-significant (variable not entered into the multiple logistic regression model).
[‡‡] Early dry = November to January; late dry = February to April; early wet = May to July.

Table 6.4 Frequency of polyparasitism with intestinal nematode infections in women in the first trimester of pregnancy in Western Sierra Leone.

Infections	Frequency (%)
No infections	28 (15.2)
Single infections	
A. lumbricoides	3 (1.6)
N. americanus	18 (9.8)
T. trichiura	28 (15.2)
Double infections	
A. lumbricoides, N. americanus	3 (1.6)
A. lumbricoides, T. trichiura	2 (1.1)
N. americanus, T. trichiura	71 (38.6)
Triple infections	
A. lumbricoides, N. americanus, T. trichiura	31 (16.8)

Table 6.5 Association between infection with *Necator americanus*, *Ascaris lumbricoides* and *Trichuris trichiura* in women in the first trimester of pregnancy in Western Sierra Leone.

Infection	Subset of women	*n*	Odds ratio for *N. americanus* infection (95% CI)	Wald	*p*
A. lumbricoides	*T. trichiura* uninfected	52	1.56 (0.28, 8.57)	0.26	0.61
	T. trichiura infected	132	6.11 (1.37, 27.25)	5.63	0.018
T. trichiura	*A. lumbricoides* uninfected	145	3.94 (1.89, 8.24)	13.35	0.0003
	A. lumbricoides infected	39	15.49 (1.81, 132.44)	6.26	0.012

Table 6.6 Risk factors for polyparasitism with intestinal nematodes in women in the first trimester of pregnancy in Western Sierra Leone.

Risk factor		n	Frequency of poly-parasitism (%)	Univariate analysis[†]			Multivariate analysis[‡]		
				Odds ratio[§] (95% CI)	χ^2	p	Odds ratio[§] (95% CI)	Wald	p
Education	none	120	76 (63.3)	2.76 (1.31, 5.82)	7.89	0.019	-	-	NS[††]
	primary	25	16 (64.0)	2.84 (1.00, 8.05)					
	secondary/Tertiary	39	15 (38.5)						
Occupation	farmer	25	21 (84.0)	4.46 (1.46, 13.57)	6.76	0.0093	-	-	NS[††]
	other	159	86 (54.1)						
Partners occupation	farmer	29	24 (82.8)	4.16 (1.51, 11.48)	7.41	0.0065	-	-	NS[††]
	other	155	83 (53.5)						
Residence	rural	117	82 (70.1)	3.94 (2.09, 7.42)	17.48	<0.0001	3.41 (1.77, 6.54)	13.57	0.0002
	peri-urban	67	25 (37.3)						
Socio-economic status	low	89	64 (71.9)	3.10 (1.68, 5.72)	12.34	0.00044	2.60 (1.37, 4.94)	8.55	0.0035
	high	95	43 (45.3)						
Season at first trimester assessment[‡‡]	early dry	80	57 (71.3)		10.07	0.0065	-	-	NS[††]
	late dry	65	32 (49.2)	0.39 (0.20, 0.78)					
	early wet	39	18 (46.2)	0.35 (0.16, 0.77)					

[†] Chi-square tests of independence.
[‡] Multiple logistic regression.
[§] Reference category that with the lowest proportion, except for season at first trimester assessment, for which the reference category is that with the highest proportion..
[††] Non-significant (variable not entered into the multiple logistic regression model).
[‡‡] Early dry = November to January; late dry = February to April; early wet = May to July.

Table 6.7 Prevalence of intestinal nematode infections in women in the first trimester of pregnancy and non-pregnant women matched for household in Western Sierra Leone.

Infection	Frequency (prevalence %)†		McNemar's χ^2	*p*
	Pregnant	Non-pregnant		
Ascaris lumbricoides	28 (19.3)	30 (20.7)	0.03	0.87
Necator americanus	96 (66.2)	95 (65.5)	<0.01	1.00
Trichuris trichiura	101 (69.7)	87 (60.0)	3.02	0.082

† 145 matched pairs

Table 6.8 Intensity of intestinal nematode infections in women in the first trimester of pregnancy and non-pregnant women matched for household in Western Sierra Leone: includes all women, regardless of infection status.

Infection	Geometric mean intensity (±SD) † (eggs per gram faeces)		Percentage *NP/P*	Paired t-value	*p*
	Pregnant *P*	Non-Pregnant *NP*	(95% CI)		
Ascaris lumbricoides	3.0 (-2.7, +31.0)	2.8 (-2.6 +24.6)	95.3 (58.3, 155.9)	0.19	0.85
Necator americanus	31 (-30, +476)	21 (-19, +213)	64.8 (40.1, 104.7)	1.79	0.076
Trichuris trichiura	24 (-21, +219)	14 (-13, +149)	61.4 (90.9, 98.1)	2.06	0.042

† 145 matched pairs

Table 6.9 Intensity of intestinal nematode infections in women in the first trimester of pregnancy and non-pregnant women matched for household in Western Sierra Leone: includes pairs of infected women only.

Infection	Number of pairs	Geometric mean intensity (±SD) (eggs per gram faeces)		Percentage NP/P (95% CI)	DF	Paired t-value	*p*
		Pregnant (P)	Non-Pregnant (NP)				
Ascaris lumbricoides	10	320 (-284, +2551)	154 (-142, +1755)	48.1 (7.8, 297.7)	19	0.91	0.39
Necator americanus	72	224 (-197, +958)	111 (-80, +289)	49.5 (31.8, 76.8)	71	3.19	0.002
Trichuris trichiura	66	111 (-81 +300)	90 (-68, +281)	81.0 (54.9, 119.4)	65	1.08	0.28

Table 6.10 Baseline (first trimester) prevalence and intensity of intestinal nematode infections in all pregnant women in Western Sierra Leone who gave both baseline and post-treatment stool samples.

Infection	Albendazole (*n*=82)	Placebo (*n*=90)
Ascaris lumbricoides		
frequency (%)	15 (18.3)	22 (24.4)
geometric mean intensity† (± SD)	496 (-411, +2409)	166 (-145, +1138)
Necator americanus		
frequency (%)	55 (67.1)	60 (66.7)
geometric mean intensity† (± SD)	194 (-154, +749)	181 (-143, +685)
Trichuris trichiura		
frequency (%)	59 (72.0)	65 (72.2)
geometric mean intensity† (± SD)	114 (-79, +256)	77 (-57, +217)

† Estimated indirectly as eggs per gram faeces.

Table 6.11 Cure rate for intestinal nematode infections associated with albendazole (400 mg) or placebo in pregnant women in Western Sierra Leone.

Infection	Albendazole			Placebo			Chi-square χ^2	p
	Number excreting eggs		Cure rate (%)	Number excreting eggs		Cure rate (%)		
	Baseline†	Post-treatment‡		Baseline†	Post-treatment‡			
Ascaris lumbricoides	15	1	93.3	22	19	13.6	19.71	<0.0001
Necator americanus	55	3	94.5	59	57	3.4	91.25	<0.0001
Trichuris trichiura	58	21	63.8	64	58	9.4	37.13	<0.0001

† First trimester of pregnancy

‡ Two to four weeks after treatment, which took place at the beginning of the second trimester of pregnancy.

Table 6.12 Egg reduction rate (ERR) of intestinal nematode infections associated with albendazole (400 mg) or placebo in pregnant women in Western Sierra Leone.

Infection	Albendazole Geometric mean eggs per gram (± SD)				Placebo Geometric mean eggs per gram (± SD)				t-value[††]	DF	*p*
	n[†]	Baseline[‡]	Post-treatment[§]	ERR (95 CI)	*n*[†]	Baseline[‡]	Post-treatment[§]	ERR (95 CI)			
A. lumbricoides	22	502.7 (-413.9, +2365.5)	1.7 (-0.2, +10.9)	99.7 (98.9, 99.9)	15	170.6 (-148.0, +1118.1)	123.1 (-111.5, +1181.6)	27.9 (-19.8, 56.6)	8.75	19.30	<0.001
N. americanus	55	196.9 (-155.5, +740.9)	1.2 (-0.7, +1.7)	99.4 (99.0, 99.6)	59	186.0 (-146.8, +694.9)	178.3 (-147.4, +850.9)	4.2 (-37.6, 33.2)	16.55	100.86	<0.001
T. trichiura	58	119.6 (-81.5, +225.5)	4.8 (-4.3, +39.3)	96.0 (92.7, 97.8)	64	80.4 (-58.9, +220.3)	63.0 (-52.8, +327.1)	21.6 (-10.4, 44.3)	8.61	91.47	<0.001

[†] Sample includes only pregnant women who were infected at baseline.
[‡] First trimester of pregnancy
[§] Two to four weeks after treatment, which took place at the beginning of the second trimester of pregnancy.
[††] Comparison of the ERR associated with albendazole and placebo treatment.

Table 6.13 Baseline (first trimester) prevalence and intensity of intestinal nematode infections in the longitudinal cohort of pregnant women in Western Sierra Leone.

Infection	Albendazole (*n*=61)	Placebo (*n*=64)
Ascaris lumbricoides		
frequency (%)	12 (19.7)	13 (20.3)
geometric mean intensity† (± SD)	506 (-434, +3052)	95 (-78, +422)
Necator americanus		
frequency (%)	40 (65.6)	42 (65.6)
geometric mean intensity† (± SD)	193 (-157, +840)	123 (-94, +397)
Trichuris trichiura		
frequency (%)	45 (73.8)	48 (75.0)
geometric mean intensity† (± SD)	114 (-78, +247)	74 (-53, +183)

† Estimated indirectly as eggs per gram faeces.

Table 6.14 Baseline (first trimester) prevalence and intensity of intestinal nematode infections in the longitudinal cohort of pregnant women in Western Sierra Leone by intervention group.

Infection	Intervention group†			
	FeA (n=32)	FeP_A (n =35)	$P_{Fe}A$ (n =29)	$P_{Fe}P_A$ (n =29)
Ascaris lumbricoides				
frequency (%)	8 (25.0)	9 (25.7)	4 (13.8)	4 (13.8)
geometric mean intensity‡ (± SD)	292 (-256, +2083)	81 (-65, +338)	1519 (-1031, +3207)	137 (-119, +869)
Necator americanus				
frequency (%)	23 (71.9)	25 (71.4)	17 (58.6)	17 (58.6)
geometric mean intensity‡ (± SD)	235 (-194, +1113)	115 (-86, +339)	147 (-117, +577)	137 (-109, +520)
Trichuris trichiura				
frequency (%)	26 (81.3)	26 (74.3)	19 (65.5)	22 (75.9)
geometric mean intensity‡ (± SD)	125 (-85, +262)	76 (-47, +127)	100 (-70, +233)	73 (-57, +259)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.
‡ Estimated indirectly as eggs per gram faeces.

Table 6.15 Incidence of intestinal nematode infections in pregnant women in Western Sierra Leone between the post-treatment assessment and the third trimester of pregnancy.

Infection	Albendazole		Placebo		Fisher's Exact Test
	Number uninfected at post-treatment	Number infected in third trimester (incidence %)	Number uninfected at post-treatment	Number infected in third trimester (incidence %)	*p*
Ascaris lumbricoides	60	1 (1.7)	54	4 (7.4)	0.19
Necator americanus	58	2 (3.4)	23	1 (4.3)	1.00
Trichuris trichiura	42	11 (26.2)	17	0 (0.0)	0.024

Table 6.16 Percentage reduction in the baseline (first trimester) geometric egg counts of intestinal nematode infections in women in Western Sierra Leone at each assessment during pregnancy following treatment with albendazole (400 mg) or placebo.

Infection	Assessment	Percentage reduction (±SD) in baseline geometric egg count[†]		t-value	DF	*p*
		Albendazole	Placebo			
Ascaris lumbricoides	post-treatment	99.6 (-3.7, +0.3)	44.8 (-158.1, +40.9)	6.46	23.00	<0.001
	second trimester	99.6 (-3.7, +0.3)	44.5 (-168.8, +41.8)	6.43	23.00	<0.001
	third trimester	99.4 (-10.4, +0.5)	49.8 (-172.2, +38.9)	4.73	15.97	<0.001
Necator americanus	post-treatment	99.3 (-4.2, +0.6)	-19.5 (-378.4, +90.8)	13.55	70.71	<0.001
	second trimester	99.3 (-5.6, +0.7)	-23.9 (-409.0, +95.1)	12.59	68.32	<0.001
	third trimester	99.2 (-6.2, +0.7)	-6.8 (-453.4, +86.5)	11.45	73.46	<0.001
Trichuris trichiura	post-treatment	95.0 (-44.7, +4.5)	28.7 (-220.9, +53.9)	6.66	72.35	<0.001
	second trimester	93.1 (-58.3, +6.2)	23.9 (-247.8, +58.2)	6.08	74.46	<0.001
	third trimester	87.6 (-100.4, +11.0)	23.6 (-281.2, +60.1)	4.58	78.17	<0.001

[†] Includes only pregnant women who were infected at baseline. Sample sizes: *Ascaris lumbricoides*, albendazole *n*=12, placebo *n*=13; *Necator americanus*, albendazole *n*=40, placebo *n*=42; *Trichuris trichiura*, albendazole *n*=45, placebo *n*=48.

Table 6.17 Clinical symptoms reported by pregnant women in Western Sierra Leone within 14 d of intervention with iron-folate supplements and albendazole or respective placebos.

Clinical symptoms	Frequency of reported symptoms in each treatment group†			
	FeA (*n*=39)	FeP_A (*n*=45)	$P_{Fe}A$ (*n* =42)	$P_{Fe}P_A$ (*n* =44)
Headache	2	1	1	3
Abdominal pain	3	1	2	3
Chest pain		1		
General body pain‡	2	1	1	2
Fever	1			2
Cough	1	3		1

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.
‡ Includes back pain, sacral pain, side pain.

Table 6.18 Cure rates and egg reduction rates for intestinal nematodes infections associated with albendazole in pregnant women in Western Sierra Leone and in 80 previous trials.

Nematode	Cure rate (%)		Egg reduction rate (%)	
	Present study†	Previous trials‡	Present study†	Previous trials‡
A. lumbricoides	93.5	85-100	99.7	85-100
N. americanus	94.5	57-100	99.4	73-100
T. trichiura	66.4	10-67	96.0	73-87

† Pregnant women in Western Sierra Leone (*n*=184).
‡ Findings of 80 previous trials with albendazole (WHO, 1996a).

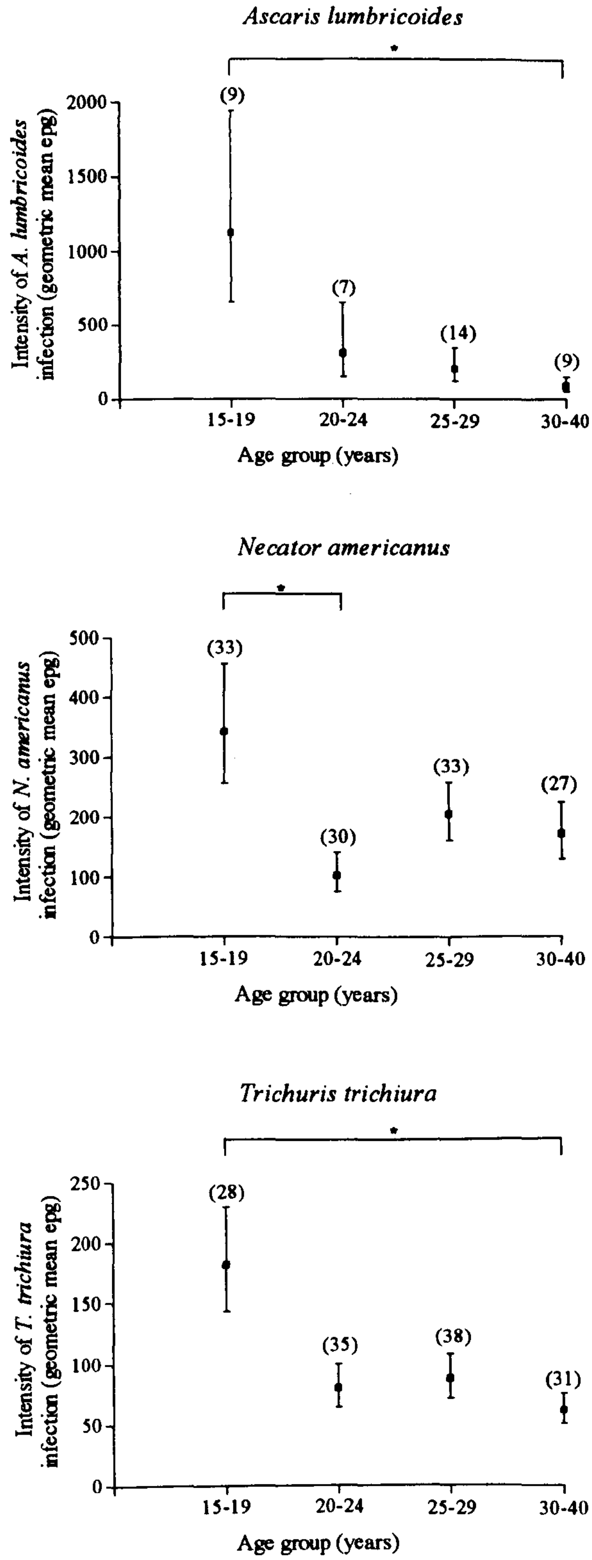

Figure 6.1 Geometric mean intensities of *Ascaris lumbricoides*, *Necator americanus* and *Trichuris trichiura* by age group in women in the first trimester of pregnancy in Western Sierra Leone.
Sample sizes are indicated in parentheses (n); error bars indicate standard error; * = $p<0.05$.

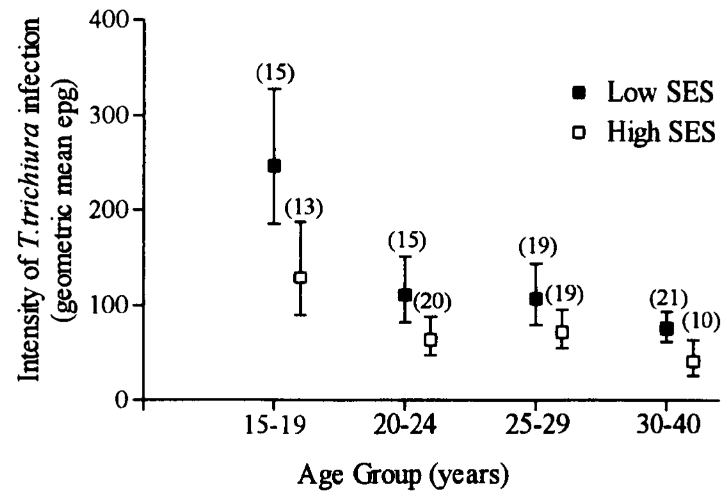

Figure 6.2 Geometric mean intensity of *Trichuris trichiura* by age group and socio-economic status (SES) in women in the first trimester of pregnancy in Western Sierra Leone.
Sample sizes are indicated in parentheses (n); error bars indicate standard error.

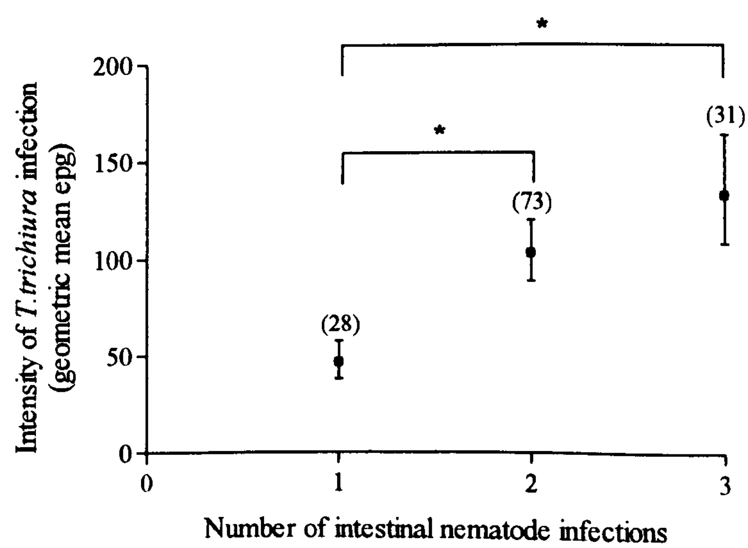

Figure 6.3 Geometric mean intensity of *Trichuris trichiura* by polyparasitism with intestinal nematode infections in women in the first trimester of pregnancy in Western Sierra Leone.
Sample sizes are indicated in parentheses (n); error bars indicate standard error; * = $p<0.05$.

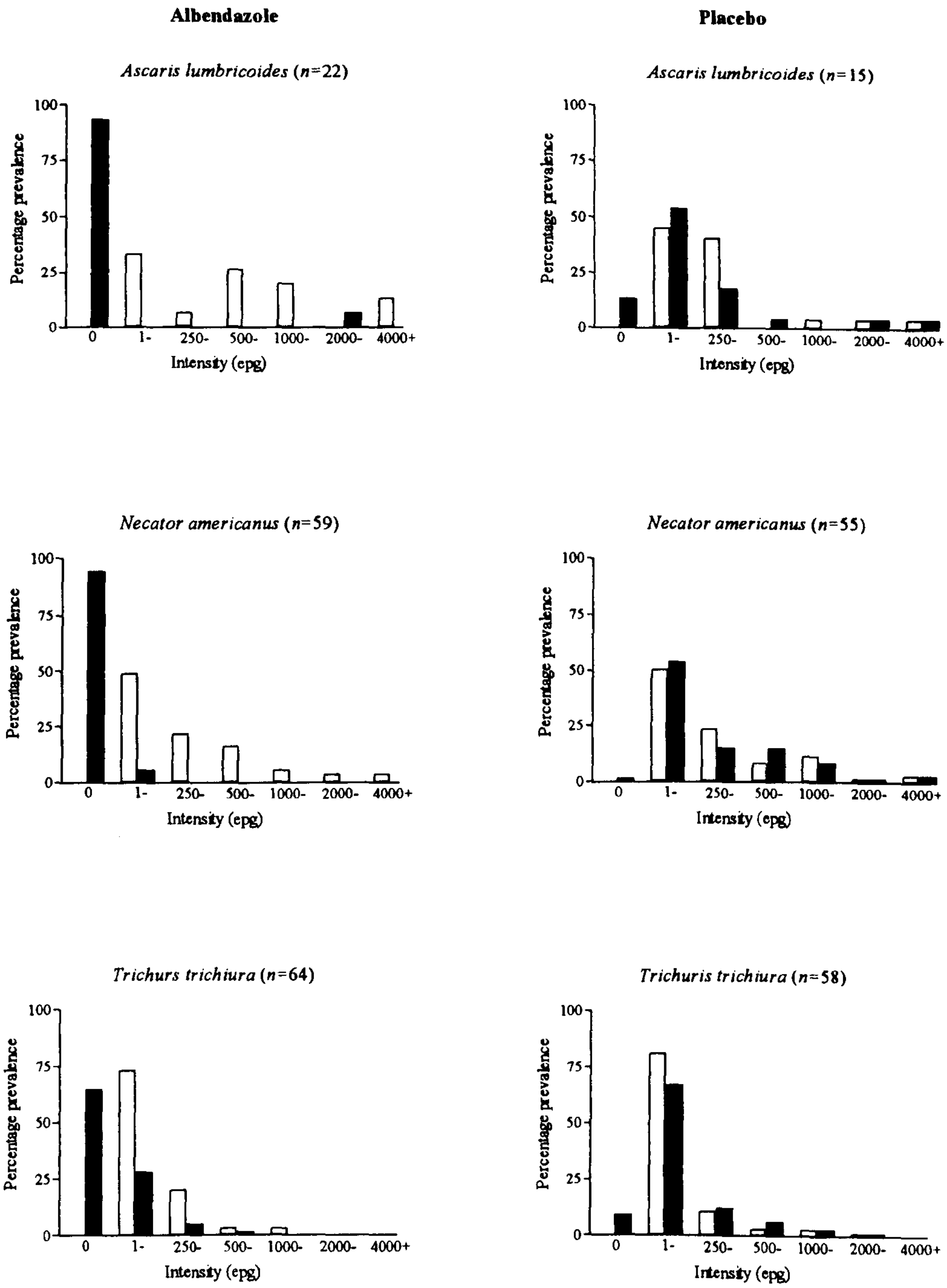

Figure 6.4 Distribution of egg counts of *Ascaris lumbricoides*, *Necator americanus* and *Trichuris trichiura* at baseline (first trimester) and 2-4 weeks after intervention with albendazole (400 mg) or placebo in pregnant women in Western Sierra Leone.
White bar = baseline; black bar = post-treatment. Includes only pregnant women who were infected at baseline.

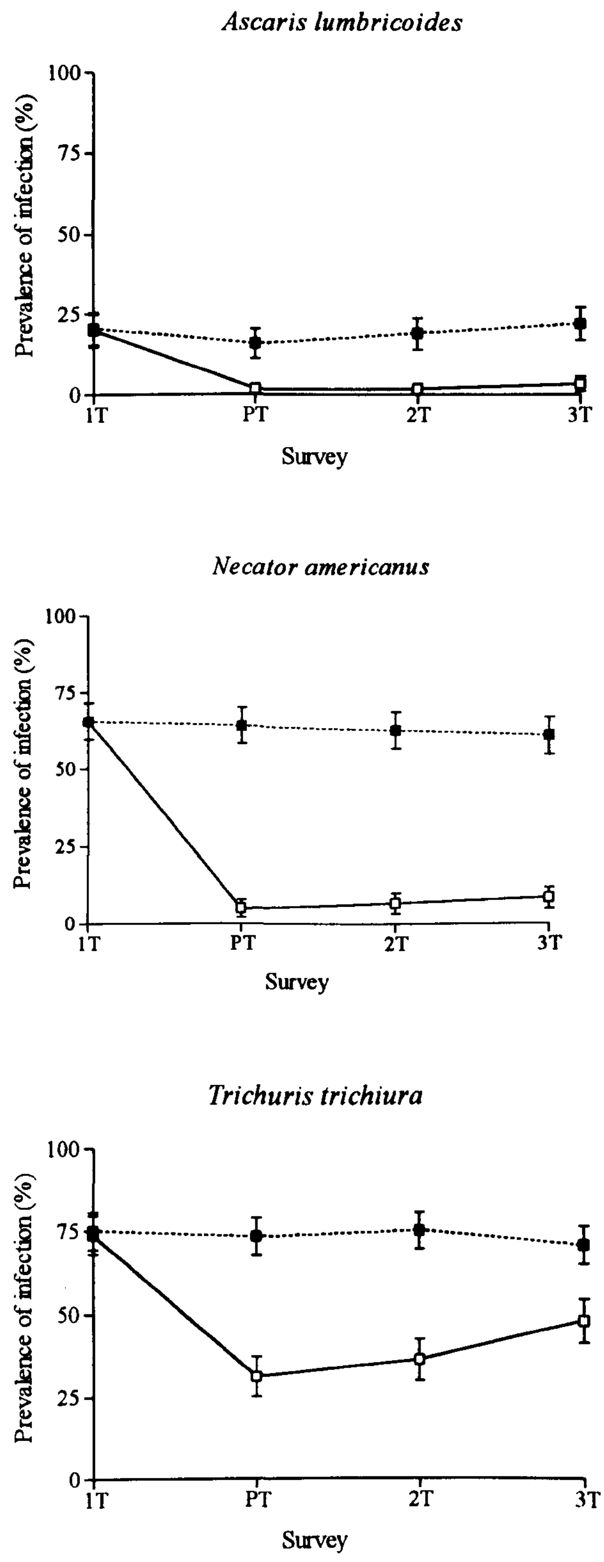

Figure 6.5 Prevalence of *Ascaris lumbricoides*, *Necator americanus* and *Trichuris trichiura* infection in 125 women during pregnancy in Western Sierra Leone by treatment with albendazole (400 mg) or placebo.

Solid line = albendazole; broken line = placebo. Error bars indicate standard error. 1T = first trimester (baseline); PT; post-treatment (2-4 weeks after intervention); 2T = second trimester; 3T = third trimester.

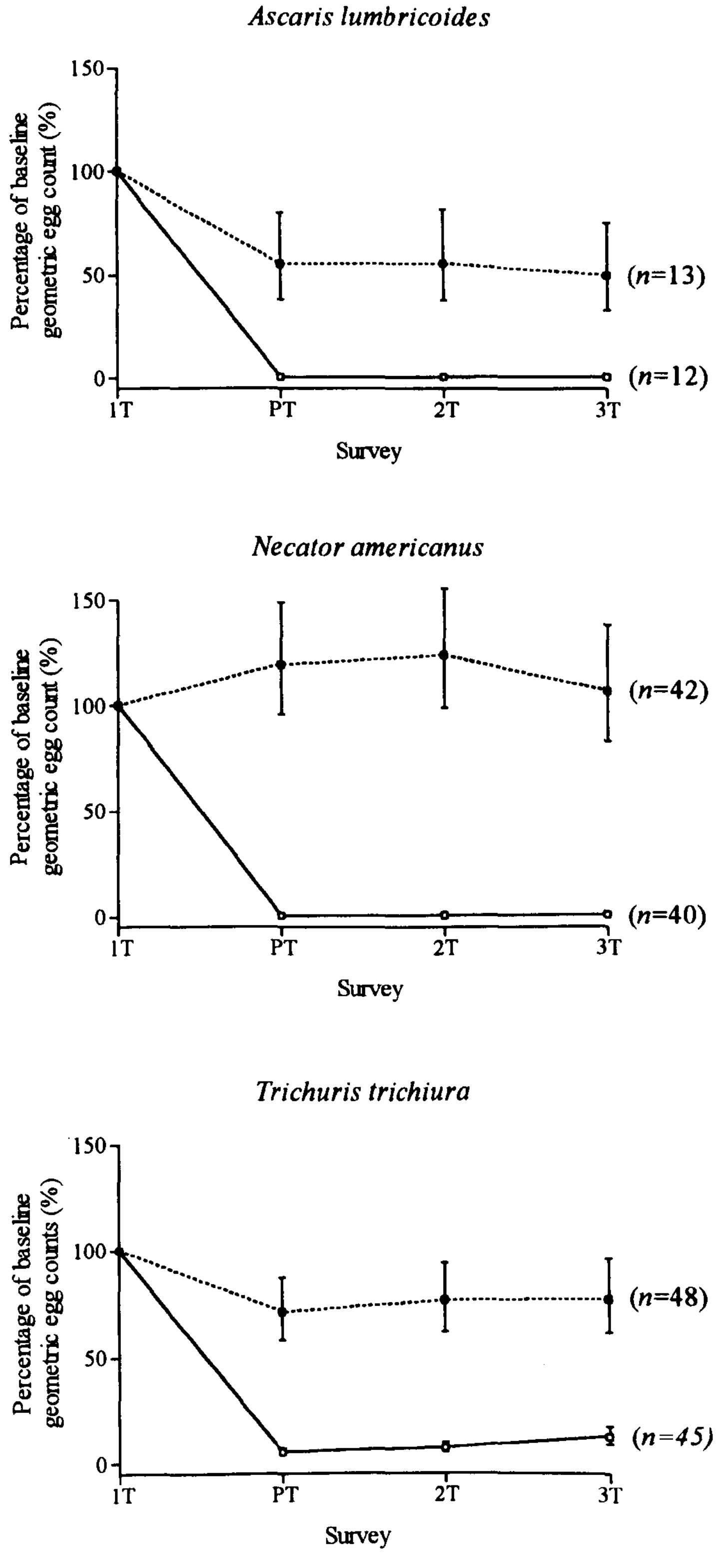

Figure 6.6 Percentage reduction in baseline geometric mean egg counts of *Ascaris lumbricoides, Necator americanus* and *Trichuris trichiura* infection in women during pregnancy in Western Sierra Leone by treatment with albendazole (400 mg) or placebo. Includes only pregnant women who were infected at baseline (first trimester). Solid line = albendazole; broken line = placebo. Error bars indicate standard error. 1T = first trimester (baseline); PT; post-treatment (2-4 weeks after intervention); 2T = second trimester; 3T = third trimester.

Chapter 7

Maternal anthropometry

7.1 INTRODUCTION

7.1.1 Anthropometric assessment of nutritional status during pregnancy

Anthropometric assessment of nutritional status during pregnancy provides information on maternal body size and composition. Anthropometry is particularly suitable for field studies because the measurements are non-invasive, relatively inexpensive, simple to perform and are applicable to large sample sizes (Heymsfield & Casper, 1987; Dwyer, 1991). Most measurements reflect present energy or protein stores, such as weight, limb circumferences and skinfold measures of subcutaneous fat. The exception is height, which is an indicator of past nutritional status. Combinations of these measurements can be used to derive measures of fat and muscle mass, such as mid-upper arm fat and muscle area. Anthropometry cannot identify specific nutrient imbalances and is insensitive to disturbances in nutritional status over short periods of time, but it can assist in the identification of mild to severe malnutrition and can be used to monitor periodic changes in growth and/or body composition (Gibson, 1990a). Changes in anthropometric measurements and indices during pregnancy reflect alternations in maternal body composition that may result from the accumulation or mobilisation of maternal energy stores, the development of maternal reproductive tissue, increases in extracellular fluid blood volume and fetal growth (Adair & Bisgrove, 1991).

There were three central reasons for the collection of anthropometric data in this study: firstly, to provide an assessment of maternal nutritional status, secondly to examine the impact of intestinal nematode infections on maternal body size and composition, and thirdly, to control for maternal body size and composition in the analysis of the intervention effects on maternal iron deficiency and anaemia (Chapter 8) and neonatal outcomes (Chapter 9).

7.1.2 Aims and objectives

The aim of the work described in this chapter was to characterise the nutritional status of the women in the first trimester of pregnancy as reflected by anthropometric indicators of chronic undernutrition and chronic energy deficiency, and to investigate the efficacy of albendazole and iron-folate supplements as interventions to improve maternal growth during pregnancy.

The specific objectives were:

1. To measure the height, weight, body mass index, mid-upper arm circumference, skinfold measures of subcutaneous fat, upper arm muscle area and upper arm fat area of the women in the first trimester of pregnancy.

2. To relate anthropometric variables to intensity of intestinal nematode infection after adjusting for potentially confounding socio-demographic factors, socio-economic status, reproductive characteristics and season at assessment.
3. To determine the impact of iron-folate supplements (36 mg iron and 5 mg folate) and albendazole treatment (400 mg) on the change in maternal anthropometric variables between baseline (first trimester) and the third trimester.

7.2 METHODS

7.2.1 Anthropometric indices of body fat and muscle mass

Anthropometric measures of weight (WT), height (HT), mid-upper arm circumference (MUAC), biceps skinfold (BSF) and triceps skinfold (TSF) were converted to anthropometric indices as follows:

(i) Body mass index

Body mass index (BMI) is defined as body weight in kilograms divided by the square of height in metres (WT/HT^2 kg/m^2).

(ii) Mid-upper arm fat area and mid-upper arm muscle area

Estimates of mid-upper arm fat area (AFA) and mid-upper arm muscle area (AMA) are frequently based on only the triceps skinfold (TSF) and mid-upper arm circumference (MUAC), even though subcutaneous fat is not evenly distributed around the mid-upper arm. The TSF is consistently larger than the biceps skinfold (BSF), and the average of these values provides a better estimate of AMA and AFA (Himes *et al.*, 1980). Therefore, AFA and AMA were estimated from the mean mid-upper arm skinfold (MASF) and MUAC and using the formulae below:

$$MASF = (BSF+TSF)/2$$

$$AMA = (MUAC - \pi MASF)^2/4\pi$$

$$AFA = (MUAC^2/4\pi) - AMA$$

7.2.2 Anthropometric reference data and definition of chronic undernutrition and chronic energy deficiency

There are no local anthropometric reference data for Sierra Leone, and no widely accepted international anthropometric reference standards for pregnant women in Sierra Leone or elsewhere. The usefulness of reference data compiled from women in developed countries for developing countries is questionable (WHO, 1995a). Height, BMI and MUAC are recommended for the assessment of past and present undernutrition in pregnant women, but there are no universal cut-off values for screening purposes.

Height is used as a measure of chronic undernutrition (Villar *et al.*, 1989a). The appropriate maternal height cut-off point for predicting poor pregnancy outcomes probably falls between 140 cm and 150 cm (Krasovec and Anderson, 1991a; Working Group, 1991b). Following other studies conducted in sub-Saharan Africa (Krasovec, 1991b), maternal height <150 cm was used in the present study. Maternal height declines slightly during pregnancy due postural and other changes associated with carrying the fetus (Suitor, 1994). However, this decline is probably minimal during the first trimester, when the rate of weight gain is low.

Body mass index is recommended as a measure of chronic energy deficiency (CED) in early pregnancy before there is significant weight gain (Bailey & Ferro-Luzzi, 1995). In non-pregnant women, CED is defined as a BMI <18.5 kg/m^2 (James *et al.*, 1988). This value was used in the present analysis, although it may underestimate the prevalence of CED due to weight gain during the first trimester.

MUAC remains relatively stable during pregnancy in developing countries (Shah, 1991), and the use of MUAC for assessing CED in pregnant women has been suggested (James *et al.*, 1994). Values between 21 cm and 23.5 cm have been used for screening purposes (Krasovec & Anderson, 1991a; Shah, 1991). A previous survey in Sierra Leone used MUAC <23 cm to screen pregnant women (USAID, 1978), and in order to facilitate comparison, this value was also used in the present study.

7.2.3 Statistical analysis

(i) Baseline (first trimester) analysis

The distributions of first trimester WT, BMI, MUAC, BSF, TSF, AFA and AMA were skewed to high values and therefore the data was transformed using natural logarithms. Geometric mean values were estimated as the antilog of the mean of the transformed data. The individual skinfolds and MUAC were not included in further analysis as limb fat area provides a better estimate of total body fat than skinfold thicknesses at the same site (Himes *et al.*, 1980) and limb muscle area more accurately reflects the protein reserves than circumferences (Gibson, 1990b).

The association between infection with intestinal nematodes and baseline WT, HT, BMI, AFA and AMA was examined using t-tests (prevalence of infection) and linear regression (intensity of infection). The intensity of intestinal nematode infection was estimated indirectly as the natural logarithm of egg counts per gram faeces, $\log_e(\text{epg}+1)$. The association between intestinal nematode infection and maternal anthropometry was investigated further using multiple linear regression to adjust for potentially confounding maternal covariates.

These covariates included age, place of residence (rural or peri-urban), socio-economic status, gravidity, parity, inter-birth interval and the season at assessment. Variables were included in the multiple linear regression models in a stepwise fashion, with an entry p-value of 0.05 and a removal p-value of 0.06. The results are shown only for significant factors as non-significant factors were not retained in the final multiple linear regression model.

(ii) Longitudinal analysis

Only the longitudinal cohort (n=125) was included in the longitudinal analysis, as only these subjects provided all necessary measurements and samples at each assessment during pregnancy.

Because the women were pregnant at the time of enrolment, no pre-pregnancy anthropometric data was available. Inter-population changes in maternal weight during the first three months of pregnancy are highly variable (Working Group, 1991b), and thus predictions of pre-pregnancy values based on the baseline data are unlikely to be accurate. Therefore, the longitudinal analysis is based on the change (Δ) in anthropometric values between baseline (the first trimester) and third trimester assessment. The distribution of the ΔWT and ΔBMI for untransformed data was approximately normal. The ΔAFA and ΔAMA was calculated as the natural logarithm of the third trimester value minus the natural logarithm of the baseline value.

Analysis of variance (ANOVA) models were constructed for ΔWT, ΔBMI, ΔAFA and ΔAMA. The main intervention effects (iron-folate supplements and albendazole treatment) and the interaction effect for the interventions were examined with and without adjustment for potentially confounding maternal and background covariates. These covariates included age, place of residence, socio-economic status, gravidity, parity, inter-birth interval, the season at baseline, baseline anthropometry values and the baseline prevalence and intensity of intestinal nematode infections. The main effects and covariates were added to the ANOVA model simultaneously.

The intervention groups are defined as follows: FeA = iron-folate supplements and albendazole; FeP_A = iron-folate supplements and albendazole placebo; $P_{Fe}A$ = iron-folate placebo and albendazole; $P_{Fe}P_A$ = iron-folate placebo and albendazole placebo.

7.3 RESULTS

7.3.1 Baseline (first trimester) analysis

(i) First trimester maternal anthropometry

Maternal anthropometry at the first trimester assessment is summarised in Table 7.1 for all 184 pregnant women. Ten (5.4%) women were less than 150 cm tall, 15 (8.2%) had BMI values less than 18.5 kg/m^2, and 15 (8.2%) women had MUAC values less than 23 cm. Three (1.6%) had both low height and low MUAC, and 7 (3.8%) had low BMI and low MUAC. None of the women had low values for both height and MUAC.

(ii) Intestinal nematode infection and maternal anthropometry

Univariate analysis (Table 7.2) indicated that subjects infected with *N. americanus* had significantly lower AFA than uninfected subjects. Furthermore, AFA was negatively associated with the intensity of *N. americanus* infection. Rural residents and subjects whose first trimester assessment was in the early dry season (November to January) also had significantly lower AFA. When all these variables were entered into a multiple regression model, the season in which the subjects were measured accounted for most of the explainable variation (adjusted R^2=5.5%) and adding further variables (prevalence and intensity of *N. americanus* infection and place of residence) did not improve the fit significantly. The geometric mean AFA of subjects measured in the early dry season (n=80) and late dry or early wet season (n=104) was 8.68 (SD -2.77, +4.06) cm^2 and 10.28 (SD -2.59, +3.47) cm^2 respectively.

There were no remarkable findings for *A. lumbricoides* infection or *T. trichiura* infection.

7.3.2 Longitudinal analysis

The anthropometric variables at baseline (first trimester) and at follow-up in the second and third trimesters are presented in Table 7.3. At baseline, subjects receiving iron-folate supplements had significantly lower AFA than those receiving the iron-folate placebo (Two-way ANOVA: iron-folate supplements $F_{1,121}$=4.40, p=0.038; albendazole treatment $F_{1,121}$=0.19, p=0.66; interaction $F_{1,121}$=0.01, p=0.93), but otherwise there were no significant differences between the intervention groups in any of the anthropometric variables. The four intervention groups were comparable with respect to known socio-demographic factors, socio-economic status and reproductive characteristics (Chapter 4) and the baseline prevalence and intensity of intestinal nematode infections (Chapter 6).

Between baseline and the third trimester, there was a significant increase in maternal weight (Paired t-test, t=33.31, p<0.0001) and BMI (Paired t-test, t=32.17, p<0.0001), and a significant decrease in AFA (Paired t-test, t=2.94, p=0.0004) and AMA (Paired t-test, t=5.46, p<0.0001). Neither intervention with iron-folate supplements or albendazole were significantly associated with the ΔWT, ΔBMI, ΔAFA or ΔAMA either before or after controlling for other potentially confounding maternal or background covariates.

7.4 DISCUSSION

The anthropometric findings indicate a mild degree of undernutrition in the population. Pre-pregnancy anthropometric measurements were not obtained in the present study, and the nutritional status of the study population was characterised using baseline data collected in the first trimester. Chronic undernutrition (HT <150 cm) was found in 5.4% of women and CED (BMI <18.5 kg/m^2) in 8.2% of women. The latter value is likely to underestimate the true extent of CED due to weight gain during the first trimester. Low MUAC (<23 cm) was also observed in 8.2% of women, but only 3.8% had both low BMI and MUAC values. The prevalence of low MUAC was lower than that observed in a national survey of pregnant women (11%) conducted 20 years ago (USAID, 1978). However, the previous survey included women in the second and third trimesters of pregnancy and these values may not, therefore, be comparable. The same survey also reported that the prevalence of low MUAC was less than half this value in non-pregnant women (5%), which suggests that pre-pregnancy MUAC values are not maintained during pregnancy.

Compared with well-nourished British women of three months gestation, the mean height of the women Western Sierra Leone was 3.0% lower, the mean weight 9.0 % lower, the mean biceps skinfold 21.4% lower and the mean triceps skinfold 39.9% lower (Taggart *et al.*, 1967). However, the mean height and weight of the study population in the first trimester was similar to values reported in women of three months gestation in the Gambia (158 cm and 51.4 kg respectively) (Lawrence *et al.*, 1987b).

There was an overall increase in BMI and weight from the first trimester to the third trimester assessments in the longitudinal cohort. Both AFA and AMA increased between the first and second trimester, but then declined between the second and third trimester. Similar findings were observed in rural Kenyan women, among whom mean weight increased continually over pregnancy, while both fat and muscle area increased between the first and second trimesters and then decreased between the second and third trimesters (Jansen *et al.*, 1984). These findings reflect the maternal energy balance, and suggest that both fat and lean body mass were deposited during the first two trimesters and mobilised in later pregnancy. Loss of maternal fat and lean body mass is not infrequent in developing countries (Krasovec, 1991a), where dietary intake is inadequate for the level of physical activity that is maintained by pregnant women.

Anthropometric indicators identify women with nutritional problems but provide no information on the underlying causal mechanisms. An understanding of these mechanisms is essential for the design of appropriate interventions (Krasovec & Anderson, 1991a). In this study, the contribution of intestinal nematode infections to maternal body size and composition during pregnancy was investigated. None of anthropometric measurements examined was found to be associated with the prevalence or intensity of intestinal nematode infection in the first trimester of pregnancy. However, cross-sectional analyses are limited by the fact that anthropometric variables reflect both long-term and short-term nutritional status, while a single stool examination only provides information on infection status at one point in time and may not reflect the previous history of infection (Willett *et al.*, 1979). A subject who has a history of continual reinfections with intestinal nematode infections is clearly not comparable to a subject who is experiencing a first infection episode. For this reason, longitudinal investigations after placebo-controlled anthelminthic intervention are the preferred study design (Stephenson, 1987b). In this population, intervention with albendazole did not appear to have any impact on the change in anthropometric values between the first and third trimesters. Thus, both the cross-sectional and longitudinal analyses suggest that intestinal nematode infections do not pose a threat to maternal body size and composition in these women. It is possible that physiological or behavioural adaptations to infection may have concealed a nutritional effect. For example, the symptoms associated with intestinal nematode infection may have caused a reduction in physical activity, thus conserving energy and compensating for nutrient imbalance due to infection (Durnin, 1993).

These findings should be interpreted in the light of a number of caveats. Firstly, the multivariate analyses did not control for a number of important factors that affect maternal anthropometry, such as maternal diet, physical activity level and endemic infections other than intestinal nematodes. The dietary intake data (see Chapter 5) clearly indicates that the intake of a substantial proportion of the pregnant women was inadequate, but this data was not suitable for multiple regression analyses due to the semi-quantitative nature of the 24-h dietary recall methodology. The physical activity level of these women is unknown, but is likely to be considerably higher for those women who are not excused from labour intensive activities during pregnancy, such as farming and heavy domestic duties. Secondly, the estimated AFA and AMA may not accurately reflect total body fat and muscle mass. The accuracy of the estimates is affected by oedema, which may occur during the latter stages of pregnancy, and alterations in energy balance are known to affect the rate of fat accumulation differently between different areas of the body (Heymsfield & Casper, 1987; Gibson, 1990b).

There is limited information from other disadvantaged populations on the impact of intestinal nematode infections on maternal anthropometry. Three non-intervention studies in Southeast Asian pregnant women found no evidence that a positive infection status for intestinal parasites including hookworms, *A. lumbricoides* and *T. trichuria* was associated with significantly lower weight gain during pregnancy (D'Alauro *et al.*, 1985; Roberts *et al.*, 1985; Saowakontha *et al.*, 1992). However, there was no indication that the authors controlled for any potentially confounding maternal factors in the analyses, such as dietary intake and physical activity level. In addition none of these studies quantified the parasite load. The extent to which intestinal nematodes affect maternal body size and composition is likely to be a function of the intensity of infection, which determines morbidity (Stephenson, 1987b; Durnin, 1993; Nesheim, 1993). In the present study, the intensities of *A. lumbricoides, N. americanus*, and *T. trichiura* infections were predominantly low. The acute signs and symptoms of infection which precipitate anorexia are probably not common in chronic low grade infections (Durnin, 1993).

Intestinal nematode infections are only likely to cause undernutrition in individuals and communities of marginal nutritional status who are unable to buffer the losses associated with infection (Stephenson, 1987b). The extent of undernutrition among the pregnant women in the Sierra Leonean and Southeast Asian study populations may have been inadequate to reveal a significant impact of infection. This is supported by the findings of a prospective study in Guatemala, where the prevalence of chronic undernutrition (defined as maternal height <1.47 m) was 22% (Villar *et al.*, 1989a), considerably higher than in the present study population, although this difference may reflect genetic rather than nutritional differences. In the Guatemalan study, women with intestinal helminth infections had significantly lower pregnancy weight gain (8.7 ± 5.0 kg, *n*=2,155) than those without any intestinal helminth or protozoal infection (9.1 ± 5.0 kg, *n*=8,331). Furthermore, infection with *A. lumbricoides* and/or *T. trichiura* was significantly associated with increased odds on intra-uterine growth retardation *only* in chronically undernourished women. As the study was cross-sectional, the authors were unable to infer whether infection aggravated nutritional status, or whether undernutrition increased susceptibility to infection.

In the present study, iron-folate supplements did not appear to influence the change in weight, BMI, fat stores or lean body mass in the pregnant women. It is possible that the period of intervention in the present study was of insufficient duration to mediate a positive effect of iron-folate supplements on maternal anthropometry. (Similarly, a significant effect of albendazole may have been observed if the follow-up period had been longer.) Both iron and folate are necessary for the synthesis of DNA and are therefore essential for cell growth and replication. Defective DNA synthesis caused by deficiency of these nutrients may affect all dividing cells in the body, including the gastrointestinal tract (Davidson & Townley, 1977; Prasad & Prasad, 1991). Folate is also a major coenzyme for one-carbon metabolism and the biosynthesis of some amino acids (Wagner, 1995). Iron deficiency is associated with reduced appetite and depressed immune function, which may increase morbidity and reduce growth further (Oski, 1979; Chwang *et al.*, 1988; Prasad & Prasad, 1991).

Studies in Kenya (Latham *et al.*, 1990; Lawless *et al.*, 1991), Indonesia (Chwang *et al.*, 1988), United Kingdom (Aukett *et al.*, 1986) and USA (Judisch *et al.*, 1966) have shown that iron deficient children and infants experience improved growth following iron supplementation. There is also evidence for improved appetite (Latham *et al.*, 1991). There is a lack of evidence from intervention studies to indicate that either iron and/or folate supplementation improves growth in pregnant women. A prospective non-intervention study among pregnant women in the United States found that iron-deficiency anaemia and anaemia due to other causes were both associated with an increased risk of inadequate weight gain for gestation (Scholl *et al.*, 1992). In the same study, dietary iron intake, controlled for energy and protein intake, was also associated with poor weight gain. However, a common underlying mechanism may account for the association between the indicators of iron status and weight gain in this study, and the relationship needs to be confirmed using intervention studies.

One final observation of note is the highly significant association between first trimester AFA and the season at the first trimester assessment. Pregnant women who were assessed in the early dry season (November to January) had lower fat stores than those assessed in the late dry season (February to April) or early wet season (May to July). A causal relationship between maternal fat stores and season cannot be affirmed using cross-sectional analyses, as the association may be coincidental or due to some unknown confounding factor. However, this finding is in agreement with other studies in West Africa which have indicated that seasonality is a strong determinant of maternal anthropometry. During the wet season in the Gambia, pregnant and lactating women gain less weight (Prentice *et al.*, 1981). Furthermore, there is a reduction in the body fat stores, in sharp contrast to the accumulation of maternal fat reserves that occurs in the dry season (Lawrence *et al.*, 1987a). The effect of dietary energy supplements on weight gain and maternal fat deposition is greater when these supplements are given during the wet season (Lawrence *et al.*, 1987a; Ceesay *et al.*, 1997). The loss of maternal body condition occurs during the wet season in the Gambia because there is a rapid decline in food intake caused by diminishing food stocks prior to the new harvest, which coincides with the most intense period of agricultural activity and increased transmission of parasitic infections (Roberts *et al.*, 1982; Lawrence & Whitehead, 1988; Tomkins, 1993). Western Sierra Leone has a similar monomodal seasonal pattern to the Gambia, although seasonal alterations in energy balance are probably less profound as there are no acute seasonal shortages in food intake. Cassava and yams are grown as reserve crops, replacing rice during the wet season (Aitken, 1990), and imported rice is widely available in the Western Area. Nonetheless, the wet season is associated with increased physical activity, even among pregnant women (see Chapter 5). A tentative explanation for the low fat stores among the first trimester Sierra Leonean women in the early dry season is that their fat stores, which were mobilised during the wet season due to negative energy balance, have not yet been replaced.

There was no association between season and the change in anthropometric measures between the first and third trimester, which is contrary to the longitudinal observations in the Gambian pregnant women (Prentice *et al.*, 1981; Lawrence *et al.*, 1987a; Ceesay *et al.*, 1997). This may reflect the less severe effects of season on energy balance in pregnant women in Western Sierra Leone. However, it is possible that the subdivision of the year in Western Sierra Leone into early/late wet and dry seasons may be a simplification of the seasonal alterations in food intake, activity and morbidity. In addition, the full annual seasonal cycle was not covered by subjects in the present study as they were recruited over a six month period from mid December to June.

7.5 SUMMARY

1. The nutritional status of the women in their first trimester, as reflected by anthropometric variables, and the significance of intestinal nematode infections as determinants of maternal body size and composition was investigated in Western Sierra Leone.
2. In the first trimester of pregnancy, chronic undernutrition (height <150 cm) was found in 5.4% of women and chronic energy deficiency (body mass index <18.5 kg/m^2) in 8.2% of women.
3. Women in the first trimester who were infected with *N. americanus* had a lower mean AFA than uninfected subjects. Furthermore, AFA was negatively associated with the intensity of *N. americanus* infection. However, the association between AFA and *N. americanus* infection was not significant after controlling for season at the first trimester assessment.
4. Women in the first trimester of pregnancy who were assessed in the early dry season had less fat stores than women enrolled in the late dry or early wet season, possibly because their fat stores, which were mobilised in the wet season due to negative energy balance, had not yet been replaced.

5. Iron-folate supplements and albendazole treatment were not significantly associated with the ΔWT, ΔBMI, ΔAFA or ΔAMA, either before or after controlling for other potentially confounding maternal and background factors.
6. Intestinal nematode infections do not appear to pose a threat to maternal body size and growth during pregnancy in these communities. This may reflect the mild degree of underlying chronic undernutrition and energy deficiency in these women. However, it is feasible that the impact of infection on anthropometric measures of nutritional status may have been concealed by physiological or behavioural adaptations to infection.

Table 7.1 Anthropometry of women in the first trimester of pregnancy in Western Sierra Leone (*n*=184).

Anthropometrical variable	Geometric mean (±SD)†
Weight (kg)	53.52 (-7.22, +8.35)
Height (cm)	158.28 (-5.80, +5.80)
Body mass index (kg/m^2)	21.39 (-2.57, +2.92)
Mid-upper arm circumference (cm)	26.05 (-2.62, +2.91)
Biceps skinfold (mm)	5.82 (-1.59, +2.20)
Triceps skinfold (mm)	9.44 (-2.32, +3.08)
Upper arm fat area (cm^2)	9.54 (-2.78, +3.92)
Upper arm muscle area (cm^2)	44.26 (-7.84, +9.53)

† Mean (±SD) is given for height.

Table 7.2 Determinants of upper arm fat area (AFA) among women in the first trimester of pregnancy in Western Sierra Leone (*n*=184).

Variable	Univariate analysis[†]			Multivariate analysis[‡]		
	Coefficient (SE)[§]	t-value	*p*	Coefficient (SE)[§]	t-value	*p*
Necator americanus infection	-0.114 (0.053)	2.13	0.035	-	-	NS[‡‡]
Necator americanus intensity[††]	-0.020 (0.009)	2.25	0.026	-	-	NS[‡‡]
Rural residence	-0.135 (0.052)	2.60	0.010	-	-	NS[‡‡]
Assessment in early dry season[§§]	-0.169 (0.050)	3.41	0.0008	-0.169 (0.050)	3.41	0.0008

† Linear regression (continuous variables) and t-tests (categorical variables).
‡ Multiple linear regression.
§ Data are presented in natural logarithms units.
†† Intensity estimated indirectly as eggs per gram of faeces (epg); data was log-transformed, $\log_e$(epg+1).
‡‡ Non-significant (variable not entered into the multiple linear regression model).

Table 7.3 Anthropometrical variables of pregnant women in Western Sierra Leone at baseline (first trimester) and at follow-up in the second and third trimesters following intervention with daily iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo (*n*=125).

Intervention†	Number of pregnant women	Mean (±SD)			Mean difference between first and third trimesters (95% CI)
		First trimester	Second trimester	Third trimester	
Weight (kg)					
FeA	32	53.25 (5.62)	56.53 (5.52)	60.66 (5.66)	7.41 (6.73, 8.09)
FeP_A	35	55.47 (8.94)	59.03 (9.54)	63.52 (9.59)	8.05 (6.97, 9.14)
$P_{Fe}A$	29	55.69 (8.94)	58.98 (8.97)	63.05 (9.30)	7.36 (6.40, 8.33)
$P_{Fe}P_A$	29	57.50 (10.77)	60.74 (10.33)	65.26 (10.35)	7.76 (6.79, 8.73)
Total	125	55.42 (8.74)	58.78 (8.79)	63.08 (8.92)	7.66 (7.20, 8.12)
Body mass index (kg/m^2)					
FeA	32	20.96 (1.78)	22.25 (1.71)	23.88 (1.88)	2.93 (2.65, 3.21)
FeP_A	35	21.79 (3.31)	23.20 (3.61)	24.97 (3.69)	3.18 (2.75, 3.61)
$P_{Fe}A$	29	22.08 (3.31)	23.39 (3.30)	25.01 (3.47)	2.93 (2.54, 3.32)
$P_{Fe}P_A$	29	22.73 (3.62)	24.02 (3.30)	25.83 (3.59)	3.11 (2.68, 3.53)
Total	125	21.31 (3.10)	23.78 (3.14)	24.32 (3.27)	3.04 (2.85, 3.23)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 7.3 Anthropometrical variables of pregnant women in Western Sierra Leone at baseline (first trimester) and at follow-up in the second and third trimesters following intervention with daily iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo (*n*=125) (continued).

Intervention[†]	Number of pregnant women	Geometric mean (±SD)			Mean difference between first and third trimesters[‡] (95% CI)
		First trimester	Second trimester	Third trimester	
Upper-arm fat area (cm^2)					
FeA	32	9.19 (-2.31, +3.08)	9.70 (-2.46, +3.29)	8.97 (-2.16, +2.84)	97.56 (95.04, 100.16)
FeP_A	35	9.40 (-4.00, +4.40)	9.95 (-3.09, +4.47)	9.18 (-2.88, +4.20)	97.61 (93.05, 102.39)
$P_{Fe}A$	29	10.44 (-3.12, +4.45)	10.95 (-3.39, +4.92)	10.14 (-3.22, +4.72)	97.11 (93.69, 100.66)
$P_{Fe}P_A$	29	10.80 (-3.38, +4.92)	11.03 (-3.31, +4.74)	10.42 (-3.08, +4.38)	96.48 (92.47, 100.67)
Total	125	9.89 (-2.96, +4.22)	10.35 (-3.05, +4.33)	9.62 (-2.84, +4.03)	97.22 (95.39, 99.08)
Upper-arm muscle area (cm^2)					
FeA	32	44.88 (-5.37, +6.10)	45.47 (-5.94, +6.83)	43.90 (-5.88, +6.79)	97.84 (96.59, 99.10)
FeP_A	35	43.93 (-8.84, +11.06)	44.48 (-8.64, +10.72)	43.25 (-8.29. +10.26)	98.45 (96.94, 99.99)
$P_{Fe}A$	29	44.33 (-8.92, +11.16)	44.85 (-9.10, +11.41)	43.31 (-8.65, +10.81)	97.70 (95.77, 99.65)
$P_{Fe}P_A$	29	47.64 (-8.73, +10.68)	47.50 (-8.63, +10.55)	46.14 (-8.36, +10.22)	96.85 (94.89, 98.85)
Total	125	45.11 (-8.13, +9.91)	45.41 (-8.16, +9.94)	44.09 (-7.85, +9.56)	97.75 (96.95, 98.56)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

‡ Mean difference (D) for the log-transformed data is presented as $100(e^D)$, and corresponds to the third trimester values as a proportion of the first trimester values.

Chapter 8

Iron deficiency and anaemia

8.1 INTRODUCTION

8.1.1 Assessment of iron deficiency and anaemia during pregnancy

Iron is an important nutrient to consider in relation to parasitism in pregnant women, as iron deficiency is the most common nutritional deficiency in the world, the most frequent cause of anaemia in pregnant women, and consequently, a public health problem in many developing countries (ACC/SCN, 1991). Intestinal nematode infections, and in particular hookworms, are the predominant cause of abnormal iron loss in the developing world. The extent to which intestinal nematode infections contribute to poor iron status and low haemoglobin levels during pregnancy is dependent on three main factors: the amount and availability of iron in the diet (Chapter 5); the species and intensity of infection (Chapter 6); and the magnitude of the mother's iron stores (Gilles *et al.*, 1964). Alterations in the intake, excretion or utilisation of iron due to parasitic infection will result in iron deficiency and iron-deficiency anaemia if the host is unable to replace the iron by increased dietary intake or by mobilisation of existing body iron stores. Iron is known to be limiting in human diets, and iron stores in pregnant women in developing countries are frequently low due to poor dietary iron intake, frequent and closely spaced pregnancies and parasitism (WHO, 1993).

The relationship between intestinal nematode infection and maternal iron deficiency and anaemia during pregnancy can be examined by measuring the effect of interventions to control these disorders. In this study, haemoglobin (Hb) concentration was used to assess the prevalence and severity of anaemia. As iron-deficiency anaemia was of particular interest in this study, a measure of iron deficiency was also required. Haemoglobin concentration has traditionally been used as an index of iron deficiency (Carriaga *et al.*, 1991), but other factors such as folate deficiency, concurrent infection and haemoglobinopathies may lower Hb concentration regardless of iron status (Cook & Finch, 1979). Furthermore, Hb concentration cannot be used to measure changes in the magnitude of iron stores prior to the onset of anaemia, as levels only become abnormal after body iron stores are severely depleted (Cook *et al.*, 1974). Serum ferritin (SF) concentration was used to detect iron deficiency and to assess the impact of intervention on body iron stores. Serum ferritin concentration is an indirect measure of body iron stores (Cook *et al.*, 1974; Lipschitz *et al.*, 1974) and is generally considered to be the most sensitive indicator of iron deficiency (Cook & Finch, 1979; Cook & Skikne, 1982). In subjects with low Hb concentration indicative of anaemia, low SF concentration is diagnostic of iron-deficiency anaemia, while a normal or high SF concentration indicates that some other factor is contributing in part or in whole to anaemia.

As the diagnosis of anaemia and iron deficiency is based on Hb concentration and SF concentration respectively, it is necessary to consider the physiological changes in these parameters during pregnancy. In healthy iron replete pregnant women, Hb concentration remains stable until about the 16th week and then progressively falls until 30-36 weeks, after which it may stabilise or rise again near term (Bentley, 1985; Hytten, 1985; Scholl & Hediger, 1994). This natural reduction in Hb concentration, which amounts to about 5 g/l (WHO, 1993), occurs because there is a earlier and proportionately larger increase in plasma volume (45%) relative to the red blood cell mass (18%). Serum ferritin concentration falls dramatically in the second and third trimesters as iron stores are mobilised (Puolakka *et al.*, 1980; Bentley, 1985). However, some of the decrease (≥15 μg/l) may also be physiological (Carretti *et al.*, 1992; Whittaker & Lind, 1993). These normal physiological responses may confound the diagnosis of iron deficiency and anaemia during pregnancy. In the present study, the interpretation of the baseline data study will not be greatly affected by haemodilution, which is minimal during the first trimester (Scholl & Hediger, 1994). It is assumed that the effects of haemodilution on the change in Hb and SF concentration across pregnancy is similar in the intervention groups.

8.1.2 Aims and objectives

The aim of the work described in this chapter was to characterise the nutritional status of the women in the first trimester of pregnancy as reflected by biomedical indicators of iron deficiency and anaemia, and to investigate the efficacy of albendazole and iron-folate supplements as interventions to improve maternal iron stores (SF concentration) and Hb concentration during pregnancy.

The specific objectives were:

1. To determine the prevalence of iron deficiency, anaemia and iron-deficiency anaemia in the women in the first trimester of pregnancy.
2. To relate Hb and SF concentrations of the women in the first trimester of pregnancy to socio-demographic factors, socio-economic status, reproductive characteristics, the prevalence and intensity of intestinal nematode infections, maternal anthropometry and season at assessment.
3. To determine the impact of daily iron-folate supplements (36 mg iron and 5 mg folate) and albendazole treatment (400 mg) on the change in Hb concentration and SF concentration between baseline (first trimester) and the third trimester.

8.2 METHODS

8.2.1 Definitions of anaemia, iron deficiency and iron-deficiency anaemia

The World Health Organization (WHO) defines anaemia as Hb <110 g/l in pregnant women and Hb <120 g/l in non-pregnant women (WHO, 1968). Severe anaemia is defined as Hb <70 g/l. Recent studies, which indicate that apparently healthy iron replete individuals of African extraction have Hb values 5 to 10 g/l lower than those of European extraction, have been used in support of race-specific anaemia criteria (Pan & Habicht, 1991; Perry *et al.*, 1993; John-Spear & Yip, 1994). In this study, the WHO criteria were adopted, as the latter findings have not been validated for the Sierra Leonean population. Furthermore, there is insufficient information as to whether these race-specific differences reflect normal physiological factors, pathological processes associated with red cell production or genetic factors related to Hb synthesis (Perry *et al.*, 1993). The WHO criteria are most widely used, and are therefore more useful for comparative purposes.

A SF measurement below 12 μg/l is often used as the cut-off point for iron deficiency, but this value is less accurate diagnostically in individuals who have clinical disorders that cause an increase in SF concentration independently of iron status (Cook & Skikne, 1989). Infections such as malaria, inflammation, liver disease and cancers may increase SF concentration several-fold (Lipschitz *et al.*, 1974; Cook & Finch, 1979; Bradley-Moore *et al*, 1985; Kent *et al.*, 1994). In populations where these disorders are common, Cook & Skikne (1989) suggests that values <20 μg/l be considered diagnostic of iron deficiency. In this study, iron deficiency is defined as a SF <20 μg/l and iron-deficiency anaemia is defined as the same value in an anaemic subject. Subjects who had normal values for both Hb (≥110 g/l) and SF (≥20 μg/l) were considered to be free from anaemia and iron deficiency.

8.2.2 Data analysis

(i) Baseline (first trimester) analysis

The distribution of SF values was skewed and therefore the data was transformed using natural logarithms. Intestinal nematode egg counts were transformed using natural logarithms, $\log_e(\text{epg}+1)$, where epg is the egg count per gram faeces.

The association between maternal factors and both baseline Hb concentration and SF concentration was examined using univariate and multivariate analyses. The following maternal factors were examined: age, place of residence, socio-economic status, gravidity, parity, inter-birth interval, the season at the first trimester assessment, first trimester anthropometry (height, weight, body mass index, upper arm fat area and upper arm muscle area) and first trimester prevalence and intensity of intestinal nematode infections. The univariate analyses included t-tests and one-way analysis of variance (ANOVA). Multiple linear regression was employed to relate several variables to baseline Hb or SF concentration. Variables were included in the multiple linear regression models in a stepwise fashion, with an entry p-value of 0.05 and a removal p-value of 0.06. The results are shown only for significant factors as non-significant factors were not retained in the final multiple linear regression model.

The Hb concentration of pregnant women was compared to non-pregnant women matched for the same household (see Chapter 6 for details of matching) using paired t-tests. The prevalence of anaemia in the two groups was compared using McNemar's Test.

(ii) Longitudinal analysis

The efficacy of iron-folate supplements and albendazole treatment was evaluated in terms of the change in the prevalence of anaemia, the prevalence of iron deficiency, the prevalence of iron-deficiency anaemia, Hb concentration (ΔHb) and log-transformed SF concentration (ΔSF) between baseline (first trimester assessment) and the third trimester assessment. The ΔHb was calculated as Hb concentration at the third trimester assessment minus Hb concentration at baseline. The ΔSF was calculated as the natural logarithm of SF concentration at the third trimester value minus the natural logarithm of SF concentration at baseline.

McNemar's test was used to test for significant changes in the prevalence levels of anaemia, iron deficiency and iron-deficiency anaemia between baseline and the third trimester assessment for each intervention group. Paired t-tests were used to test for significant changes in Hb concentration and SF concentration for each intervention group.

ANOVA models were constructed for ΔHb and ΔSF. The effect of intervention (iron-folate supplements and albendazole treatment) on ΔHb and ΔSF was examined before and after adjustment for potentially confounding maternal and background covariates. The maternal and background variables examined included age, place of residence, socio-economic status, gravidity, parity, inter-birth interval, the season at baseline, baseline prevalence and intensity of intestinal nematode infections, baseline anthropometry (weight, height, body mass index, upper arm fat area and upper arm muscle area), gestational age at baseline and the third trimester assessment, the change in anthropometry between baseline and the third trimester assessment, baseline Hb concentration and baseline SF concentration. The change in the prevalence and intensity of intestinal nematode infections between baseline and third trimester assessment was not included in the analysis of intervention effects as this would falsely eliminate any beneficial effect of intervention mediated through the removal of intestinal nematode infections. Covariates were entered simultaneously with the intervention effects into the ANOVA models. Only covariates that remained significant in the ANOVA model were retained. The two-way interactions between the intervention effects and covariates were examined, but none was found to be significant.

The intervention groups are defined as follows: FeA = iron-folate supplements and albendazole; FeP_A = iron-folate supplements and albendazole placebo; $P_{Fe}A$ = iron-folate placebo and albendazole; $P_{Fe}P_A$ = iron-folate placebo and albendazole placebo.

8.3 RESULTS

8.3.1 Baseline (first trimester) analysis

(i) First trimester haemoglobin and serum ferritin concentration.

At baseline (n=184), the mean Hb concentration was found to be 106.6 (SD 14.3) g/l and the geometric mean SF concentration was found to be 23.5 (SD -15.5, +45.7) μg/l. As Hb and haematocrit values were highly correlated (Pearson correlation coefficient = 0.81, n=179, p<0.001), and as a more complete set of Hb data was obtained due to breakage of some haematocrit capillary tubes, only Hb concentration was considered in further analysis.

One hundred and eight (58.7%) pregnant women were judged to be anaemic and 69 (37.5%) were diagnosed as iron deficient. Only one individual was severely anaemic. Iron-deficiency anaemia was indicated in 39 (21.2%) pregnant women, constituting 36.1% of all anaemia cases. Thirty (16.3%) women were iron deficient without anaemia. The remaining 46 (25%) women were free from iron deficiency and anaemia.

The use of SF <50 μg/l as indicative of iron depletion for pregnant women in populations with high prevalence of infectious or inflammatory disorders has been suggested (WHO, 1989), but has yet to be fully evaluated (WHO/UNICEF/UNU, 1995). If this criterion is adopted, 81 (44.0%) pregnant women would be diagnosed with iron-deficiency anaemia, constituting 75.0% of all anaemia cases, and an additional 57 (31.0%) women would be diagnosed with iron deficiency without anaemia.

(ii) Association between haemoglobin concentration and socio-demographic, socio-economic, reproductive, parasitic and anthropometric variables.

Univariate analysis indicated that Hb concentration was negatively associated with the intensity of *N. americanus* infection (Figure 8.1), and positively associated with body weight. When both these factors were entered into a multiple linear regression model, the intensity of *N. americanus* infection (p=0.020) remained significant after adjusting for body weight (p=0.033) (adjusted R^2=4.7%) (Table 8.1). Note however, that there was no difference in the Hb concentration or prevalence of anaemia between subjects who were infected and uninfected with *N. americanus* infection (Table 8.2).

(iii) Association between serum ferritin concentration and socio-demographic, socio-economic, reproductive, parasitic and anthropometric variables.

Serum ferritin concentration was negatively associated with the intensity of *N. americanus* infection (Figure 8.2). Serum ferritin concentration was also significantly lower in grandmultiparae (parity >6), women with an inter-birth interval ≤12 months, women who had not used modern methods of contraceptives, and women who were assessed in the early dry season (November to January) or early wet season (May to July) as compared to the late dry season (February to April). When all these factors were entered into a multiple linear regression model, the effect of *N. americanus* intensity was not significant after adjusting for grandmultiparae (p=0.038), inter-birth interval (p=0.0035), use of modern contraceptive methods (p=0.035) and season at the first trimester assessment (early dry p<0.0001; early wet p=0.0024) (adjusted R^2=22.1%) (Table 8.3). The subset of iron replete women was examined separately, as variation in SF concentration among iron depleted women is limited by the lack of iron stores. However, in the multivariate analysis, the effect of *N. americanus* intensity was also not significant in iron replete women. There was no difference in the SF concentration or prevalence of iron deficiency or iron-deficiency anaemia between subjects who were infected and uninfected with *N. americanus* infection (Table 8.2).

(iv) Comparison of first trimester haemoglobin concentration with non-pregnant women

The mean Hb concentration of non-pregnant women matched for household with the pregnant subjects was 105.7 (SD 16.5) g/l (n=145). One hundred and seventeen (80.7%) of the non-pregnant women were anaemic (Hb <120 g/l).

There was no significant difference in the Hb concentration of pregnant subjects and their non-pregnant counterparts (p=0.71) (Table 8.4). However, the prevalence of anaemia in non-pregnant women was significantly higher (McNemar's Test, χ^2 =14.50, p=0.0001) (Table 8.5). This anomalous result may be due to the lower cutoff value for anaemia in pregnant women (Hb <110 g/l) as compared to non-pregnant women (Hb <120 g/l). The basis for a lower cut-off value for anaemia in pregnant women is that haemodilution lowers Hb values independent of iron status during pregnancy. However, haemodilution is not usually apparent until the second trimester of pregnancy (Bentley, 1985), and therefore the non-pregnant cut-off value for anaemia may be more appropriate during the first trimester. If the non-pregnant cut-off value for anaemia is employed for both pregnant and non-pregnant women, the prevalence of anaemia in similar in both groups (McNemar's Test, χ^2 =0.03, p=0.87) (Table 8.6).

8.3.2 Longitudinal analysis

(i) Prevalence of iron deficiency, anaemia and iron-deficiency anaemia.

The proportion of subjects with iron deficiency, anaemia and iron-deficiency anaemia at baseline (first trimester) and at follow-up in the second and third trimesters is given in Figure 8.3. At baseline, the prevalences of iron deficiency, anaemia and iron-deficiency anaemia were statistically similar in all intervention groups. The four intervention groups were comparable with respect to known socio-demographic factors, socio-economic status and reproductive characteristics (Chapter 4), the baseline prevalence and intensity of intestinal nematodes (Chapter 6) and baseline anthropometric variables except for a lower upper arm fat area among subjects who were allocated iron-folate supplements (Chapter 7).

In all intervention groups except FeA, there was an increase in the prevalence of anaemia (FeA, $p=0.61$; FeP_A, $p=0.039$; $P_{Fe}A$, $p=0.0001$; $P_{Fe}P_A$, $p=0.0020$) (Table 8.7) and iron-deficiency anaemia (FeA, $p=1.00$; FeP_A, $p=0.013$; $P_{Fe}A$, $p<0.0001$; $P_{Fe}P_A$, $p=0.0001$) between baseline and the third trimester (Table 8.8). The prevalence of iron deficiency increased in intervention groups $P_{Fe}A$ and $P_{Fe}P_A$ but not FeA or FeP_A (FeA, $p=1.00$; FeP_A, $p=0.27$; $P_{Fe}A$, $p<0.0001$; $P_{Fe}P_A$, $p=0.0005$) (Table 8.9).

(ii) Haemoglobin concentration

Measures of Hb concentration at baseline (first trimester) and at follow-up in the second and third trimesters are presented in Table 8.10. The distribution of haemoglobin concentration at baseline and at follow-up in the third trimester following intervention is shown in Figure 8.4. At baseline, the distribution of Hb concentration was statistically similar in all intervention groups. There was a decrease in mean Hb concentration between baseline and the third trimester in all intervention groups except FeA. The magnitude of the decline in each intervention group increased in the order $FeA<FeP_A<P_{Fe}A<P_{Fe}P_A$. In all intervention groups except FeA, this decline was statistically significant ($p<0.001$).

Intervention alone (iron-folate supplements, albendazole treatment and the interaction effect) accounted for 21.6% (adjusted R^2) of the variation in ΔHb (Table 8.11). An ANOVA model, which included iron-folate supplements ($p<0.0001$), albendazole treatment ($p=0.0034$), the intervention interaction effect ($p=0.28$) and the covariates baseline Hb concentration ($p<0.0001$) and the season at baseline (early dry season $p=0.055$; late dry season $p=0.020$), explained 48.4% (adjusted R^2) of the variation in ΔHb. Adding further maternal or background variables did not improve the fit significantly. The intervention interaction effect was not significant, and therefore the effects of the iron-folate supplements and albendazole were additive. The mean reduction in baseline Hb concentration in subjects who received the iron-folate placebo was 13.67 g/l more that those who received the iron-folate supplements, while the reduction in baseline Hb concentration in subjects who received the albendazole placebo was 6.55 g/l more than those who received the albendazole. The reduction in baseline Hb concentration was also less among those whose first trimester assessment was taken in the early (6.13 g/l) or late dry season (7.64 g/l) as compared to the early wet season. Baseline Hb concentration was negatively associated with ΔHb.

(iii) Serum ferritin concentration

Measures of SF concentration at baseline (first trimester) and at follow-up in the second and third trimesters are presented in Table 8.10. The distribution of serum ferritin concentration at baseline and at follow-up in the third trimester following intervention is shown in Figure 8.5. At baseline, the distribution of SF concentration was statistically similar in all intervention groups. There was a decrease in mean SF concentration between baseline and the third trimester in all intervention groups except FeA. The magnitude of the decline in each intervention groups increased in the order $FeA<FeP_A<P_{Fe}A<P_{Fe}P_A$. The decline was significant in intervention groups $P_{Fe}A$ and $P_{Fe}P_A$ only ($p<0.001$).

Intervention alone (iron-folate supplements, albendazole treatment and the interaction effect) accounted for 14.5% (adjusted R^2) of the variation in ΔSF (Table 8.12). An ANOVA model, which included iron-folate supplements ($p<0.0001$), albendazole treatment ($p=0.76$), the intervention interaction effect ($p=0.85$) and the covariates baseline SF concentration ($p<0.0001$) and the season at baseline (early dry season $p=0.005$; early wet season $p=0.014$), explained 55.8% (adjusted R^2) of the variation in ΔSF. Adding further maternal or background variables did not improve the fit significantly. Subjects who received iron-folate supplements experienced a significantly lower decline in baseline SF concentration than those receiving the placebo, but the albendazole treatment was not associated with a significant effect. The reduction in baseline SF was less among those whose first trimester assessment was taken in late dry season as compared to the early dry season and early wet season. Baseline SF concentration was negatively associated with ΔSF.

(iv) Reported side effects of intervention.

Two subjects reported symptoms of nausea, which they attributed to the iron-folate supplements. After reassurance from the field nurse, both subjects agreed to continue taking the supplements.

8.4 DISCUSSION

8.4.1 Iron deficiency and anaemia among pregnant women in Western Sierra Leone

Although poor pregnancy outcomes are attributed to anaemia in Sierra Leone (MNDEP/UNICEF, 1989), adequate information on the prevalence, epidemiology and aetiology of anaemia in women of child-bearing age is lacking. In a recent tabulation of the prevalence of anaemia in women published by the World Health Organization (WHO, 1992a), the only available data for Sierra Leone dates back to a study reported in 1977. The prevalence of anaemia in pregnant women in the study was 45%, although the cutoff value that was used to diagnose anaemia in this study is unknown (WHO, 1977).

Mild or moderate anaemia was diagnosed in 58.2% of the study population comprising women in the first trimester of pregnancy in Western Sierra Leone. This prevalence value falls within the severe category designated by WHO (prevalence >40%) (ACC/SCN, 1997) and is close to the estimated value for pregnant women in West Africa (56%) (WHO, 1992a). Only one pregnant woman had a Hb concentration less than 70 g/l and thus severe anaemia was not a common feature of early pregnancy in these communities.

Anaemia was also very common (80.7%) in non-pregnant women of child-bearing age, matched for household with the pregnant subjects. This prevalence level was greater than the estimated value for non-pregnant women in West Africa (47%) (WHO, 1992a), and suggests that the anaemia problem among women of child-bearing age is high for the region. Pregnancy is normally associated with an increased risk of anaemia, and therefore it is unusual to observe a higher prevalence of anaemia among non-pregnant women. However, it is questionable whether a lower cutoff point for anaemia is appropriate during the first trimester of pregnancy, and these findings may therefore not reflect a true difference in the prevalence of anaemia between pregnant and non-pregnant women. The rationale for a lower cut-off point for anaemia in pregnant women is that haemodilution lowers Hb concentration independently of iron status during pregnancy. However, there is little increase in the plasma volume during the first trimester (Hytten, 1985) and Hb concentration remains stable until about the 16th week (Bentley, 1985). Furthermore, iron requirements are actually at their lowest during the first trimester and iron status may even improve, as the iron conserved by the cessation of menstruation compensates for the slow rate of fetal and maternal growth during this period (Perlas *et al.*, 1992). Therefore, the non-pregnant cut-off value for anaemia (Hb <120 g/l) may be more appropriate during the first trimester of pregnancy. In developing countries, pregnant women rarely attend antenatal facilities in the first trimester, and it is perhaps for simplicity that WHO recommend only one cut-off value for the entire duration of pregnancy. There was no significant difference in the Hb concentration of pregnant and non-pregnant women matched for household, and if the same cut-off value was used to define anaemia for both groups (Hb <120 g/l), there was no significant difference in the prevalence of anaemia.

Iron deficiency was clearly an important aetiological factor of anaemia among these pregnant women in Western Sierra Leone. Iron deficiency was diagnosed in 37.5% of women in the first trimester of women. Iron deficiency was associated with anaemia (iron-deficiency anaemia) in 21.2% of first trimester pregnant women, constituting just over one-third of anaemia cases. A further 16.3% of the study population were iron deficient but without anaemia: these women were in precarious iron balance, with no buffer available to supply the additional iron required for pregnancy or to compensate for blood loss due to infections. It is likely that these values underestimate the true extent of iron deficiency and iron-deficiency anaemia in these communities, as infectious and inflammatory processes are common in tropical settings and may elevate SF concentration independently of iron status. The cut-off value used to diagnosis iron deficiency in the present study was set at 20 μg/l rather than 12 μg/l, which is more commonly used in populations of developed countries. However, subjects who have SF concentration between 20 μg/l and 100 μg/l may also have deficient iron stores if they have infectious or inflammatory disorders, although the probability of iron deficiency declines rapidly above 60 μg/l (Cook & Skikne, 1989). It has been suggested by WHO (1989) that a cut-off value of 50 μg/l be employed for pregnant women in settings where there is a high prevalence of infections and inflammatory disorders. This cut-off value, which has yet to be fully evaluated (WHO/UNICEF/UNU, 1995), almost doubles the estimate of the prevalence of iron-deficiency anaemia (44.0%) and non-anaemic iron deficiency (31.0%) in these Sierra Leonean women. It also increases the proportion of anaemia cases associated with iron deficiency from 36.1% to 75.0%.

Alternative direct or indirect measures of iron deficiency would not have offered a significant advantage over the use of SF concentration in this study. Investigation for the presence or absence bone marrow iron stores is the most reliable test for iron deficiency (Massey, 1992), but this method is particularly invasive and ethically questionable for non-therapeutic purposes (Stephenson, 1987b). Other iron biochemistry tests such as the measurement of transferrin saturation, serum iron and erythrocyte protoporphryin are also affected by inflammatory and infectious processes (Cook & Finch, 1979). The use of multiple iron biochemistry tests to diagnosis iron deficiency would improve the specificity of the diagnosis (Cook *et al.*, 1986), but would also decrease the sensitivity and thereby underestimate the true prevalence in the population (Hallberg *et al.*, 1993). Serum transferrin receptor, a relatively new index of tissue iron deficiency, is probably a more reliable measure of iron status as it is unaffected by infections or inflammation (Ferguson *et al.*, 1992). It may therefore be useful in countries where these disorders are prevalent (Kuvibidila *et al.*, 1994). However, this parameter has yet to be validated in pregnant women in the tropical context (van den Broek, 1996).

Iron deficiency is a major aetiological factor of anaemia among non-pregnant (Rougereau *et al.*, 1982) and pregnant women in West Africa (Ogunbode *et al.*, 1979; Jackson & Latham, 1982; Powers *et al.*, 1985; Hercberg *et al.*, 1987; Daouda *et al.*, 1991; Preziosi *et al.*, 1997). Poor dietary practices are usually the predominant cause of iron deficiency in tropical low-income countries, and the 24-h dietary recall data for these women suggests that the dietary intake of iron is likely to be inadequate for a substantial proportion of these women, even without the additional faecal iron losses associated with parasitic infection (Chapter 5). The positive association between Hb concentration and body weight may also suggest an underlying dietary mechanism for anaemia in this population. Women who are lighter may ingest less food and thus less haematopoietic nutrients, including iron. Alternatively, the association between Hb concentration and low body weight could be the direct result of poor growth induced by iron deficiency (Oski, 1979; Prasad & Prasad, 1991), although there was no evidence that iron-folate supplements improved weight, fat or muscle gain during pregnancy in these women (see Chapter 7). The association between body weight and Hb concentration is not particular to this population: studies in the United States have observed a positive relationship between pre-pregnancy weight and Hb concentration (Garn, 1991).

In this population, the iron cost of pregnancy appears to be an important determinant of low iron stores, as reflected by low SF concentration. Grandmultiparity (parity >6) and a short time interval between the previous and present pregnancy (≤12 months) were all associated with lower SF concentration, while previous use of modern methods of contraception was associated with higher values. For reasons that have been discussed, these findings should be interpreted with some caution due to the possible influence of processes unrelated to iron status on SF concentration. However, it is logical that continuous childbearing will have a detrimental effect on maternal iron status. Each pregnancy incurs a substantial iron loss (approximately 790 mg: Hallberg, 1988) and closely spaced pregnancies allow little opportunity to recover iron stores. It can take up to two years for women to regain pre-pregnancy iron status if iron supplements are not taken (Taylor *et al.*, 1982b). In grandmultiparae, the cumulative iron losses associated with repeated pregnancies are augmented by the increase in menstrual blood loss with parity (Andrade *et al.*, 1991). In this population, anovulatory contraceptives are most popular (E.Fullah, personal communication[1]), and these methods decrease menstrual blood loss by about 50% (Nilsson & Solvell, 1967), as well preventing the iron losses associated with an unplanned pregnancy.

Necator americanus was the only intestinal nematode found to be related to Hb concentration in the pregnant women in Western Sierra Leone. Haemoglobin concentration was negatively associated with the intensity of *N. americanus* infection, as estimated indirectly by faecal egg counts. The extent to which *N. americanus* causes anaemia depends on the intensity of infection and host iron balance, which is a function of dietary iron intake and body iron stores (Gilles *et al.*, 1964). The intensities of *N. americanus* infection among women in the present study were generally low (at baseline, only 10.3% of pregnant women had egg counts greater than 1,000 epg). However, the 24-h dietary intake data and SF data for these women suggest a substantial proportion consume an inadequate amount of utilisable iron and have depleted iron stores. In women with no *N. americanus* infection, 57.4% were anaemic, 29.5% had iron deficiency, and 16.4% had iron-deficiency anaemia, indicating a high extent of underlying anaemia and iron deficiency in these communities. These women will have a limited ability to buffer the blood losses associated with *N. americanus* infection, and in this context, small hookworm burdens are not meaningless. Previous studies in non-pregnant populations indicate that a wide range of intensities, equivalent to burdens of 40 to 160 worms, are associated with anaemia (Hb <110 g/l) (Lwambo *et al.*, 1992). The threshold worm load for morbidity in pregnant women has not been defined (Bundy *et al.*, 1995), although theoretical analysis suggests that anaemia may be precipitated by lower worm loads during pregnancy when there is increased burden on iron stores (Crompton & Whitehead, 1993). The estimation of a threshold *N. americanus* intensity for anaemia is not relevant in this population in Western Sierra Leone, as the mean Hb concentration of pregnant women was below the level associated with anaemia in both uninfected and infected individuals, and therefore infection was not a prerequisite for anaemia. In any case, such threshold intensities are of dubious value, as the development of iron deficiency and anaemia depends not only on the worm load, but on the iron balance of the host (Gilles *et al.*, 1964).

Although the inverse relationship between intensity of hookworm infections and Hb concentration has been well documented in non-pregnant populations (Roche & Layrisse, 1966), there is little work that has focused specifically on pregnant women. Most previous studies have only examined the effect of infection status and not the intensity of infection. Cross-sectional studies in pregnant women in Thailand (D'Alauro *et al.*, 1985; Saowakontha *et al.*, 1992) and South Africa (Thomson, 1997) reported that the Hb concentration, haematocrit or the prevalence of anaemia did not differ significantly between pregnant women who were infected and uninfected with hookworm infection. These studies did not quantify the

[1] Maternal Child Health Aide, Saint Andrew's Clinic for Children, Sierra Leone.

intensity of infection, which limits the usefulness of the findings. The magnitude of intestinal blood loss is directly related to the intensity of hookworm infection (Gilles *et al.*, 1964; Roche & Layrisse, 1966; Martinez-Torres *et al.*, 1967). Infected individuals are taken to include all those with evidence of hookworm eggs in a stool sample, regardless of whether the egg count per gram is 1 or 10,000. As lightly infected individuals tend to predominate in a community (Croll *et al.*, 1982; Bundy *et al.*, 1985b; Schad & Anderson, 1985), these subjects would 'dilute' any effect of infection status on Hb concentration. In the present study, which showed a significant association between intensity and Hb concentration, there was no apparent difference in mean Hb concentration of those uninfected and infected with *N. americanus*.

A study in Liberia, which borders Sierra Leone, also reported a negative correlation between the intensity of hookworm infection and the Hb concentration of pregnant women in their third trimester (Jackson & Jackson, 1987). The species of hookworm was not determined, although a previous studies in Liberia (Hsieh, 1972) indicated that *N. americanus* is the predominant species. As in present study, the intensities of hookworm infection in the Liberian pregnant women were low: the median egg count was 26 epg and only two women (n=138) had egg counts greater than 1,000 epg. The intensity of infection with hookworm is also a dominant risk factor for anaemia in multigravidae in coastal Kenya (Shulman *et al.*, 1996), but there was no correlation between the intensity of hookworm infection and blood Hb concentration among pregnant women in the Seychelles (WHO, 1996b). In the latter study, only 14% of women were anaemic (Hb <110g) and only 8.6% were infected with hookworm. Holland (1987a) suggests that the prevalence of hookworm infection needs to exceed 50% in order to demonstrate a relationship between infection and indicators of iron status.

Serum ferritin concentration was not associated with the intensity of *N. americanus* infection after controlling for other maternal factors both in the iron-replete and the entire group of pregnant women. This is slightly surprising given that the effect of *N. americanus* infection on Hb concentration is most likely to be mediated through a reduction in iron stores, and the onset of iron-deficiency anaemia will not occur until these stores are depleted. A possible explanation for this finding is that the association between *N. americanus* infection and Hb concentration is also mediated through mechanisms that are unrelated to iron nutrition. For example, anorexia associated with *N. americanus* infection may decrease the intake of other haematopoietic nutrients such as folate. In addition, hookworm infection has also been linked with malabsorption, although it is now thought that concurrent protein-energy malnutrition accounts for this association (Holland, 1987a). The relationship between SF concentration and *N. americanus* infection may also be confounded by variable dietary iron intake by the subjects. It is also possible that the SF values do not accurately reflect the magnitude of the iron stores in this population due concomitant infections and inflammatory disorders.

It is apparent that iron deficiency did not account for all the anaemia observed in this population. Two-thirds of anaemia cases were not associated with iron deficiency (or one-quarter, if SF <50 μg/l is indicative of iron deficiency). Although this study did not examine aetiological factors other than iron deficiency, it is clear from other studies in West Africa that multiple factors frequently contribute to anaemia in this region (van den Broek, 1996). These may include other nutritional deficiencies, sickle cell disease, malaria and other inflammatory disorders including acquired immunodeficiency syndrome (AIDS).

Folate status has been shown to decline during pregnancy in the Gambia (Bates *et al.*, 1986) and was associated with maternal anaemia in Benin (Hercberg *et al.*, 1987). The dietary intake data for the present study population indicated that some pregnant women are likely to be receiving inadequate folate in their diet. However, folate deficiency was not found to be an important determinant of maternal anaemia in pregnant women in Liberia (Jackson & Latham, 1982), the West African country that probably most closely resembles Sierra Leone in terms of the climate, agricultural practices and maternal dietary habits. Vitamin B_{12} deficiency is rarely seen in West Africa (van den Broek, 1996) and is rare in pregnancy, as deficiency is associated with infertility (Letsky, 1995). Riboflavin deficiency has been observed among pregnant women in the Gambia (Reddy *et al.*, 1987) and may contribute to low Hb values. However, supplementary riboflavin (5 mg) given with or without iron sulphate (30 mg) for 6 weeks to pregnant women in the Gambia showed no convincing haematological response (Powers *et al.* 1985). In most settings where malaria is endemic, the Hb concentration of pregnant women with and without malaria parasitaemia is significantly different only among primigravidae (McGregor, 1984). This is not always the case, and subsequent pregnancies may also be affected (Bouvier *et al.*, 1997). Malaria is an important determinant of maternal anaemia in the Gambia (Greenwood *et al.*, 1989) and Mali (Bouvier *et al.*, 1997) but was not found contribute to anaemia in Benin where *Plasmodium falciparum* was found in 99% of women (Hercberg *et al.*, 1987) or in Liberia (Jackson & Jackson, 1987). A recent study in coastal Kenya reported that malaria is the main cause of anaemia among primigravidae, whilst hookworm intensity rather than malaria was the predominant determinant of anaemia among multigravidae (Shulman *et al.*, 1996). Sickle cell anaemia (HbSS) causes chronic haemolytic anaemia (Fleming, 1989), but is rare among pregnant women, as few sufferers survive to adulthood in Africa (WHO, 1993). The heterozygous sickle cell trait (HbAS) does not appear to contribute significantly to anaemia among pregnant women in Liberia (Jackson & Latham, 1982; Jackson & Jackson, 1987), Benin (Hercberg *et al.*, 1987) or the Gambia (Powers *et al.*, 1985). It has been predicted that AIDS will become an increasingly important determinant of maternal anaemia in Africa (Fleming 1989). However, none of the women in the present study were found to be infected with human immunodeficiency virus (HIV) (Chapter 10). Nonetheless, pregnant women in developing countries are exposed to numerous other infections that may result in anaemia, such as urinary tract and respiratory tract infections, tuberculosis and amoebiasis (van den Broek, 1998). Inflammatory processes associated with these infections may be a source of error in diagnosing iron-deficiency and iron-deficiency anaemia, as serum ferritin is an acute phase protein and is raised independently of iron status in inflammation.

Seasonality is an important determinant of nutritional status (Ferro-Luzzi & Branca, 1993), and was associated with the iron stores (SF concentration) of pregnant women in their first trimester in Western Sierra Leone (Figure 8.6). The iron stores of pregnant women who were assessed in the late dry season (February to April) were significantly greater than those of women who were assessed in the early dry (November to January) or early wet season (May to July). The Hb concentration of women who were assessed in late dry season was also highest, although the inter-seasonal differences were not significant (Figure 8.7).

In West Africa, the seasonal pattern of rainfall affects the availability of food items, the incidence of parasitic infections and the physical activity level of those who farm the land (Bates *et al.*, 1982; Roberts *et al.*, 1982; Lawrence & Whitehead, 1988; Bates *et al.*, 1994). These seasonal fluctuations may influence iron metabolism and Hb values in women. As discussed in Chapter 5, the quantity of food intake probably does not vary greatly between the seasons in Western Sierra Leone, but the quality of the diet may vary greatly depending on the quantity of haem iron and promoters and inhibitors of non-haem iron absorption in the diet. During the dry season, there is increased intake of fruits rich in vitamin C and vitamin A. Vitamin C promotes non-haem iron absorption (Bothwell *et al.*, 1989), while vitamin A is linked to improved iron metabolism (Panth *et al.*, 1990; Suharno *et al.*, 1993). The intake of fish, which is the predominant source of haem iron in these communities, may also increase in the dry season when fishing activities are increased. The high temperatures and comparative low humidity are less conducive to the survival and development of the infective *N. americanus* larvae (Chandiwana, 1990; Smith, 1990; Pawlowski *et al.*, 1991). Thus, in the dry season, the intake of absorbable iron is likely to peak, the incidence of new *N. americanus* infections is likely to reduce, and there is less demand for agricultural labour. These features of the dry season may explain why women assessed in the late dry season had higher iron stores than their peers. The same group of women also experienced least decline in SF concentration between the first and third trimester, presumably due to their higher Hb concentration and iron stores. However, it is also possible that infectious processes associated with diseases such as malaria, which is associated with the onset of the rains in Sierra Leone

(Blacklock & Gordon, 1925; Aitken, 1990), elevated their SF values independently of iron status during the wet season.

Pregnant women who were enrolled in the early wet season experienced a significantly greater decline in Hb concentration between the first and third trimester than those enrolled in the early or late dry season. These women had the largest proportion of their pregnancy in the wet season, which is associated with increased anaemia in West Africa (Tomkins, 1993; Bouvier *et al.*, 1997). In this season, the availability of citrus fruits and mangoes declines rapidly, and therefore non-haem iron absorption may be reduced. There is increased demand for manpower in the fields, and less attention is given to fishing activities. The high temperatures and high humidity provide favourable conditions for the survival and development of infective hookworm larvae and for *Anopheles* species to breed. The transmission of hookworm infection fluctuates in regions where rainfall is seasonal (Pawlowski *et al.*, 1991) and in these pregnant women, a higher prevalence of infection was observed among women who were enrolled in the early dry season, the three months immediately following the previous wet season (Chapter 6). The incidence of malaria increases during the rains in Sierra Leone (Aitken, 1990) and the prevalence of placental malaria infection has been shown to peak in the early wet season in Freetown (Blacklock & Gordon, 1925).

In summary, it appears that iron deficiency is related to the demands of pregnancy and seasonal fluctuations in the quality of food intake and/or parasitic infection. Further studies are needed to clarify the contribution of other aetiological factors to maternal anaemia in Western Sierra Leone, such as folate deficiency, sickle cell disorders and malaria, but in light of the available evidence, it is likely that poor dietary intake of absorbable iron, aggravated by *N. americanus* infection, are major determinants of maternal anaemia in these communities.

8.4.2 Effect of intervention with iron-folate supplements and albendazole treatment on haemoglobin concentration and iron stores during pregnancy

The baseline analysis indicates that both iron deficiency and *N. americanus* infection are associated with Hb concentration in these pregnant women. However, these cross-sectional findings cannot be used in support of a causal relationship as it is possible that the association may be coincidental or that it is mediated by a common underlying, as yet unidentified, factor. The best approach to confirm the contribution of iron deficiency and *N. americanus* to maternal anaemia is to measure the impact of intervention to control these conditions on the change in Hb concentration over time using a randomised placebo-controlled design (Yip, 1994). This approach has the advantage that it does not rely on iron biochemical tests, the results of which are likely to be affected by inflammatory or infectious processes. The longitudinal component of the study was designed to provide a means of confirming the contribution of iron deficiency and to determine the relative benefit of iron-folate supplements and albendazole treatment for the control of maternal anaemia during pregnancy. The impact of intervention on SF concentration was also examined, although these results must be interpreted with caution as the SF values may not accurately reflect iron stores in this setting. It is also necessary to point out that the results may be biased by the exclusion of 59 (32.0%) of subjects who were enrolled at baseline but did not provide all the necessary samples and measurements. Of particular concern are the eight subjects who were withdrawn from the study after the second trimester assessment due to anaemia (Hb <80 g/l). These subjects were not evenly distributed among the intervention groups: one subject received FeP_A, three received $P_{Fe}A$ and four received $P_{Fe}P_A$. Loss of subjects from the placebo groups, in particular the iron-folate placebo group, may have caused an underestimation of the effects of intervention.

To my knowledge, this is the first study which has used a randomised placebo-controlled factorial design to examine the benefits of albendazole treatment and iron-folate supplements simultaneously in pregnant women. There are a limited number of studies that have investigated the effects of anthelminthic treatment on haematological status. A small-scale study in Ivory Coast (West Africa) examined the impact of pyrantel pamoate on Hb concentration in 32 pregnant women, 25 of whom were infected with *Ascaris lumbricoides* and 15 with hookworm (Welffens-Ekra *et al.*, 1990). Thirty days after anthelminthic treatment, which cleared all but one infection, the improvement in Hb concentration was greater among those who had hookworm infection than among those who had *Ascaris* only, but no significant tests were conducted. More recently, a small scale study (*n*=115) in Sri Lanka examined the impact of one course of mebendazole (100 mg, twice daily for 3 days) administered after the first trimester of pregnancy on Hb concentration and iron status in pregnant women (Atukorala *et al.*, 1994). Mebendazole treatment plus iron-folate supplements resulted in a marked increase in Hb concentration and a significant improvement in iron status compared with iron-folate supplements alone. As all of these women received daily iron-folate supplements, the effect of mebendazole treatment alone could not be determined. The latter study has frequently been quoted in support of hookworm control as a strategy to control maternal anaemia during pregnancy. However, the prevalence and intensity of intestinal nematode infections were not measured in these women. Therefore, it is not clear which infections were present in these women or whether the distribution of parasites was similar in the intervention and control groups. The results cannot be related to the severity of infection, and it is difficult to extrapolate the results of this study to other communities where the prevalence and intensity of intestinal nematode infections are known. A separate parasitological survey among 146 non-pregnant women in Sri Lanka found evidence of hookworm infection in 41.4%, the majority of which were mild infections with mean egg counts <1,000 epg (de Silva *et al.*, 1994). This would suggest, but not confirm, that most infections in the Sri Lankan pregnant women were low in intensity.

In the present study, the intervention groups were similar at baseline with respect to social, demographic and reproductive characteristics (Chapter 5), the prevalence and intensity of intestinal nematode infections (Chapter 6), measures of body size and composition (Chapter 7), prevalence of iron deficiency, anaemia and iron-deficiency anaemia, Hb concentration and SF concentration, with the exception of upper arm fat area. One other exception was the season at baseline, and the effects of intervention were controlled for this variable in the multivariate analyses.

No intervention group was associated with a significant improvement in Hb concentration or SF concentration, or a reduction in the baseline prevalence of anaemia, iron deficiency or iron-deficiency anaemia between baseline and the third trimester. However, the baseline Hb concentration, prevalence of anaemia and prevalence of iron-deficiency anaemia did not change significantly in the group of subjects who received both iron-folate supplements and albendazole (intervention group FeA), while the baseline SF concentration and prevalence of iron deficiency did not change significantly in the groups of subjects who received iron-folate supplements (intervention groups FeA and FeP_A).

Pregnant women who received iron-folate supplements and/or albendazole treatment experienced less decline in Hb concentration between baseline and the third trimester than the respective controls. Iron-folate supplements, but not albendazole treatment, also reduced the decline in baseline SF concentration. The lack of impact of albendazole treatment on the change in SF concentration may indicate that iron savings associated with treatment were not sufficient to allow net storage of iron. Alternatively, the effects of albendazole may be confounded by concomitant infections, inflammatory processes or heterogeneous dietary iron intake across the intervention groups.

These findings confirm that both iron-folate deficiency and intestinal nematode infections are important aetiological factors of anaemia during pregnancy in these communities. On a purely statistical basis, they indicate that both iron-folate supplementation and albendazole treatment improve the haematological status of the pregnant women relative to those who do not receive these interventions. However, it is important to assess whether the magnitude of the observed differences is physiologically important as well as statistically significant. After controlling for Hb concentration and season at baseline, the mean benefit of iron-folate supplements and albendazole treatment relative to their respective controls was 13.7 g/l Hb and 6.6 g/l Hb respectively. These effects were additive, and therefore the mean benefit was 20.2 g/l Hb in women who received both iron-folate supplements and albendazole treatment. The Hb benefit induced by the iron-folate supplements is similar to the haematological improvement a pregnant woman with Hb=87.2 g/l (the mean third trimester Hb concentration of women who received both placebos) and a total blood volume of 5.3 L at 30 weeks (Taylor & Lind, 1979) would theoretically gain from two pints of blood from a donor with Hb=125.0 g/l. The Hb benefit in the latter scenario is 12.7 g/l, assuming 250 ml of fluid from each pint of donated blood is not retained in the circulatory system. Similarly, the Hb benefit induced by albendazole treatment is similar to that the same woman would gain from one pint of blood from the same donor (6.6 g/l), and the Hb benefit associated with iron-folate supplements plus albendazole treatment is similar to a transfusion of three pints (19.0 g/l). Expressed in these terms, it is clear that the Hb benefit induced by both iron-folate supplements and albendazole treatment is far from negligible. The effect of albendazole treatment may be even greater in the rural provinces of Sierra Leone, where higher intensities of *N.americanus* infection have been reported (see Appendix 2).

There is a lack of similar intervention studies with which to compare these findings. Controlled studies in both children and adults have demonstrated that anthelminthic treatment increases Hb concentration by 2-6 g/l after six months, a similar follow-up period to that used in the present study (Working Group on Fortification of Salt with Iron, 1982; Stephenson *et al.*, 1989a). The intervention study in Sri Lanka (Atukorala *et al.*, 1994) reported that the Hb concentration of pregnant women 14 weeks after a single course of mebendazole was 1.62 g/l higher than pregnant women who received the placebo (adjusted for initial Hb concentration and the amount of iron supplements received). The effect of anthelminthic treatment per week of intervention in the Sri Lanka study was 0.12 g/l, less than half the rate in the present study (0.28 g/l/wk). These rates are not directly comparable, as the gestational age of the women at baseline and follow-up was different in the two studies, and the gestational-dependent effect of haemodilution may confound the comparison. A search of previous iron-folate supplementation studies among pregnant women failed to find a study which used a similar dose of iron and folate and similar follow-up period as that in the present study. The intervention period of most studies in developing countries rarely exceeds three months, and haemodilution is likely to confound a comparison. Comparison with supplementation studies in developed countries is not ideal, as fewer pregnant women in these countries are iron or folate deficient.

If the primary purpose of interventions to control maternal anaemia is to normalise Hb concentration in as short a time as possible, iron-folate supplements would appear to have the advantage over albendazole treatment in this setting. However, there are other factors that must also be considered. Iron-folate supplements may induce a faster haematological response, but this response may be short-lived. Once supplementation is discontinued, the Hb concentration will decline if the causes of anaemia (including gastrointestinal blood loss) are not addressed. The haematological responses observed in the present study occurred over 24 weeks of daily supplementation under semi-supervised conditions with adequate follow-up, and is likely to represent the maximum achievable impact. It is unlikely that a national maternal anaemia control programme would be able to achieve the same duration of supplementation or degree of compliance that was possible under the semi-supervised conditions of this field trial. Indeed, there is plenty of evidence to suggest that national supplementation programmes are failing to repeat the efficacy that has been achieved under field condition (ACC/SCN, 1991; Yip, 1996; Viteri, 1997). The most common reasons for women not taking iron supplements are the lack of access to antenatal care and the insufficient supply and distribution of supplements (ACC/SCN, 1991; Galloway & McGuire, 1994). Other important obstacles include poor patient compliance due to the side effects of iron preparations and frustration due to the frequency and number of pills to be taken (Galloway & McGuire, 1994).

The clear advantage that albendazole treatment has over iron-folate supplementation is the ease of administration. In this population, one course administered at the first antenatal clinic after the first trimester of pregnancy induced a relative improvement in Hb concentration that, while less than the daily iron-folate supplements, was not dependent on further treatment during the pregnancy. In this respect, hookworm control may be more effective than iron-folate supplementation, albeit less efficacious under controlled conditions. Albendazole treatment also has other benefits. This anthelminthic is broad-spectrum and can rid the body of multiple parasites. It may improve food intake by relieving the symptoms of intestinal nematode infection that reduce appetite and by eliminating the physiological causes of anorexia induced by infection. In addition, it may reduce the risk of complications associated with ectopic migration of nematode larvae or worms (Pinus, 1985; MacLeod, 1988). Furthermore, integration of anthelminthic treatment into strategies for the control of maternal anaemia may encourage clinic attendance and participation in iron-folate supplementation programmes.

Despite the diverse benefits of albendazole treatment, it is clear that the poor haematological status of women in this community cannot be corrected with anthelminthics alone. A substantial number of pregnant women with no evidence of *N. americanus* infection were iron deficient and had iron-deficiency anaemia, indicating substantial underlying iron deficiency. Iron-folate supplementation is therefore an essential component of anaemia control in these pregnant women. However, for maximum benefit it is recommended that iron-folate supplements be complemented with one course of albendazole in the second trimester of pregnancy in these communities.

While intervention with both iron-folate supplements and albendazole treatment prevented a deterioration in haematological status, they did not normalise the Hb or SF concentrations or even elicit a convincing improvement. This may imply that: (1) there were other important aetiological factors of anaemia that were left untreated and limited the haematological response (2) the frequency and/or dose of the supplements were insufficient (3) the severity of anaemia was such that there was insufficient time to normalise Hb concentration. The possibility that other aetiological factors such as dietary deficiencies, malaria and sickle cell disease, contributed to the low Hb values in this population was discussed in Section 8.4.1. It is unlikely that the single dose treatment with albendazole administered after the first trimester of pregnancy was insufficient in these women as reinfection with intestinal nematodes was slow following treatment (see Chapter 6). However, there is a possibility that some women were infected with large numbers of fourth-stage hookworm larvae, which are voracious feeders on blood but do not produce eggs and would not have been detected using stool analysis (Nelson, 1990). It is also unlikely that erythropoiesis was limited by folate deficiency as a daily dose of 5 mg folate is considered sufficient to replenish any subject's body stores (Fleming, 1989). It is more likely that the low dose of ferrous gluconate limited the haematological response.

The dose of ferrous gluconate provided 36 mg of iron each day, which is less than one third of the amount recommended by the World Health Organization (120 mg) for areas where the prevalence of iron-deficiency anaemia is high (ACC/SCN, 1991). A low dose was purposely chosen in response to recent research which has indicated that lower doses or less frequent regimens may induce similar haematological responses as daily regimens. It is suggested that iron blockage of the intestinal mucosal cells following an iron dose limits further absorption until these cells are replaced (Viteri *et al.*, 1995). Therefore, the absorptive capacity for iron is increased if the frequency of iron supplementation matches the turnover time of the intestinal mucosal cells. Low dose or less frequent supplementation is desirable as it is economically advantageous and may improve patient compliance by reducing the gastro-intestinal side effects associated with oral iron. In the present study, the iron dose was well tolerated and only two subjects reported mild gastro-intestinal symptoms. Recent studies have indicated that once weekly and twice weekly supplementation schedules are as equally efficacious as daily supplementation in pregnant women (Lui *et al.*, 1995; Chew *et al.*, 1996; Ridwan *et al.*, 1996), but less attention has been given to the use of lower daily dose regimens. In this population, it is likely that the low iron dose was insufficient over the follow-up period of 24 weeks. The duration of iron-folate supplementation is rarely this long in developing countries, where attendance during the first and even second trimester of pregnancy is uncommon. Over a shorter period of supplementation, it is highly likely that the iron dose should be higher. This is particularly relevant for women who have a large proportion of their pregnancy during the wet season, which was associated with greater declines in Hb concentration.

The poor iron status at baseline may have limited normalisation of Hb and SF concentration within the time frame of pregnancy. Due to the high iron demands of pregnancy, maternal iron-folate supplementation is not a practical means of normalising these levels, but is used primarily to prevent further depletion of iron stores and to maintain Hb levels above the level associated with poor maternal and fetal outcomes (Viteri, 1997).

Maternal anaemia is detrimental in the post-partum period when the mother must care, nurse and breast-feed her infant (Milman *et al.*, 1991). In developing countries, where repeated cycles of pregnancy and lactation place a heavy burden on the iron stores, the effect of intervention on maintaining satisfactory iron status after delivery is also relevant. A single course of albendazole treatment two months prior to delivery may not accrue substantial haematological benefits during the course of the pregnancy, but the benefits achieved by stopping the leakage of iron from the intestine may persist after parturition. Studies have indicated that iron supplementation during pregnancy improves maternal Hb and SF concentration two to six months after delivery in Africa (Preziosi *et al.*, 1997) and in Western populations (Puolakka *et al.*, 1980; Milman *et al.*, 1991; Godel *et al.*, 1992; Taylor *et al.*, 1982a; Milman *et al.*, 1994). In the present study, it was not possible to interpret the data on maternal Hb and SF concentration that was collected one month after delivery, as there were a large number of intervention-related drop-outs from the study after the third trimester and birth assessments due to anaemia. The effect of the interventions on postnatal maternal iron deficiency and anaemia could be the focus of further research.

8.4.3 Implications for the control of maternal iron deficiency and anaemia during pregnancy

The World Health Organization has recently recommended that anthelminthic therapy be included in strategies to control maternal anaemia during pregnancy in areas where hookworm infection is endemic (prevalence >20-30%) and where anaemia is prevalent (WHO, 1996b). This recommendation does not stipulate the definition of 'endemic' with respect to the intensity of infection, or the appropriate frequency of anthelminthic treatment. The present study has indicated that in the Western Sierra Leonean setting where there is profound iron deficiency, removal of relatively mild intestinal nematode infections with a single course of albendazole at the beginning of the second trimester of pregnancy in addition to iron-folate supplements minimised the decline in first trimester Hb and SF concentration. In this and similar settings, one course of albendazole treatment administered after the first trimester of pregnancy together with iron-folate supplements should be included in strategies to control maternal anaemia.

Because the factors that contribute to iron deficiency and anaemia differ in importance both within and between countries, it is difficult to extrapolate these findings to all communities where the endemicity of intestinal nematodes is similar. In adequately nourished women with carefully monitored antenatal care, it may be unnecessary to treat intestinal parasites during pregnancy (D'Alauro *et al.*, 1985). Any interventions must therefore be made on a sound epidemiological understanding of the factors that contribute to iron deficiency and anaemia in pregnant women in a particular area.

In both Sri Lanka and the Seychelles, hookworm control is already included in the national strategies for the prevention and control maternal anaemia during pregnancy (Ministry of Health and Women's Affairs, 1994; WHO, 1996b). Such national strategies are still beyond the limited health care budget of many African countries. Nonetheless, it is important that health care professionals are aware of the benefits of anthelminthic intervention during pregnancy so that they can inform and advise those antenatal patients who can afford treatment.

8.5 SUMMARY

1. The distribution of iron deficiency and anaemia in women in their first trimester of pregnancy, and the efficacy of iron-folate supplements and albendazole as control measures against iron deficiency and anaemia during pregnancy, was investigated in Western Sierra Leone.
2. In the first trimester of pregnancy, 58.7% of women were anaemic (Hb <110 g/l). Anaemia was associated with iron deficiency in 36.1% of anaemia cases if SF <20 μg/l is diagnostic of iron deficiency, or 75.0% if SF <50 μg/l is diagnostic of iron deficiency.
3. First trimester Hb concentration was negatively associated with the intensity of *N. americanus* infection and was positively associated with body weight.
4. The Hb concentration of women in the first trimester was similar to that of non-pregnant women residing in the same household. However, the prevalence of anaemia (Hb <120 g/l) in non-pregnant women (80.7%) was higher than in pregnant women. This finding suggests that the WHO recommended cut-off point for anaemia in pregnant women is questionable during the first trimester.

5. First trimester SF concentration was significantly lower among grandmultiparae, those who delivered ≤12 months previously and women who had never used modern methods of contraceptives. Women who were assessed in the late dry season had significantly higher SF concentration than those assessed in the early dry season or early wet season.
6. Intervention with daily iron-folate supplements and/or albendazole treatment was not effective in decreasing the prevalence of iron deficiency, anaemia or iron-deficiency anaemia between baseline and the third trimester assessment. However, women who received iron-folate supplements experienced less decline in baseline Hb and SF concentration than unsupplemented controls. Albendazole treatment was also effective in reducing the decline in Hb concentration. After controlling for Hb concentration and season at baseline, the mean benefit of iron-folate supplements and albendazole treatment relative to their respective controls was 13.7 g/l Hb and 6.6 g/l Hb respectively.
7. In this and similar settings, one course of albendazole treatment administered after the first trimester of pregnancy together with iron-folate supplements should be included in strategies to control maternal anaemia.

Table 8.1 Determinants of haemoglobin concentration (g/l) in women in the first trimester of pregnancy in Western Sierra Leone (*n*=184).

	Univariate analysis[†]			Multivariate analysis[‡]		
	Coefficient (SE)	t-value	*p*	Coefficient (SE)	t-value	*p*
Intensity of *N. americanus* infection[§]	-0.94 (0.37)	2.50	0.013	-0.87 (0.37)	2.35	0.020
First trimester weight (kg)	0.30 (0.13)	2.32	0.022	0.27 (0.13)	2.15	0.033

† Linear regression.
‡ Multiple linear regression.
§ Intensity estimated indirectly as eggs per gram of faeces (epg); data was log-transformed, $\log_e$(epg+1).

Table 8.2 Haemoglobin concentration, serum ferritin concentration and the prevalence of anaemia, iron deficiency and iron-deficiency anaemia by infection status for *Necator americanus* in women in the first trimester of pregnancy in Western Sierra Leone.

		Infection status for *N. americanus*[†]	
		Uninfected (*n*=61)	Infected (*n*=123)
Haemoglobin concentration (g/l)	mean (±SD)	107.8 (14.6)	106.0 (14.2)
Serum ferritin concentration (μg/l)	mean (±SD)	28.3 (-18.1, +50.2)	21.5 (-14.3, +43.0)
Anaemia[‡]	frequency (%)	35 (57.4)	73 (59.3)
Iron deficiency[§]	frequency (%)	18 (29.5)	51 (41.5)
Iron-deficiency anaemia[††]	frequency (%)	10 (16.4)	29 (23.6)

[†] No significant difference between uninfected and infected women for any variable.
[‡] Haemoglobin concentration <110 g/l
[§] Serum ferritin concentration <20 μg/l
[††] Haemoglobin concentration <110 g/l and serum ferritin concentration <20 μg/l

Table 8.3 Determinants of serum ferritin concentration[†] (μg/l) in women in the first trimester of pregnancy in Western Sierra Leone (*n*=184).

		Univariate analysis[‡]			Multivariate analysis[§]		
		Coefficient (SE)	t-value	*p*	Coefficient (SE)	t-value	*p*
Intensity of *N. americanus* infection[††]		-0.073 (0.028)	2.60	0.010	-	-	NS[‡‡]
Grandmultipara (parity >6)		-0.775 (0.306)	2.53	0.012	-0.582 (0.279)	2.09	0.038
Inter-birth interval ≤12 months		-0.695 (0.231)	3.00	0.0030	-0.633 (0.214)	2.96	0.0035
Use of modern contraceptives		0.757 (0.240)	3.16	0.0018	0.493 (0.232)	2.13	0.035
Season at assessment[§§]:	D_1	-0.964 (0.166)	5.80	<0.0001	-0.877 (0.163)	5.39	<0.0001
	D_3	-0.391 (0.202)	1.94	0.054	-0.618 (0.200)	3.08	0.0024

[†] Serum ferritin concentration was log-transformed.
[‡] Linear regression.
[§] Multiple linear regression.
[††] Intensity estimated indirectly as eggs per gram of faeces (epg); data was log-transformed, $\log_e$(epg+1).
[‡‡] Non significant (p >0.05).
[§§] Dummy variables (entered simultaneously in univariate analysis): early dry season (November to January) D_1=1, D_3=0; late dry season (February to April) D_1=0, D_3=0; early wet season (May to July) D_1=0, D_3=1.

Table 8.4 Haemoglobin concentration in women in the first trimester of pregnancy and non-pregnant women matched for household in Western Sierra Leone.

Mean (SD) haemoglobin concentration g/l (n=145)		Mean difference (95% CI)	t-value	p
Pregnant	Non-pregnant			
106.3 (13.1)	105.7 (16.5)	0.6 (-2.6, 3.9)	0.37	0.71

Table 8.5 Prevalence of anaemia in women in the first trimester of pregnancy (haemoglobin concentration <110 g/l) and non-pregnant women (haemoglobin concentration <120 g/l) matched for household in Western Sierra Leone.

		Number (prevalence %) of non-pregnant women		
		Non-anaemic	Anaemic	Total
Number (prevalence %) of pregnant women	Non-anaemic	14 (9.7)	44 (30.3)	58 (40.0)
	Anaemic	14 (9.7)	73 (50.3)	87 (60.0)
	Total	28 (19.3)	117 (80.7)	145 (100.0)

Table 8.6 Prevalence of anaemia in women in the first trimester of pregnancy (haemoglobin concentration <120 g/l) and non-pregnant women (haemoglobin concentration <120 g/l) matched for household in Western Sierra Leone.

		Number (prevalence %) of non-pregnant women		
		Non-anaemic	Anaemic	Total
Number (prevalence %) of pregnant women	Non-anaemic	7 (4.8)	19 (13.1)	58 (17.9)
	Anaemic	21 (14.5)	98 (67.6)	119 (82.1)
	Total	28 (19.3)	117 (80.7)	145 (100.0)

Table 8.7 Anaemia status (haemoglobin concentration <110 g/l) of pregnant women in Western Sierra Leone at baseline (first trimester) and at follow-up in the third trimester following intervention with iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo.

		Number (%) of women in third trimester			McNemar's Test (Binomial)
		Non-anaemic	Anaemic	Total	*p*
Number (%) of women at baseline	**FeA**†				
	Non-anaemic	9 (28.1)	6 (18.8)	15 (46.9)	0.61
	Anaemic	9 (28.1)	8 (25.0)	17 (53.1)	
	Total	18 (56.2)	14 (43.8)	32 (100.0)	
	FeP_A†				
	Non-anaemic	4 (11.4)	10 (28.6)	14 (40.0)	0.039
	Anaemic	2 (5.7)	19 (54.3)	21 (60.0)	
	Total	6 (17.1)	29 (82.9)	35 (100.0)	
	P_{Fe}A†				
	Non-anaemic	0 (0.0)	15 (51.7)	15 (51.7)	0.0001
	Anaemic	0 (0.0)	14 (48.3)	14 (48.3)	
	Total	0 (0.0)	29 (100.0)	29 (100.0)	
	$P_{Fe}P_A$†				
	Non-anaemic	1 (3.4)	10 (34.5)	11 (38.0)	0.0020
	Anaemic	0 (0.0)	18 (62.1)	18 (62.1)	
	Total	1 (3.4)	28 (98.6)	29 (100.0)	

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 8.8 Iron-deficiency anaemia (IDA) status (haemoglobin concentration <110 g/l and serum ferritin concentration <20 µg/l) of pregnant women in Western Sierra Leone at baseline (first trimester) and at follow-up in the third trimester following intervention with iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo.

		Number (%) of women in third trimester			McNemar's Test (Binomial)
		No iron-deficiency anaemia	Iron-deficiency anaemia	Total	*p*
Number (%) of women at baseline	**FeA**†				
	No iron-deficiency anaemia	19 (59.4)	5 (15.6)	24 (75.0)	1.00
	Iron-deficiency anaemia	6 (18.8)	2 (6.3)	8 (25.0)	
	Total	25 (78.1)	7 (21.9)	32 (100.0)	
	FeP_A†				
	No iron-deficiency anaemia	18 (51.4)	12 (34.3)	30 (85.7)	0.013
	Iron-deficiency anaemia	2 (5.7)	3 (8.6)	5 (14.3)	
	Total	20 (57.1)	15 (42.9)	35 (100.0)	
	$P_{Fe}A$†				
	No iron-deficiency anaemia	3 (10.3)	22 (75.9)	25 (86.2)	<0.0001
	Iron-deficiency anaemia	0 (0.0)	4 (13.8)	4 (13.8)	
	Total	3 (10.3)	26 (89.7)	29 (100.0)	
	$P_{Fe}P_A$†				
	No iron-deficiency anaemia	6 (20.7)	17 (58.6)	23 (79.3)	<0.0001
	Iron-deficiency anaemia	1 (3.4)	5 (17.3)	6 (20.7)	
	Total	7 (24.1)	22 (75.9)	29 (100.0)	

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 8.9 Iron deficiency status (serum ferritin concentration <20 µg/l) of pregnant women in Western Sierra Leone at baseline (first trimester) and at follow-up in the third trimester following intervention with iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo.

		Number (%) of women in third trimester			McNemar's Test (Binomial)
		Iron sufficient	Iron deficient	Total	*p*
Number (%) of women at baseline	**FeA**†				
	Iron sufficient	11 (34.4)	7 (21.9)	18 (56.3)	1.00
	Iron deficient	6 (18.8)	8 (25.0)	14 (43.8)	
	Total	17 (53.1)	15 (46.9)	32 (100..0)	
	FeP_A†				
	Iron sufficient	14 (40.0)	9 (25.7)	23 (65.7)	0.27
	Iron deficient	4 (11.4)	8 (22.8)	12 (34.3)	
	Total	18 (51.4)	17 (48.6)	35 (100.0)	
	P_{Fe}A†				
	Iron sufficient	3 (10.3)	16 (55.2)	19 (65.5)	<0.0001
	Iron deficient	0 (0.0)	10 (34.5)	10 (34.5)	
	Total	3 (10.3)	26 (86.7)	29 (100.0)	
	$P_{Fe}P_A$†				
	Iron sufficient	6 (20.7)	15 (51.7)	21 (72.4)	<0.0001
	Iron deficient	1 (3.4)	7 (24.1)	8 (27.6)	
	Total	7 (24.1)	22 (75.9)	29 (100.0)	

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 8.10 Haemoglobin and serum concentration of pregnant women in Western Sierra Leone at baseline (first trimester) and at follow-up in the second and third trimesters following intervention with iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo (n=125).

Intervention†	Number of women	Mean (±SD)‡			Mean difference between first and third trimester (95% CI)§	Paired t-test t value	p
		First trimester	Second trimester	Third trimester			
Haemoglobin (g/l)							
FeA	32	108.5 (11.8)	107.0 (11.4)	107.3 (14.3)	-1.1 (-7.2, 5.0)	0.38	0.71
FeP_A	35	108.0 (10.9)	99.2 (11.4)	97.5 (12.5)	-10.5 (-14.7, -6.4)	5.15	<0.001
$P_{Fe}A$	29	108.6 (12.9)	93.4 (8.3)	88.9 (12.3)	-19.6 (-25.8, -13.5)	6.54	<0.001
$P_{Fe}P_A$	29	109.0 (12.5)	95.1 (9.1)	87.2 (11.5)	-21.8 (-27.2, -16.4)	8.29	<0.001
Serum ferritin (μg/l)							
FeA	32	20.0 (-13.8, +44.6)	19.7 (-11.6, +27.5)	20.9 (-11.9, +27.7)	104.8 (65.6, 167.6)	0.20	0.84
FeP_A	35	27.3 (-18.5, +57.3)	18.9 (-11.4, +29.0)	20.4 (-12.0, +28.9)	74.8 (52.0, 107.4)	1.63	<0.001
$P_{Fe}A$	29	22.4 (-14.6, +42.1)	11.1 (-7.6, +23.5)	7.6 (-4.7, +12.3)	34.0 (22.3, 51.7)	5.27	<0.001
$P_{Fe}P_A$	29	27.7 (-10.1, +61.2)	16.5 (-10.4, +28.1)	8.9 (-5.7, +15.7)	32.0 (20.8, 49.2)	5.43	<0.001

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

‡ Geometric mean (±SD) is given for serum ferritin concentration

§ Mean difference (D) for the log-transformed data (serum ferritin) is presented as $100(e^D)$, and corresponds to the third trimester values as a percentage of the first trimester values.

Table 8.11 Effect of iron-folate supplements and albendazole treatment on the change in haemoglobin (ΔHb) concentration between baseline (first trimester) and the third trimester in pregnant women in Western Sierra Leone (*n*=125).

ANOVA models		ΔHb g/l Parameter estimates B (SE)	F	*p*
1. Main intervention effects only				
Iron-folate supplements		14.87 (2.67)	31.08	<0.0001
Albendazole treatment		5.80 (2.67)	4.72	0.032
Intervention interaction		3.62 (2.67)	1.84	0.18
2. Main intervention effects and covariates†				
Iron-folate supplements		13.67 (2.22)	38.05	<0.0001
Albendazole treatment		6.55 (2.19)	8.97	0.0034
Intervention interaction		2.46 (2.25)	1.19	0.28
Baseline haemoglobin concentration (g/l)		-0.72 (0.09)	61.05	<0.0001
Season at baseline‡:	D_1	6.13 (3.16)	3.75	0.055
	D_2	7.64 (3.25)	5.54	0.020

† Covariates entered simultaneously with the main intervention effects into the ANOVA model.

‡ Dummy variables: early dry season (November to January) $D_1=1$, $D_2=0$;
late dry season (February to April) $D_1=0$, $D_2=1$;
early wet season (May to July) $D_1=0$, $D_2=0$.

Table 8.12 Effect of iron-folate supplements and albendazole treatment on the change in log-transformed serum ferritin concentration (ΔSF) between baseline (first trimester) and the third trimester in pregnant women in Western Sierra Leone (*n*=125).

ANOVA models		ΔSF μg/l Parameter estimates B (SE)	F	*p*
1. Main intervention effects only				
Iron-folate supplements		0.988 (0.207)	2.87	<0.0001
Albendazole treatment		0.199 (0.207)	0.93	0.34
Intervention interaction		0.139 (0.207)	0.45	0.50
2. Main intervention effects and covariates†				
Iron-folate supplements		0.945 (0.152)	38.66	<0.0001
Albendazole treatment		0.047 (0.151)	0.10	0.76
Intervention interaction		0.030 (0.155)	0.04	0.85
Baseline serum ferritin concentration (μg/l)‡		-0.760 (0.073)	108.75	<0.0001
Season at baseline§:	D_1	-0.519 (0.181)	8.18	0.005
	D_3	-0.558 (0.224)	6.23	0.014

† Covariates entered simultaneously with the main intervention effects into the ANOVA model.
‡ Transformed using natural logarithms.
§ Dummy variables: early dry season (November to January) D_1=1, D_3=0;
late dry season (February to April) D_1=0, D_3=0;
early wet season; (May to July) D_1=0, D_3=1.

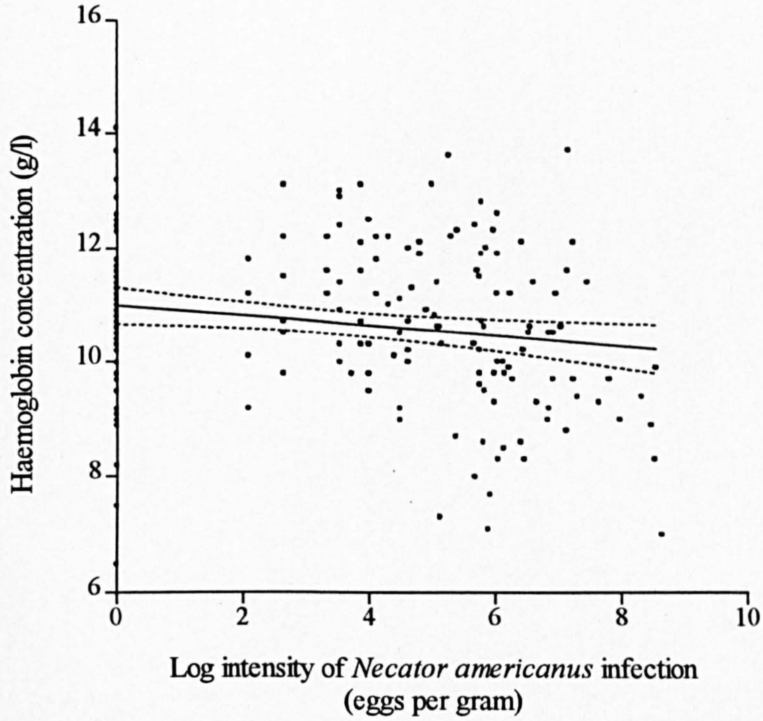

Figure 8.1 Scatterplot of haemoglobin concentration against log intensity of *Necator americanus* infection in women in the first trimester of pregnancy in Western Sierra Leone (*n*=184).
The linear regression line (solid line) is given with the 95% Confidence Interval (broken line).

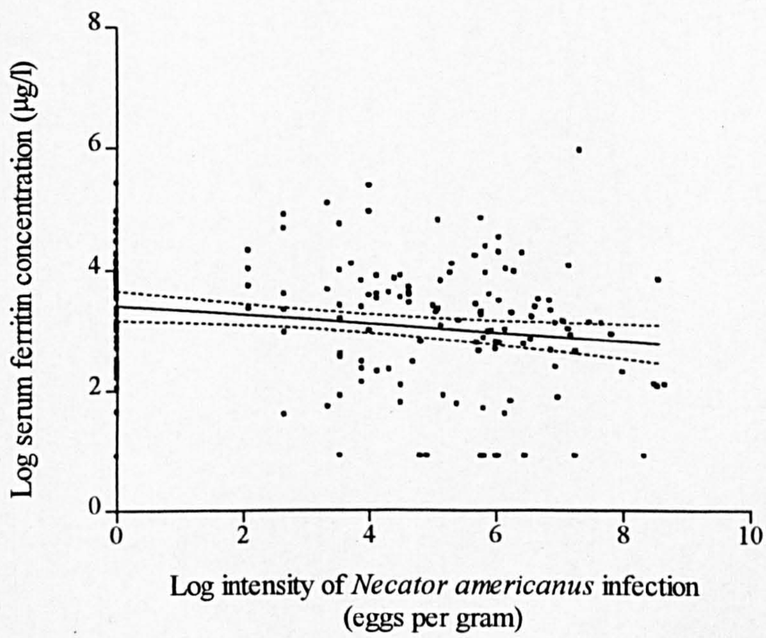

Figure 8.2 Scatterplot of log serum ferritin concentration against log intensity of *Necator americanus* infection in women in the first trimester of pregnancy in Western Sierra Leone (*n*=184).
The linear regression line (solid line) is given with the 95% Confidence Interval (broken line).

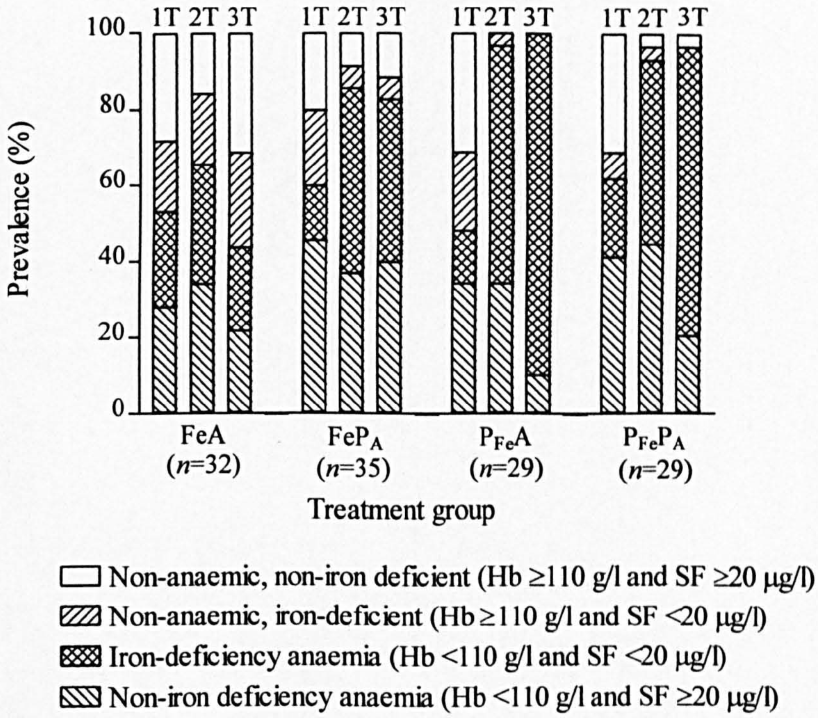

Figure 8.3 Prevalence of iron-deficiency, iron-deficiency anaemia and anaemia in each trimester by intervention group in women in the first trimester of pregnancy in Western Sierra Leone.

Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo. 1T = first trimester (baseline); 2T = second trimester; 3T = third trimester. Sample sizes are indicated in parentheses (n).

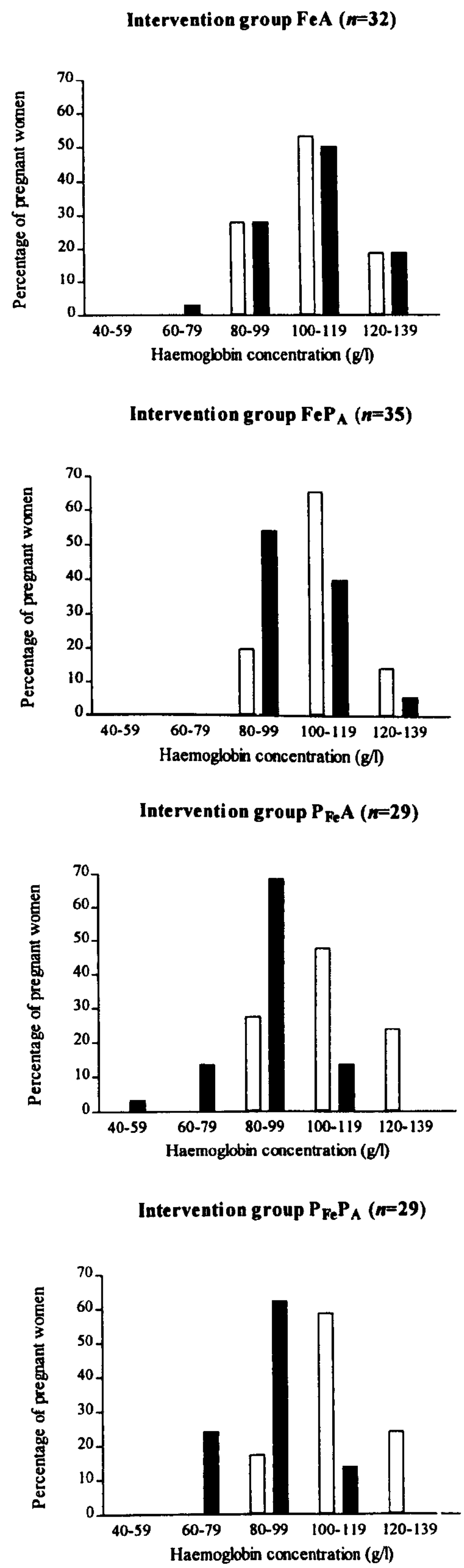

Figure 8.4 Distribution of haemoglobin concentration at baseline (first trimester) and at follow-up in the third trimester following intervention with iron-folate supplements (Fe) or iron-folate placebo (P_{Fe}) and albendazole (A) or albendazole placebo (P_A). White bar = baseline; black bar = third trimester.

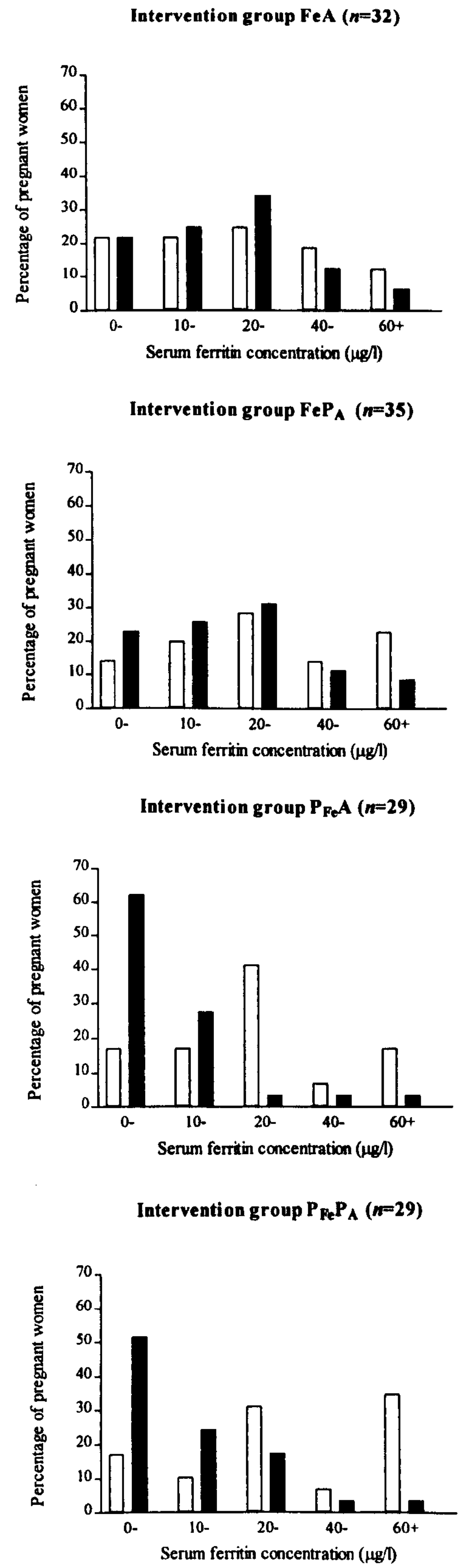

Figure 8.5 Distribution of serum ferritin concentration at baseline (first trimester) and at follow-up in the third trimester following intervention with iron-folate supplements (Fe) or iron-folate placebo (P_{Fe}) and albendazole (A) or albendazole placebo (P_A).
White bar = baseline; black bar = third trimester.

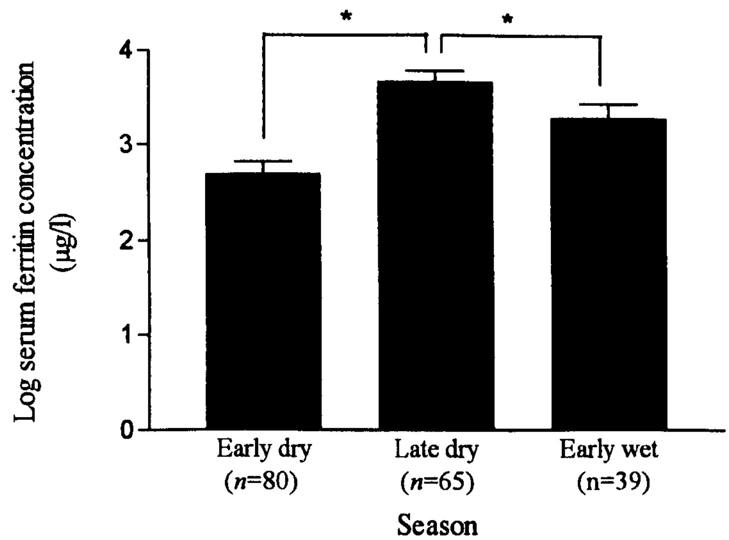

Figure 8.6 Log serum ferritin concentration of women in the first trimester of pregnancy in Western Sierra Leone by season.
Early dry = November to January; late dry = February to April; early wet = May to July. Sample sizes are indicated in parentheses (n); error bars indicate standard error; * = $p<0.050$

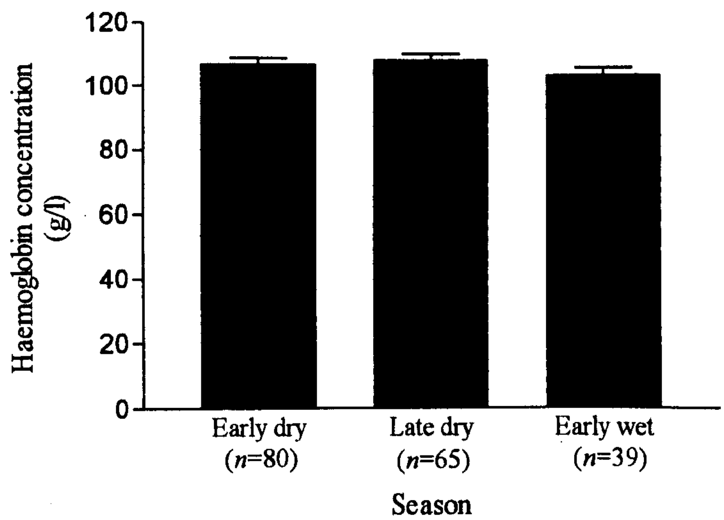

Figure 8.7 Haemoglobin concentration of women in the first trimester of pregnancy in Western Sierra Leone by season.
Early dry = November to January; late dry = February to April; early wet = May to July. Sample sizes are indicated in parentheses (n); error bars indicate standard error.

Chapter 9

Neonatal outcomes

9.1 INTRODUCTION

9.1.1 Neonatal outcomes

Prophylactic or therapeutic interventions during pregnancy can only be justified if they result in improved clinical outcomes for the mother or newborn. The most frequently measured clinical outcome for the newborn is its nutritional status at delivery, which is a critical determinant of short- and long-term growth and health sequelae. Birth-weight is considered a useful marker of fetal nutritional status (Caulfield, 1991), and is an important predictor of fetal, neonatal and postneonatal mortality and morbidity (McCormick, 1985). Each year an estimated 20.4 million infants are born with low birth-weight[1] in developing countries (de Onis *et al.*, 1998a), predominantly as a consequence of intrauterine growth retardation[2] and, to a lesser extent, preterm[3] delivery (Villar & Belizan, 1982).

Size at birth depends on a complex relationship between the genetically programmed body size and the capacity of the uteroplacental environment to adequately support its growth (Prada & Tsang, 1998). Multiple factors may contribute to low birth-weight including maternal nutritional status both prior to and during pregnancy, general morbidity and episodic illness and malaria (Kramer, 1987). Most interventions aimed at improving birth-weight in developing countries have focused on maternal nutritional status. Balanced protein-energy supplements have been shown to improve fetal growth in undernourished women, but comparatively little is understood about the impact of other nutritional interventions, including iron and folate supplements (de Onis *et al.*, 1998b).

Iron deficiency, folate deficiency and intestinal nematode infections are all implicated in the aetiology of poor growth in childhood (see Chapter 7), but their association with poor prenatal growth is less well characterised. Anaemia is associated with adverse pregnancy outcomes including lower birth-weight and preterm delivery (Harrison & Ibeiako, 1973; Singla *et al.*, 1978; Murphy *et al.*, 1986; Ogunniyi, 1990). Iron deficiency and iron-deficiency anaemia (Goepel *et al.*, 1988; Ulmer *et al.*, 1988; Bhargava *et al.*, 1991; Scholl *et al.*, 1992; Allen, 1993) and folate deficiency (Baumslag *et al.*, 1970; Rolschau *et al.*, 1979; Blot *et al.*, 1981) have been specifically linked to these outcomes. There is also evidence that maternal infection with intestinal nematodes increases the risk of fetal growth retardation (Villar *et al.*, 1989a). If poor maternal iron status, folate status and infection with intestinal nematodes contribute to adverse pregnancy outcomes, then interventions to control these disorders may result in improved birth-weight. These interventions may also benefit the health of the fetus in other ways. For example, there is increasing evidence to indicate that maternal iron status

---

[1] Birth-weight < 2500 g (WHO, 1995b)
[2] 10th percentile of birth-weight-for-gestational age (WHO, 1995b)
[3] Gestational age at delivery <37 weeks

affects fetal iron stores and the risk of iron deficiency in infancy (Kaneshige, 1981; Milman *et al.*, 1987; Colomer *et al.*, 1990; Daouda *et al.*, 1991). As hookworms and *Trichuris trichiura* can cause or aggravate iron deficiency, anthelminthic treatment may indirectly improve fetal iron status. In addition, maternal anthelminthic treatment may reduce the risk of fetal intestinal nematode infection for any species that may be vertically transmitted (MacLeod, 1988).

9.1.2 Aims and objectives

The aim of the work described in this chapter was to follow the birth outcomes of the women in terms of neonatal weight, length, haemoglobin concentration and infection with intestinal nematodes, and to examine the impact of maternal haemoglobin (Hb) concentration, iron stores and intestinal nematode infections on these outcomes.

The specific objectives were:

1. To present the neonatal outcomes in terms of weight, length, gestational age, Hb concentration and infection with intestinal nematodes.
2. To examine the association between these outcomes and maternal Hb concentration, maternal serum ferritin (SF) concentration and the prevalence and intensity of intestinal nematode infections during pregnancy.

9.2 METHODS

9.2.1 Limitations of the data

The subjects in the study were carefully monitored throughout pregnancy and, following ethical guidelines drawn up by the World Health Organization (WHO, 1981), any woman with Hb <80 g/l at any stage of the study was withdrawn and treated with appropriate therapy. After the third trimester assessment, 13 women (10.4%) were withdrawn from the study due to low Hb concentration and one woman left the study area before delivery. All but one of these drop-outs had received the iron-folate placebo, and half had received the albendazole placebo (Table 9.1). These drop-outs were clearly biased with respect to intervention with the iron-folate placebo (Multiple logistic regression: iron-folate Wald=7.91, p=0.0049; albendazole Wald<0.01, p=0.93). The interpretation of the neonatal outcomes is compromised by the intervention-related drop-outs. It is possible that the women who received iron-folate placebo and remained in the study until delivery were a biased subset of the original sample. For example, this group of women may have had access to a higher quality diet or may have experienced lower malaria parasitaemia than their contemporaries.

The analysis of neonatal outcomes is also limited by the time interval between delivery and the neonatal assessment. Due to the disparate location of subjects and poor communication facilities, only 23.8% of neonates were assessed within 48 hours of delivery. Most neonates (72.4%) were assessed within the first week after delivery and all were assessed within two weeks of delivery. During the first two weeks of life, changes in neonatal body weight (Alberman, 1984; Caulfield, 1991) and Hb concentration (Stockman & Oski, 1978) can be rapid and inconsistent among neonates. For example, the feeding practices employed by the mother during the first few days may affect the extent of neonatal hydration and consequently values of body weight and Hb concentration (Alberman, 1984; Stockman & Oski, 1978). This problem was anticipated during the design of the study, and an additional postnatal assessment was included at one month postpartum to provide a more comparable analysis of neonatal outcome. However, a further 29 women were withdrawn from the study at the birth assessment due to low Hb concentration, and once these women were withdrawn from the study, few subjects could be followed-up at one month postpartum.

9.2.2 Data analysis

The impact of intervention with iron-folate supplements and albendazole treatment on neonatal outcomes was not evaluated, as it is likely that the limitations imposed by the biased drop-outs and the variable time interval between delivery and neonatal assessment distorted the intervention effects. Instead, exploratory analyses were performed to identify maternal factors associated with the neonatal outcomes. This analysis was conducted using multiple linear regression. Variables were analysed in the forward stepwise method with an entry *p*-value of 0.05 and a removal *p*-value of 0.06. The maternal factors examined included age, socio-economic status, place of residence, primigravidity, inter-birth interval, infection with intestinal nematodes (prevalence and intensity), anthropometry (weight, height, body mass index, upper arm fat area and upper arm muscle area) and haematology (Hb and SF concentration). The effect of gestational age at delivery, the sex of the infant, the season at delivery and the age of the neonate at assessment was also examined.

9.3 RESULTS

9.3.1 Neonatal weight, length, haemoglobin concentration and infection with intestinal nematodes.

Of the 111 women who remained in the study until parturition, 3 (2.7%) delivered prematurely (<37 weeks) and 6 (5.4%) delivered still-born infants (Table 9.2). Two of the preterm infants were still-born. There were no multiple births. The frequency of these adverse outcomes was too small for meaningful statistical analysis.

The baseline (first trimester) characteristics of the 105 women who delivered live infants are presented in Table 9.3. The mean (SD) weight, length and Hb concentration, gestational age at delivery, proportion of male infants and age at assessment for these neonates is shown in Table 9.4. The age at assessment and proportion of male neonates was similar in all intervention groups.

The results of the multiple linear regression analysis for the neonatal outcomes are shown in Table 9.5.

Neonatal weight increased with gestational age ($p<0.0001$), maternal weight gain between baseline and the third trimester assessment ($p=0.007$), change in maternal Hb concentration between baseline and the third trimester assessment ($p<0.001$), and was higher among male neonates ($p=0.033$) and lower among primigravidae ($p=0.016$). These variables accounted for 48.3% (adjusted R^2) of the variation in neonatal weight.

Neonatal length increased with gestational age ($p<0.001$), maternal weight gain between baseline and the third trimester assessment ($p=0.017$) and maternal height ($p=0.007$). These variables accounted for 30.0% (adjusted R^2) of the variation in neonatal length.

Gestational length was shorter among primigravidae ($p=0.004$, adjusted R^2= 6.7%) and neonatal Hb concentration decreased with the age of the neonate at assessment ($p<0.001$, adjusted R^2=13.9%). No other factors were found to be associated with gestational length or neonatal Hb concentration.

Stool samples were obtained from 101 neonates; none were found to contain intestinal nematode eggs.

9.4 DISCUSSION

There is little information on the occurrence and aetiology of adverse pregnancy outcomes in Sierra Leone. Many deliveries occur in the home and are attended by traditional birth attendants who, like the mothers themselves, are often illiterate (Edward, 1989). Adverse pregnancy outcomes, which are sometimes attributed to adulterous behaviour or supernatural forces, are rarely reported as the childless mother has no motivation to attend a clinic. Many clinics lack basic equipment such as infant weighing scales and there are limited resources to ascertain the cause of poor outcomes. It has, however, been estimated that low birth-weight affects 13% of all newborns in Sierra Leone (WHO, 1992b). Approximately 60% of these newborns are growth retarded and born at term (de Onis *et al.*, 1998a). The remaining 40%

are born prematurely, although it is likely that many also experienced subnormal intrauterine growth (de Onis *et al.*, 1998a).

Measurements of weight, length, gestational age at delivery, Hb concentration and intestinal nematode infection were obtained from neonates in Western Sierra Leone. Estimates of low birth-weight and intrauterine growth retardation could not be obtained for these neonates because they were measured up to 14 days after delivery. The mothers of the neonates received daily iron-folate supplements or iron-folate placebo and a single course of albendazole treatment or albendazole placebo after the first trimester of pregnancy. Unfortunately, it was not appropriate to evaluate the effects of intervention on the neonatal outcomes due to the limitations attached to the neonatal data. However, exploratory analyses on maternal determinants of neonatal outcomes was performed to provide a stimulus for further research. The main focus of these analyses was the relationship between the neonatal outcomes and maternal Hb concentration, SF concentration and intestinal nematode infections during pregnancy.

9.4.1 Maternal haemoglobin and serum ferritin concentration during pregnancy and neonatal outcomes

In a multiple regression model that controlled for maternal and neonatal covariates, the change in maternal Hb concentration between baseline (first trimester) and the third trimester was found to be positively associated with neonatal weight. Thus, the greater the decline in baseline maternal Hb concentration, the lower the neonatal weight. For each gram per litre decrease in baseline maternal Hb concentration, mean neonatal weight decreased by 7 g. It is feasible that the iron-folate supplements and/or albendazole treatment may have mediated these findings, given that women who received these interventions experienced less decline in baseline Hb concentration (Chapter 8). The role of iron and folate in cellular growth, metabolism and immune function (see Chapter 7) could underlie a beneficial effect of adequate iron-folate nutrition on neonatal weight. However, the findings do not support a causal relationship between maternal Hb concentration (or intervention) and neonatal weight because the change in maternal Hb concentration may be a marker of other causal processes that affects neonatal weight, such as dietary intake or placental malaria.

The findings justify further investigations in this setting to establish the efficacy of interventions to correct or control maternal anaemia during pregnancy on neonatal outcomes, particularly birth-weight. There is an absence of well-designed intervention trials in developing countries that has created uncertainty about the potential effects of iron and folate on size at birth (Hemminki & Starfield, 1978; Kramer, 1987; de Onis *et al.*, 1998b). Previous trials in these regions are few and frequently lack important design criteria such as randomisation and blinding of intervention allocation, appropriate controls and sufficient sample size. In a recent review, de Onis *et al.* (1998b) concluded that the available evidence from adequately designed randomised clinical trials does not support a beneficial effect of iron supplements for the prevention of intrauterine growth retardation. However, this conclusion was based on the results of two randomised clinical trials conducted in well-nourished women from developed countries (Dawson & McGanity, 1987; Hemminki & Rimpela 1991). The effects of supplements are more likely to be observed in developing countries where the prevalence of iron and folate deficiency is highest. The lack of information from developing countries may reflect the ethical barriers to research in these regions, where mass supplementation of pregnant women is recommended (ACC/SCN, 1991).

9.4.2 Maternal intestinal nematode infection during pregnancy and neonatal outcomes

Maternal intestinal nematode infection was not found to be associated with any of the neonatal outcomes examined. The efficacy of albendazole treatment on neonatal outcomes has not to my knowledge been demonstrated, but there have been several non-intervention studies. As described in Chapter 7 (Section 7.4), the nutritional effects of infection are a function of the intensity of infection, which determines morbidity (Stephenson, 1987b; Durnin, 1993; Nesheim, 1993), and the initial nutritional status of the host. A study in Guatemala indicated that up to 10% of IUGR rates may be attributed to parasitic infection, but only among chronically malnourished women (defined as maternal height <1.47 m) (Villar *et al.*, 1989a). The lack of relationship between infection and neonatal outcomes in the present study and in studies among Southeast Asian pregnant women (D'Alauro *et al.*, 1985; Roberts *et al.*, 1985; Saowakontha *et al.*, 1992) may be explained by low intensity maternal infections relative to maternal nutritional status.

The case for routine antenatal anthelminthic treatment in Sierra Leone would be strengthened in the light of evidence to suggest a vertical route of transmission for intestinal nematode infections. If vertical transmission does occur, infants could be directly vulnerable to maternal carriage of infection. Several studies have reported evidence for congenital *Ascaris lumbricoides* infection (Chu *et al.*, 1972; da Costa-Macedo & Ray, 1990; Yu & Shen, 1990). Vertical transmission of either *T. trichiura* or *Necator americanus* is not expected as the life history of *T. trichiura* is not known to involve systemic migration and *N. americanus* does not enter a period of parental hypobiosis, a prerequisite for vertical transmission (Schad, 1991). A previous study in Sierra Leone examined the infants of 189 women for evidence of intestinal nematode infection, and the presence of *A. lumbricoides* in one mother-infant pair was reported (Wilson *et al.*, 1991). The authors suggest that the infant may have acquired this infection from his mother. However, this infant was seven months at the time of screening and the possibility that the infection was acquired from the external environment cannot be excluded.

Examination of 101 infant stool samples collected within 35 days of delivery did not provide evidence for congenital intestinal nematode infection. This result does not preclude the occurrence of vertical transmission of *A. lumbricoides* infection in this cohort of pregnant women or in Sierra Leone. A congenital infection may have escaped diagnosis if (1) the duration of the fetal/infant infection was less than the prepatent period of the nematode (2) the development of adult worms is delayed in the fetus or infant or is suppressed by passive immunity from the mother (MacLeod, 1988). The number of neonatal stool samples was small, and the reduction in the prevalence and intensities of maternal infection due to intervention with albendazole may have reduced the risk of vertical transmission (MacLeod, 1988). The follow-up period was insufficient to examine the possibility of transmammary *A. lumbricoides* transmission.

9.4.3 Other maternal factors and neonatal outcomes

One important component of maternal nutritional status that bears significantly on pregnancy outcome is energy balance, which is reflected in maternal weight gain during pregnancy. Maternal weight gain between baseline and the third trimester assessment was found to be associated with both neonatal weight and length. The mean effect of each kilogram of maternal weight gain on neonatal weight and length was 34 g and 0.16 cm respectively. Appropriate pregnancy weight gain is essential for infant outcomes (WHO, 1991c), and the causal effect of inadequate weight gain in undernourished women on birth-weight and intrauterine growth retardation is well established (Kramer, 1987; Prentice *et al.*, 1987; Adair & Bisgrove, 1991; Susser, 1991; Norton, 1994). Maternal height was also positively associated with neonate length. This relationship may reflect maternal genetic factors determining the fetal linear growth potential, skeletal maturity (in adolescent pregnancies) and maternal nutritional history (Adair & Bisgrove, 1991).

The mean weight of female neonates, adjusted for maternal variables, was 137 g lower than their male counterparts. The effect of the sex of the fetus on birth-weight is well known (Chamberlain, 1984). The neonates of primigravidae were delivered on average one week earlier than neonates of multigravidae. These neonates were also 226 g lighter, after adjustment for other variables including gestational age. Infant birth-weights are usually lower among primigravidae, particularly in developing countries where women may not reach full growth before their first pregnancy (Reinhardt, 1980). In malaria endemic areas, increased low birth-weight among primigravidae has been attributed to placental malaria infection. Pregnant women with infected placentas tend to deliver lighter babies (Brabin, 1983; Bruce-

Chwatt, 1983; McGregor, 1984). Low birth-weight due to malaria is more common among primigravidae who are most susceptible to infection (Brabin, 1983; McGregor, 1984; Greenwood *et al.*, 1989). An observational study in Segbwema in the Eastern Province of Sierra Leone, showed that the difference in the mean birth-weights of primigravidae and multigravidae increased with the rise in the transmission of malaria from 60 g in March to 380 g in July (Aitken, 1990).

Studies in some West African countries have shown that season has a marked effect on birth-weight. In the Gambia, the fall in mean birth-weight during the rains is attributed to a reduction in the availability of food, increased agriculture-related physical activity and increased transmission of malaria (Roberts *et al.*, 1982; Prentice *et al.*, 1983; Prentice *et al.*, 1987; Lawrence & Whitehead, 1988; Greenwood *et al.*, 1989; Ceesay *et al.*, 1997). Dietary supplements have been shown to have a positive effective on birth-weight in the Gambia if given in the second or third trimester of pregnancy during the wet season, when the women would otherwise have been in negative energy balance (Prentice *et al.*, 1983; Ceesay *et al.*, 1997; Prentice *et al.*, 1987). A seasonal excess of low birth-weight during the wet season has also been reported from Sierra Leone (Aitken, 1990). There are no significant seasonal food shortages in Western Sierra Leone, and it is suggested that an increase in the amount of agricultural work performed by pregnant women and the increased transmission of malaria during the rains probably account for the seasonal trends (Aitken, 1990). Data on the activity patterns of pregnant women in Sierra Leone are not available. However, it is common to see women participating in farm work during pregnancy, even in the third trimester, although they tend to perform lighter tasks (Aitken, 1990; personal observations). The incidence of malaria increases during the rains in Sierra Leone (Aitken, 1990), and the prevalence of placental malaria infection has been shown to peak in the early wet season in Freetown (Blacklock & Gordon, 1925). There was no effect of season at delivery on the weight of the neonates from Western Sierra Leone after adjustment for intervention, gestational age at assessment, primigravidae and sex of the infant. It is possible that the seasonal variation in neonatal weight is represented in the multiple linear regression model by maternal weight gain (which reflects energy balance) and primigravidae (which may reflect the effects of malaria).

Maternal Hb and SF concentration were not found to be significantly associated with neonatal Hb concentration. Most studies have indicated that fetal Hb concentration is protected from low maternal Hb levels, unless maternal anaemia is very severe (Viteri, 1994). The negative relationship between neonatal Hb concentration and the age of the neonate at assessment reflects the physiological decline in Hb concentration that occurs over the first 4-10 weeks of life in normal neonates (Stockman & Oski, 1978). The decline, which is known as 'physiological anaemia of infancy', results from a decrease in red cell mass, and to a lesser extent haemodilution. It is not associated with any disability, but is a necessary adaptation that leads to the establishment of a haematopoietic equilibrium designed to operate at a lower level than exists *in utero* (Stockman & Oski, 1978).

Over 80% of the variation in neonatal Hb concentration was unexplained by the age of the neonate. The manner in which the birth attendant delivers the infant may account for some of this variation. For example, an infant may be deprived of placental blood if he/she is held above the placenta immediately after birth (which can cause blood to transfuse from the infant back to the placenta) and if the cord is cut immediately after delivery. In Sierra Leone, there is a national programme to train traditional birth attendants and improve delivery procedures, but many pregnant women are still attended by untrained women, including 29% of the pregnant women in this study.

9.4.4 Implications of findings

In developing countries, growth-retarded or preterm infants face a disadvantaged future. Impairments in fetal growth are linked with postnatal growth retardation, neurological deficits, impaired immunocompetence and increased susceptibility to infections in infancy and childhood (Westwood *et al.*, 1983; Ashworth, 1998; Ferro-Luzzi *et al.*, 1998). Low birth-weight, and in particular fetal growth retardation, is central to the intergenerational cycle of poor growth, disease, malnutrition and poverty (de Onis *et al.*, 1998b). It is therefore essential to determine the important aetiological factors that contribute to low birth-weight in these regions, so that appropriate interventions can be implemented.

The aetiology of neonatal birth-weight is complex and may involve multiple nutritional and non-nutritional factors, many of which were not considered in the present study, such as pre-pregnancy nutritional status, hypertension and placental malaria (Chamberlain, 1984; Prada & Tsang, 1998). Therefore the analysis should be regarded as exploratory. The findings justify further investigations to establish the role of maternal anaemia in the aetiology of low birth-weight in these communities. This research is urgently needed, as anaemia affects a high proportion of pregnant women, and simple low-cost interventions with iron-folate supplements and albendazole treatment could potentially reduce the incidence of low birth-weight and its sequelae.

The direct relationship between maternal anaemia and neonatal outcomes can only be shown by measuring the effect on birth-weight after interventions to control anaemia during pregnancy. These interventions may include iron-folate supplements, albendazole treatment and malaria prophylaxis in primigravidae. Further studies should also examine iron status in the neonate as improvements in maternal iron status through iron supplementation during pregnancy have been shown to improve infant iron stores and reduce the risk of iron deficiency during infancy (Milman *et al.*, 1991; Preziosi *et al.*, 1997). Infants who are larger as a result of maternal iron supplementation may also have a greater Hb mass. There is mounting evidence to indicate that infantile iron-deficiency anaemia may cause behavioural and developmental disturbances in infancy and childhood, with implications on learning capacity and school performance (Haas & Fairchild, 1989; Parks & Wharton, 1989; Viteri, 1994). Arguments for intervention with both iron-folate supplements and albendazole treatment during pregnancy will be greatly supported if the merits of these interventions for the fetus as well as mother are more clearly defined.

9.5 SUMMARY

1. The outcomes are summarised for 105 neonates born to women in Western Sierra Leone who received daily iron-folate supplements or iron-folate placebo and a single course of albendazole treatment or albendazole placebo after the first trimester of pregnancy. The high drop-out rate among women who received iron-folate placebos precluded analysis on the impact of intervention on neonatal outcomes.
2. Neonatal weight increased with gestational age, maternal weight gain between baseline and the third trimester assessment, the change in maternal Hb concentration between baseline and the third trimester assessment, and was higher among male neonates and lower among primigravidae. The relationship between maternal Hb concentration and neonatal weight justifies further investigations to establish the role of maternal anaemia in the aetiology of low birth-weight in these communities.
3. Neonate length increased with gestational age, maternal weight gain between baseline and the third trimester assessment and maternal height.
4. Gestational length was shorter among primigravidae.
5. Neonatal Hb concentration decreased with the age of the neonate at assessment.
6. There was no evidence for the vertical transmission of *A. lumbricoides*, *N. americanus* or *T. trichiura*.

Table 9.1 Reasons for drop-outs from the study at the third trimester assessment.

Reason for drop-outs	Intervention group†				Total (n=125)
	FeA (n=32)	FeP_A (n=35)	$P_{Fe}A$ n=29)	$P_{Fe}P_A$ (n=29)	
Low haemoglobin concentration (<80 g/l)	1	0	5	7	13
Left area	0	0	1	0	1
Total	1	0	6	7	14

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 9.2 Pregnancy outcomes of women in Western Sierra Leone following intervention during pregnancy with iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo.

Pregnancy outcome		Intervention group†				Total (*n*=111)
		FeA (*n*=31)	FeP_A (*n*=35)	$P_{Fe}A$ (*n*=23)	$P_{Fe}P_A$ (*n*=22)	
Gestational age at delivery (weeks)	mean (SD)	39.8 (1.2)	39.3 (1.7)	40.2 (1.2)	40.1 (1.5)	39.8 (1.5)
Preterm deliveries (gestation <37 weeks)	frequency (%)	1 (3.2)	2 (5.7)	0 (0.0)	0 (0.0)	3 (2.7)
Still births	frequency (%)	2 (6.5)	3 (8.6)	1 (4.3)	0 (0.0)	6 (5.4)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 9.3 Baseline (first trimester) social, demographic, reproductive, parasitic, anthropometric and haematologic characteristics of pregnant women in Western Sierra Leone who delivered live-born infants following intervention with iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo (*n*=105).

Characteristic			
Social, demographic and reproductive			
Maternal age (years)		mean (±SD)	25.1 (5.6)
Rural residents		frequency (%)	74 (70.5)
Low SES		frequency (%)	54 (51.4)
Primigravidae		frequency (%)	15 (14.3)
Parasitology			
A. lumbricoides	prevalence	frequency (%)	22 (21.0)
	intensity†	geometric mean (±SD)	181 (-154, +1022)
N. americanus	prevalence	frequency (%)	70 (66.7)
	intensity†	geometric mean (±SD)	173 (-137, +652)
T. trichiura	prevalence	frequency (%)	80 (76.2)
	intensity†	geometric mean (±SD)	100 (-70, +232)
Anthropometry‡			
Weight (kg)		geometric mean (±SD)	53.8 (-6.9, +8.0)
Height (cm)		geometric mean (±SD)	158.6 (5.1)
Body mass index (kg/m^2)		geometric mean (±SD)	21.4 (-2.6, +3.0)
Upper arm fat area (cm^2)		geometric mean (±SD)	9.61 (-2.73, +3.81)
Upper arm muscle area (cm^2)		geometric mean (±SD)	44.49 (-7.70, +9.31)
Haematology			
Haemoglobin (g/l)		mean (±SD)	10.85 (1.21)
Serum ferritin (μg/l)‡		geometric mean (±SD)	24.7 (-17.8, +63.8)
Season at delivery			
Early wet (May to July)		frequency (%)	42 (40.0)
Late dry (August to October)		frequency (%)	40 (38.1)
Early dry (November to January)		frequency (%)	23 (21.9)

† Intensity estimated indirectly as eggs per gram of faeces (epg); the intensity data was log-transformed, $\log_e$(epg).
‡ Data log-transformed for all variables except height.

Table 9.4 Neonatal outcomes of infants born to women in Western Sierra Leone who received iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo during pregnancy.

Neonatal outcome		Intervention group†				Total (n=105)
		FeA (*n*=29)	FeP_A (*n*=32)	$P_{Fe}A$ (*n*=22)	$P_{Fe}P_A$ (*n*=22)	
Weight (kg)	mean (SD)	3219 (446)	3049 (510)	3105 (406)	3090 (344)	3116 (439)
Length (cm)	mean (SD)	50.20 (2.24)	49.54 (2.16)	49.50 (1.69)	49.73 (1.66)	49.75 (1.99)
Haemoglobin (g/l)	mean (SD)	163.6 (16.8)	164.5 (23.9)	160.1 (26.2)	165.5 (23.7)	163.5 (22.4)
Male neonates	frequency (%)	15 (51.7)	17 (53.1)	14 (63.6)	13 (59.1)	59 (56.2)
Gestational age at delivery (weeks)	mean (SD)	39.8 (0.9)	39.3 (1.8)	40.2 (1.2)	40.1 (1.5)	39.8 (1.5)
Age at neonatal assessment (days)	mean (SD)	5.6 (3.8)	5.7 (3.9)	4.4 (3.5)	5.9 (4.1)	5.4 (3.8)

† Fe = iron-folate supplements; A = albendazole treatment; P_{Fe} = iron-folate placebo; P_A = albendazole placebo.

Table 9.5 Factors associated with the neonatal outcomes of infants born to women in Western Sierra Leone who received iron-folate supplements or iron-folate placebo and albendazole or albendazole placebo during pregnancy (*n*=105).

Neonatal outcome	Factor	Multiple linear regression		
		Coefficient B(SE)	t-value	*p*
Weight (g)	Gestational length (weeks)	187 (26)	7.33	<0.001
	Maternal weight gain (kg)†	34 (12)	2.75	0.007
	Change in maternal Hb (g/l)‡	7 (2)	3.66	<0.001
	Male neonate	137 (63)	2.16	0.033
	Primigravidity	-226 (92)	2.46	0.016
Length (cm)	Gestational length (weeks)	0.64 (0.13)	5.04	<0.001
	Maternal weight gain (kg)†	0.16 (0.07)	2.42	0.017
	Maternal height (cm)	0.09 (0.03)	2.78	0.007
Gestational length (weeks)	Primigravidity	-1.0 (0.4)	2.92	0.004
Hb§ (g/l)	Age of neonate (days)	-2.26 (0.54)	4.21	<0.001

† Change in maternal weight between baseline (first trimester) and the third trimester.
‡ Change in maternal haemoglobin concentration between baseline (first trimester) and the third trimester.
§ Haemoglobin concentration.

Chapter 10

Microparasitic infections

10.1 INTRODUCTION

10.1.1 Microparasitic infections and pregnancy

When a bacterial, protozoal or viral infection occurs during pregnancy, the outcomes for the mother, fetus and newborn may be poor (Ng & Fok, 1996). Syphilis, toxoplasmosis, cytomegalovirus (CMV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV) are important causes of adverse pregnancy outcomes in Africa (Temmerman *et al.*, 1992; De Cock *et al.*, 1994; Sahwi *et al.*, 1995; Folgosa *et al.*, 1996; McDermott *et al.*, 1996). Maternal infection may increase the risk of abortion, miscarriage, perinatal mortality, preterm delivery and intrauterine growth retardation. All these infections can be acquired congenitally, and for infants who survive the perinatal period, the long-term sequelae may lead to premature death. The HIV pandemic in sub-Saharan Africa has increased public health concern in these infections, as HIV is associated with an increase in the frequency of severe clinical symptoms of syphilis, toxoplasmosis, CMV and HBV infections (Musher *et al.*, 1990; WHO, 1991b; Schoenbaum *et al.*, 1992).

These microparasitic infections may produce specific haematological manifestations that are superimposed on the anaemia of infection (Weatherall & Wasi, 1984). Acquired toxoplasmosis has been associated with acute haemolytic anaemia in middle-aged women (Kalderon *et al.*, 1964). In immunosuppressed individuals, CMV infection may result in a chronic or fatal haemolysis (Barrett-Connor, 1972). Chronic HBV infection may lead to development of liver disease, which is associated with elevated serum ferritin concentrations (Lipschitz *et al.*, 1974). Anaemia is observed in 70-90% of patients with acquired immune deficiency syndrome (AIDS: the disease associated with HIV); possible causes include HIV infection of bone marrow stem cells, abnormal regulation of haematopoiesis, the presence of other tumours, secondary infections and nutritional deficiencies (Fleming, 1991; Schoenbaum *et al.*, 1992).

The course of these infections is usually unaffected by pregnancy (Ingall & Musher, 1983; Junge & Deinhardt, 1985; Stagno *et al.*, 1986; Stray-Pederson, 1993; Minkoff, 1998), and asymptomatic carriage is most frequent in the mother (Spence, 1988; Evans, 1992; Ault & Faro, 1993; Gilbert, 1993). Symptoms of infection, when they occur, tend to be non-specific and misdiagnosis is common where there is reliance on clinical diagnosis. Consequently, serological analysis for immunological markers of infection is necessary to confirm the diagnosis of maternal infection.

Sero-epidemiological studies facilitate the development of appropriate intervention strategies by determining the sero-prevalence of infection and/or immunity to infection, and by identifying high risk groups who may benefit from preventative or therapeutic intervention.

Although syphilis, toxoplasmosis, CMV, HBV and HIV are ubiquitous, not all sectors of a population are at equal risk of infection. Social, demographic and cultural factors influence infection status by determining the degree of exposure to infection. Understanding the routes of transmission of these infections can help identify risky behaviours. Transmission of syphilis, CMV, HBV and HIV may occur through contact with infected bodily secretions, including blood and saliva. These infections are usually acquired during sexual intercourse and are associated with sexually promiscuous groups (D'Costa *et al.*, 1985). The use of unsterilised instruments for medical procedures or cultural practices, such as circumcision and tribal scarification, may promote the transmission of HBV and HIV (Ajuwon *et al.*, 1995), whilst close contact with infected young children is an important source of maternal and congenital CMV infection (Pass *et al.*, 1987). Toxoplasmosis is acquired through contact with the faeces of feline carriers of infection or by eating under-cooked meat that is contaminated with the parasite (Frenkel & Dubey, 1972). Poor hygiene practices and cultural habits that involve eating raw or partially cooked meat promote the transmission of toxoplasmosis (Stray-Pederson, 1993). All these microparasitic infections can also be transmitted via blood transfusions where there is inadequate screening of donor blood or recipient prophylaxis (Prince *et al.*, 1971; Gotoff, 1992; Beaman *et al.*, 1995; Traumont, 1995).

(i) Toxoplasmosis

Toxoplasmosis is caused by the protozoan *Toxoplasma gondii*. The two-host life cycle involves a sexual phase in felines and an asexual phase in all other mammals and birds (Frenkel & Dubey, 1972). *In utero* infection may result in fetal wastage, intrauterine retardation and premature delivery (Frenkel, 1991). Manifestations of severe congenital infection include chorioretinitis, hydrocephalus, cerebral calcifications and mental retardation (Sabin, 1941). Milder cases are often asymptomatic at birth but almost all congenitally infected individuals develop chorioretinitis in later life (Wilson *et al.*, 1980). Previous toxoplasmosis infection, which is associated with *T. gondii* IgG antibodies, rarely recrudesces unless the woman is immunocompromised. Therefore immunity developed following a primary infection can protect against a subsequent infection (Stray-Pederson, 1993).

(ii) Syphilis

With the worldwide medical focus turned to HIV infection, there is a danger that less attention will be paid to curable sexually transmitted diseases (STDs) such as syphilis. Syphilis, a complex systemic disease caused by infection with the spirochaete *Treponema pallidum* (Tramont, 1995), continues to increase in developing countries despite readily available and inexpensive therapy, as increasing urbanization and changes in social morals lead to increased sexual promiscuity (Perine, 1991). Fetal or perinatal death occurs in up to 40% of infants with congenital syphilis, and among survivors, symptoms of late disease may include skeletal and dental abnormalities, ocular manifestations and eighth nerve deafness (Sanchez & Wendel, 1997). Mechanisms have evolved that enable *T. pallidum* to evade host immune defense, but partial humoral immunity may develop following infection (Tramont, 1995). A positive result for the *T. pallidum* haemagglutination assay (TPHA) is indicative of a past or current syphilis infection.

(iii) Cytomegalovirus

Cytomegalovirus (CMV), a member of the Herpesviridae family of viruses, is the most common congenital viral infection and is an established cause of sensioneural hearing loss, chorioretinitis, cerebral calcifications and neurodevelopmental deficits (Nelson & Demmler, 1997). The highest risk of adverse fetal outcomes is associated with primary maternal infection (Stagno *et al.*, 1982). A recurrent maternal CMV infection may occur following reactivation of the virus or reinfection (Huang *et al.*, 1980), but sero-immunity, as indicated by the presence of CMV IgG, appears to lower the risk of fetal infection and severe fetal outcomes (Schopfer *et al.*, 1978; Stagno *et al.*, 1982).

(iv) Hepatitis B virus

Hepatitis B virus (HBV) is a member of the Hepadnaviradae family of viruses. Acute HBV infection is usually resolved within six months, while chronic infection is associated with a persistent carrier state. Mother-infant transmission can occur *in utero* (Ohto *et al.*, 1987), perinatally (Hwang *et al.*, 1985) or through close contact postnatally (Beasley & Hwang, 1983). There is an increased risk of abortion, stillbirth, preterm delivery and low birth-weight infants in women with acute infection during late pregnancy (Schweitzer, 1973; Medhat *et al.*, 1993), but otherwise pregnancy outcome is usually unaffected (Pastorek *et al.*, 1988). Infants infected in early life are more likely to develop the chronic carrier state (Coursaget *et al.*, 1987; Hsieh *et al.*, 1992) and contribute to the reservoir of infection in the community by remaining lifelong carriers of infection (Okada *et al.*, 1976; Ghendon, 1990). Over 40% of these individuals die in adulthood as a result hepatitis B-related diseases (WHO, 1988), which include chronic hepatitis, liver cirrhosis and primary hepatocellular carcinoma (Iwarson, 1985; Tabor, 1985).

Active HBV infection is indicated by a seropositive status for HBsAg, the surface antigen of the hepatitis B virus. HBeAg, the envelope antigen of the hepatitis B virus, may also be present in the serum and indicates a highly infectious state most conducive to perinatal transmission (Okada *et al.*, 1976; Beasley *et al.*, 1977). The chronic state is defined as the persistence of HBsAg in the serum for more than six months.

(v) Human immunodeficiency virus

The spread of the retroviruses HIV Type 1 and 2 to the female population is alarming as 80% of women with HIV are in their peak child-bearing years and many are unaware of their infection status (Johnson, 1992). In Africa, maternal HIV is associated with intrauterine growth retardation, prematurity, fetal and perinatal mortality (Miotti *et al.*, 1992). Vertical transmission is the leading cause of paediatric HIV, and may occur *in utero*, during delivery or through breast feeding (Mok, 1992). HIV causes progressive impairment of cellular immunity (Sever, 1989) and increases susceptibility to infections and malignancies (Schoenbaum *et al.*, 1992). Congenital infection can lead to AIDS within the first 18 months of life (Mambwe Kaoma & Scott, 1992). Antibodies to HIV identify an active infection.

10.1.2 Aims and objectives

The aim of the work described in this chapter was examine the sero-prevalence and sero-epidemiology of immunological markers to syphilis, toxoplasmosis, CMV, HBV and HIV in the pregnant women, and to assess the impact of these infections with respect to the control of maternal anaemia.

The specific objectives were:

1. To obtain current data on the sero-prevalence of immunological markers of past and/or present infection with syphilis, toxoplasmosis, CMV, HBV and HIV among a sample of asymptomatic pregnant women.
2. To relate the sero-prevalence of immunological markers of infection to maternal factors, including socio-demographic, socio-economic (SES) and reproductive characteristics and first trimester haemoglobin and serum ferritin concentration.

10.2 METHODS

10.2.1 Data analysis

The relationship between the sero-prevalence of immunological markers of infection and maternal factors was assessed using univariate and multivariate techniques. Univariate analyses for sero-prevalence data included Chi-square test (χ^2) with Yate's correction for nominal data, or Fisher's exact test where expected cell sizes were less than 5 (2 x 2 contingency tables only), Chi-square test for trends (χ^2_{trend}) for ordinal data and t-tests for continuous data. The following maternal factors were examined: age, place of residence, ethnic group, marital status, socioeconomic status, gravidity, inter-birth interval, previous use of modern contraceptive methods, history of still-births or miscarriages, and first trimester haematology (haemoglobin concentration, serum ferritin concentration and the prevalence of anaemia, iron deficiency and iron-deficiency anaemia). Multiple logistic regression was employed to relate several categorical variables to the sero-prevalence of infection and to identify interactions between the variables. Variables were analysed in the forward stepwise method with an entry *p*-value of 0.05 and a removal *p*-value of 0.06. Odds ratios with 95% confidence intervals (CI) were calculated. The referent category for each categorical variable was the category with the lowest prevalence. The multivariate results are shown only for significant factors as non-significant factors were not retained in the final logistic regression model.

10.3 RESULTS

10.3.1 Study population

Of the 184 pregnant women at baseline, the sera of 179[1] were examined for antibodies/antigens to *T. gondii*, *T. pallidum*, HBV and HIV, and the sera of 139[2] were examined for antibodies to CMV. A summary of the social, demographic and reproductive characteristics of the study sub-populations is shown in Table 10.1. The subset of subjects randomly selected for analysis of CMV IgG was statistically similar to the entire study sample with respect to these variables.

10.3.2 Sero-prevalence of immunological markers to past or present microparasitic infection

The sero-prevalence of immunological markers was found to be thus: antibodies to *T. pallidum* 7.8%; *T. gondii* IgG 76.0%; CMV IgG 82.7%; antibodies to HIV 0.0%; HBsAg 11.2%; HBeAg 3.9%; anti-HBe 6.1% (Table 10.2). Seven women (3.9%) were seropositive for both HBsAg and HBeAg; these women were experiencing an acute or chronic active infection and were highly infectious. Eleven women (6.1%) were positive for HBsAg and anti-HBe positive; these women were probably chronic carriers as the period of time during which this serological profile is observed in those with acute infection is short (Koziel, 1996). A repeat serological investigation is required after six months to distinguish between the active and chronic HBV states: persistent carriage of HBsAg after this time indicates the chronic carrier state. Neither HBeAg or anti-HBe were detected in the serum of the remaining two HBsAg positive women, presumably due to very low titres, and thus their HBV status is unknown.

---

[1] The serum sample for five women was insufficient for the serological tests after an aliquot had been reserved for serum ferritin analysis.
[2] There was insufficient CMV kit reagents to test all sera.

10.3.3 Maternal factors and sero-prevalence of immunological markers to past or present microparasitic infection

The results of univariate and multivariate analyses are presented in Table 10.3.

Using univariate analysis, the sero-prevalence of *T. gondii* IgG increased significantly with maternal age group and with peri-urban residence. Multiple logistic regression indicated an interaction between maternal age group and peri-urban/rural residence. The relationship between age-group and sero-prevalence of *T. gondii* IgG was clearly different for peri-urban and rural subjects (Figure 10.1), and was investigated further by regressing maternal age group on the sero-prevalence of *T. gondii* IgG separately for peri-urban and rural subjects. Among peri-urban subjects, the odds on *T. gondii* IgG increased significantly with maternal age (p=0.018), and all subjects above 24 years were seropositive. Among rural subjects, the sero-prevalence of *T. gondii* IgG increased with maternal age, most rapidly in the third decade of life, but this increase was not significant (p=0.15).

A higher sero-prevalence of antibodies to *T. pallidum* was associated with low SES (p=0.0068). The odds on positive treponematosis serology among pregnant women of low SES was over seven times greater than those of high SES.

The sero-prevalence of CMV antibodies increased from 75.0% (n=28) among 15-19 year age group to 100.0% (n=11) among 35-40 year age group but this increase was not significant. Using univariate analysis, a higher sero-prevalence of CMV IgG was associated with the possession of at least one child and multigravidae (borderline significance). When these variables were entered into a logistic regression model, children explained most variation (p=0.029), and adding the further variables did not improve the fit significantly. The odds on positive CMV IgG serology among pregnant women with at least one child was almost three times greater than among women with no children.

Univariate analysis indicated a significantly higher HBsAg sero-prevalence among single subjects and among women who had used modern methods of contraception. When these variables were entered into a multiple logistic model, an interaction between marital status and prior use of modern methods of contraception appeared to account for all the explainable variation (p=0.0049). The interaction was caused by a comparatively higher prevalence of infection among single women who had previously used modern contraceptive methods. The sero-prevalence of HBsAg in this group was 75.0% (n=4), as compared to 9.7% (n=175) among their contemporaries. This result should be interpreted with caution due to the small number of subjects in this group.

There was no association between any microparasitic infection and a history of adverse pregnancy outcomes, first trimester haemoglobin concentration, first trimester serum ferritin concentration, or with the first trimester prevalence of anaemia, iron deficiency or iron-deficiency anaemia.

10.4 DISCUSSION

10.4.1 Sero-epidemiology of immunological markers to past or present microparasitic infection

The contribution of maternal microparasitic infections to morbidity and mortality in pregnant women and their fetuses is well established. In developing countries, these infections add to the plethora of medical and social conditions that threaten reproductive health. Poverty, ignorance and changing social morals perpetuate their transmission despite the availability of effective vaccinations and therapy and the possibility of preventative behavioural measures.

Little is understood about the sero-epidemiology of microparasitic infections in pregnant women in Sierra Leone, or the contribution of these infections to the aetiology of maternal anaemia or adverse pregnancy outcomes. A search of the medical literature over the past 30 years using computer databases (Medline and Bids Embase) did not find any published study that examined the sero-prevalence of syphilis, toxoplasmosis, CMV, HBV or HIV in pregnant women in Sierra Leone. The present study represents the first serological data on immunological markers to toxoplasmosis and CMV in Sierra Leone and adds to the scarce and unpublished data on syphilis, HBV and HIV among antenatal attendees.

(i) Toxoplasmosis

In Sierra Leone, toxoplasmosis is most commonly recognised when a patient presents with blurred vision and a diagnosis of chorioretinitis is made (McElroy, personal communication[3]). A recent study indicated that congenital toxoplasmosis is the primary cause of non-onchocercal uveitis in Sierra Leone (Ronday *et al.*, 1996). The high sero-prevalence of *T. gondii* IgG among antenatal attendees in Western Sierra Leone suggests that the environment is highly infectious for toxoplasmosis. With 76.0% immune to infection, just under one quarter of women (24.0%) were susceptible to a primary toxoplasmosis infection during the current pregnancy. Recent studies among pregnant women elsewhere in West Africa have reported sero-prevalence values ranging from 44.4% to 78.0% (Table 10.4).

In peri-urban areas, where all subjects above the age of 24 years were seropositive for *T. gondii* IgG, the risk of primary maternal infection was associated with child-bearing at an early age (≤24 years). Increasing sero-prevalence with age, also reported among pregnant women in Benin (Rodier *et al.*, 1995) and women of child bearing age in Nigeria (Olusi *et al.*,

[3] Ophthalmologist, Kissy Eye Hospital, Freetown, Sierra Leone

1996), reflects the greater opportunity for exposure to *T. gondii* with time. In rural areas, the results suggest a trend of increasing sero-prevalence with maternal age, although this was not significant. Rural areas appear less conducive to the toxoplasmosis transmission than peri-urban areas. However, the risk of primary maternal infection in the rural sub-population may remain throughout the child-bearing years, given that the sero-prevalence never reached 100% in any of the maternal age groups.

The explanation for these peri-urban/rural differences is unclear. In much of the tropics, *Toxoplasma* oocysts excreted by feline carriers in contaminated soil around the house are believed to be a major source of infection (Frenkel, 1991). Ronday *et al.* (1996) suggest that possible sources of toxoplasmosis infection in Sierra Leone are contaminated soil and ground water (presumably with the faeces of cats), as the typical diet does not include raw or undercooked meat. Contamination with cat faeces was also postulated as the source of toxoplasmosis pregnant women in Nigeria, where many households keep cats to control the rodent population (Onadeko *et al.*, 1992). In the present study, cats were not noticeably more abundant in peri-urban areas of Western Sierra Leone. However, the presence of cats is not a prerequisite for high *Toxoplasma* transmission and high transmission rates could perpetuate via congenital infection and carnivorism (Beaman *et al.*, 1995). Roast meat is frequently sold from road-side stalls in the peri-urban areas, and although meat is usually well cooked, the handling of raw meat may be an important mode of transmission. In the rural coastal areas, meat is not a common component of the diet as fish is more readily available.

(ii) Syphilis

Positive treponematosis serology indicative of past or current syphilis infection was identified in 7.8% of pregnant subjects from Western Sierra Leone. There is no antenatal data from Sierra Leone with which to compare these results. In 1996, the sero-prevalence of syphilis TPHA in blood donors (predominantly male) in Freetown was 16.8% (n=2,555) (SLRC/MOH, 1997). Comparison with data from the West African region is complicated as the rapid plasma reagin (RPR) method, which does not identify all previous infections, is frequently used to detect maternal infection. Recent findings from sero-surveys of *T. pallidum* TPHA include 2.4% among pregnant women in Benin (Rodier *et al.*, 1995) and 15.9% among pregnant women in Cameroon (Ndumbe *et al.*, 1992) (Table 10.4). Seropositive pregnant women in Sierra Leone were more likely to be of low SES, in agreement with findings from other developing countries (Behets *et al.*, 1995; Lurie *et al.*, 1995).

(iii) Cytomegalovirus

Evidence of a prior CMV infection was indicated in 82.7% of pregnant subjects from Western Sierra Leone, a slightly lower sero-prevalence than has been reported elsewhere in West Africa (Table 10.4), but nonetheless consistent with a highly infectious environment.

The high proportion of seropositive pregnant women indicates that the majority are immune to CMV during the child-bearing years and therefore the risk of primary maternal infection is low. This finding is supported by evidence from Africa, which suggests that most people are infected in early childhood (Krech & Tobin, 1981). However, the occurrence of primary maternal infection during pregnancy is not excluded as the proportion of seronegative subjects decreased from 25% in the 15-19 year group to 0% in the 35-40 year group, which suggests that seroconversion may take place during the child-bearing years. This decrease was not significant, but this may be a reflection of the sample size. In any case, pre-immune individuals are not protected from a recurrent infection resulting from reactivation of a latent infection or reinfection (Huang *et al.*, 1980). Indeed, in populations with a high seropositivity rate, the majority of congenital infections result from recurrent maternal infection (Schopfer *et al.*, 1978; Stagno *et al.*, 1982), and although the fetal effects tend to be less severe (Stagno *et al.*, 1977), these congenital infections can also be symptomatic (Ahlfors *et al.*, 1981). Of 25 cases of congenital CMV infection in the Gambia (n=178), 24 occured as the result of a secondary maternal CMV infection (Bello & Whittle, 1991). Moreover, two of these congenitally infected infants had evidence of motor neuron disease at birth.

Little is understood about the epidemiology of CMV in Africa. The predominant source of childhood infections is probably close contact with infected siblings in crowded environments (Bello & Whittle, 1991). In developed countries, seropositivity is higher among women of low SES whose environmental surrounds, such as crowding, lack of sanitation and increased contact with young children, are believed to promote transmission (Stagno *et al.*, 1982; Chandler, *et al.*, 1985; Griffiths *et al.*, 1985; Stagno *et al.*, 1986). The present study did not find a higher sero-prevalence of CMV IgG among women of low SES. However, high SES is unlikely to preclude exposure to CMV in the Sierra Leonean society where the extended family is a constant source of contact with young children. A higher sero-prevalence was found among multigravidae and among women with at least one child, although the latter characteristic appeared to account for most of the explainable variation. Therefore, increased CMV seropositivity among pregnant women is associated with the act of commencing child-bearing and the possession of a child. Child-bearing is obviously associated with sexual activity and both sexual activity and exposure to young children are regarded as the predominant sources of maternal CMV (Handsfield *et al.*, 1985; Demmler *et al.*, 1986; Pass *et al.*, 1987).

(iv) Hepatitis B virus

HBV infection is a public health problem that has received little attention in Sierra Leone. The maternal sero-prevalence of HBsAg and HBsAg in the pregnant subjects from Sierra Leone was 11.3% and 3.1% respectively. These results support the inclusion of Sierra

Leone within the geographical region of high endemicity for HBV infection, which is defined as HBsAg carrier rate equal to or exceeding 8% (Maynard *et al.*, 1989). Although we did not test for past markers among the antenatal study group, studies in Sierra Leone and other African countries have reported a sero-prevalence in excess of 70% in highly endemic areas where the sero-prevalence of HBsAg exceeds 10% (Kiire, 1990; Kiire, 1996; Hodges *et al.*, 1998).

Published data on the sero-prevalence of HBV in Sierra Leone is scarce. Previous surveys have been among specific population groups, none of which has included pregnant women. The sero-prevalence of HBsAg was 14.4% (n=2555) among blood donors in 1996 (SLRC/MOH, 1997) and 36% (n=684) among patients undergoing laboratory investigation for suspected hepatitis (Wurie *et al.*, 1996). Comparison with recent studies in West Africa indicates that the sero-prevalence of HBsAg in Sierra Leone falls within the range of values reported among pregnant women and women of child-bearing age in the region (6.4 to 25.3%) (Table 10.4). However, HBeAg positive women accounted for a higher proportion of maternal infections in Sierra Leone than observed in these studies. In the present study, 35.0% (n=20) of HBV infected mothers were seropositive for HBeAg, compared to 5.3% (n=38) of pregnant women in Cameroon (Ndumbe *et al.*, 1992), 10.3% (n=39) in Senegal (Roingeard *et al.*, 1993), 11.5% (n=26) in Nigeria (Harry *et al.*, 1994a) and 18.2% (n=44) in Ghana (Acquaye *et al.*, 1994). As maternal carriage of HBeAg is strongly correlated with the incidence of perinatal infection (Okada *et al.*, 1976; Beasley *et al.*, 1977) and postnatal infection (Beasley & Hwang, 1983), the elevated level of HBeAg carriage in Sierra Leone may be coupled with a higher rate of mother-infant transmission. The proportion of HBV pregnant women with HBeAg varies geographically and it has been suggested that maternal expression of HBeAg is genetically determined (Ghendon, 1987). In East Asia, where HBeAg positivity rates among pregnant HBV carriers may reach 40% (Kiire, 1996), the incidence of perinatal HBV infection is higher than in Africa where this rate is typically below 20% (Kew, 1996). In Sierra Leone, the maternal carriage of HBeAg is similar to values observed in East Asia, but further studies with larger numbers of HBsAg positive pregnant women are required to confirm this observation and assess its significance in terms of its contribution to perinatal and/or postnatal mother-infant HBV transmission.

Single women who had used modern methods of contraception were more likely to be seropositive for HBsAg than their contemporaries. It is not known whether these women acquired HBV infection in childhood or after becoming sexually active. Further studies are required before conclusions can be drawn as to whether women in this category are at increased risk of acquiring HBV infection during their childbearing years.

(v) Human immunodeficiency virus

The apparent absence of HIV-1 and HIV-2 among the study population from Western Sierra Leone is encouraging. Whether these results are typical of the country as a whole is difficult to ascertain as surveys are few and often lack important information such as the sample size, characteristics of the individuals screened and the methods employed. The National AIDS Control Program in Sierra Leone reported 661 cases of HIV (*n*=16,858) since its inception in 1987 and April 1996 (I.Wurie, personal communication[4]), and HIV positivity among blood donors was 0.8% (*n*=2,555) in 1996 (SLRC/MOH, 1997). There is no published data on the sero-prevalence of HIV among the antenatal population, although the STD clinic at Connaught Hospital (Freetown) reported an increase of HIV sero-prevalence among pregnant women from 0.5% in 1992 to 5.5% in 1996. Unfortunately the sample sizes for this data were not available and it is unclear whether these pregnant women were STD patients, thereby representing a select population. Although there are obvious flaws in the available data, they do appear to indicate that the lack of HIV infection among our antenatal population could be an exception rather than the norm. Rodier *et al.* (1995) also reported an absence of HIV among an antenatal population (*n*=211) in Benin, but results from other countries in the region indicate that the HIV problem is increasing among pregnant women in West Africa (Harry *et al.*, 1994b; Obisesan *et al.*, 1997), with sero-prevalences ranging from 0.8% (*n*=12,498) in Senegal (Diouf *et al.*, 1996) to 13% (*n*=1000) in Cote d'Ivoire (Brattegaard *et al.*, 1993) (Table 10.4).

The sero-prevalence of *T. pallidum* antibodies in Sierra Leone suggests a high incidence of risky sexual activity and although the sero-prevalence of HIV in the population was zero at the time of the study, there may be cause for concern in the near future. An association between syphilis and HIV infection has been demonstrated in sub-Saharan Africa (Pepin *et al.*, 1991; Cossa, 1994; Leroy *et al.*, 1995), and may reflect common behavioural risk factors or an increased susceptibility to HIV transmission associated with genital ulcerative infection (Greenblatt *et al.*, 1988; Mertens *et al.*, 1990). Syphilis infection during HIV infection is particularly severe, with an increase in the frequency of early neurosyphilis among patients (Musher *et al.*, 1990). In the event of increasing HIV seropositivity among the antenatal population in Sierra Leone, the high sero-prevalence of toxoplasmosis and CMV, which are opportunistic diseases of HIV, is also of grave concern as the environment is clearly highly infectious for these infections. Latent (chronic) toxoplasmosis and CMV infection is frequently reactivated in HIV infection, and infants of these patients may be congenitally infected at birth (Schoenbaum *et al.*, 1992). Cerebral toxoplasmosis, pneumonitis and chorioretinitis are frequently observed in HIV infected individuals with positive toxoplasmosis

---

[4] Director, Ramsy Laboratories, Freetown, Sierra Leone

serology (Beaman *et al.*, 1995) and retinitis, pneumonitis and encephalitis are more common in HIV infected individuals with CMV (Schoenbaum *et al.*, 1992). HIV may cause a poor immune response to HBV vaccine, promote reactivation of latent HBV infection, and increase the likelihood of the chronic clinical course (WHO, 1991b).

10.4.2 Implications for the control of microparasitic infections and anaemia during pregnancy

In Sierra Leone, managerial, budgetary and logistic constraints limit the implementation of a national antenatal screening programme. There are private diagnostic facilties in the major cities, but the number of individuals seeking screening is small. Access is hindered not only by distance and the cost of screening relative to per capital income, but also by the lack of referals from antenatal care providers who are unaware of the risks of vertical transmission, and the opportunity for protection. Pregnant women should be informed of the risks of maternal and fetal infections and the possibilities for preventative and therapeutic measures.

Antenatal screening is only appropriate if effective intervention or preventative measures are available and if a high incidence of fetal infection is indicated in the population (Gilbert, 1993). Screening is relevant for syphilis and toxoplasmosis as maternal infections are curable and treatment significantly reduces the risk of fetal infection and serious congenital defects (Ault & Faro, 1993). Rapid serological tests have been developed for syphilis, and women can be both tested and treated at their first visit to an antenatal clinic (Jenniskens, 1995). However, further studies are required to determine the incidence of congenital infection with syphilis and toxoplasmosis before any recommendations for antenatal screening in Sierra Leone can be justified. Antenatal CMV screening is not recommended as the sero-prevalence of CMV IgG is clearly very high, and thus few women are vulnerable to a primary infection during pregnancy. In addition, no antiviral agent with activity against CMV has been proven to be safe for administration to pregnant women, and immunotherapy is not commercially available (Whitley & Kimberlin, 1997). Furthermore, fetal damage is unpredictable and thereby complicates a decision for termination even when maternal infection is indicated (Pass *et al.*, 1987).

The high maternal carriage of HBV infection in Sierra Leone demands immediate action from antenatal care providers as HBV carriage in infancy is avoidable. Effective HBV vaccines are available, and can protect infants from acquiring the infection during the period of life when the likelihood of developing the chronic carrier state is greatest (Maynard *et al.*, 1989). In highly endemic areas, such as Sierra Leone, the World Health Organization recommends that mass neonatal vaccination be included in the Extended Programme on

Immunisation (EPI) of these countries, as the most cost effective means of controlling HBV infection and its sequelae (WHO, 1988). Although the HBV vaccine has been successfully incorporated into the EPI of several African countries (Hall *et al.*, 1989; Fortuin *et al.*, 1993; Kiire, 1996), it is unlikely that HBV vaccination will be included in the EPI of Sierra Leone unless donor partners are found (WHO, 1994). Until such a scheme becomes available, pregnant women should be made aware of the risks of perinatal HBV infection and the availability of immunotherapy for their infants. Antenatal screening is unnecessary, as infants are at a high risk of acquiring infection in early life, irrespective of maternal infection status (Maynard *et al.*, 1989; Kiire, 1996). Indeed, the most common cause of infant and childhood infection in West Africa is probably close contact with infected siblings or household contacts after six months of age (Marinier *et al.*, 1985; Martinson *et al.*, 1998).

Even though none of the women were found to be infected with HIV, this virus should not be ignored. There are indications from other African countries that the prevalence of infection is increasing among the antenatal population (Harry *et al.*, 1994b; Obisesan *et al.*, 1997), particularly in association with risky sexual activity. It has also been suggested that low-intensity wars can favour the transmission of HIV and other STDs by disrupting socio-economic status (Cossa *et al.*, 1993). The ongoing civil war in Sierra Leone has displaced a large number of women, who may be at increased risk of sexual exploitation. HIV-infected women who can afford interventions, such as antiretroviral therapy and caesarean section delivery, can markedly reduce the risk of vertical transmission (Andiman, 1998).

The reduction in exposure to infectious agents through changes in behaviour is probably the most cost effective means of preventing maternal infection, and regardless of whether screening is conducted, culturally sensitive health education should become part of standard antenatal care. Previous studies have shown that behavioural intervention, including responsible sexual practices and hygienic measures, is effective in preventing maternal infection with syphilis (Hira *et al.*, 1990), toxoplasmosis (Foulon, 1992) and CMV (Adler *et al.*, 1996) during pregnancy. Certain cultural and social influences need to be addressed if such measures are to be effective in Sierra Leone. Among a number of ethnic groups, sexual intercourse during pregnancy is encouraged as abstinence is believed to cause complications during delivery[5]. Whilst adultery among women is strongly discouraged, widespread polygamy and more lenient code of sexual conduct among males places pregnant women at risk of contracting STDs. The screening and treatment of partners is difficult in the socio-cultural context of African women, as some women risk being accused of adulterous behaviour or subjected to physical abuse (Jenniskens, 1995). Recommendations to avoid

[5] Information obtained from members of the Temne ethnic group, whilst conducting the ethnographical study (Chapter 11).

CMV infection by reducing contact with the saliva and urine of young children (Leading Article, 1989) would also be difficult to implement. Not only would these precautions demand a huge behavioural change but it is unlikely that pregnant women will be excused from domestic duties that involve close contact with young children.

In the present study, maternal iron deficiency and anaemia in the first trimester of pregnancy were not associated with markers of past or present infection with syphilis, markers of past infection with toxoplasmosis or CMV, or markers of present infection with HBV. These findings may reflect the time interval since infection with toxoplasmosis or CMV, and the relatively small number of women who were infected with syphilis or HBV infections. The duration of most infection episodes with microparasites is short, and the depressant effect on erythropoiesis may be insufficient to effect a significant haematological response (Barrett-Connor, 1972).

The haematological significance of microparasitic infections during pregnancy is not limited to their impact on maternal iron deficiency and anaemia. These infections also have important implications on the control of severe maternal anaemia. Syphilis, toxoplasmosis, CMV, HBV and HIV can all be transmitted by contaminated syringes or by transfusion of blood from an infected donor (Prince *et al.*, 1971; Gotoff, 1992; Beaman *et al.*, 1995; Tramont, 1995). Adequate control of severe maternal anaemia during pregnancy will reduce the number of women requiring parenteral iron treatment or blood transfusions. This alone will lower the incidence of these maternal infections in settings where unsterilised syringes are re-used for multiple patients and where there is inadequate screening of donor blood or recipient prophylaxis (Fleming, 1989; Fleming, 1991; WHO, 1993). In developing countries, such as Sierra Leone, congenital microparasitic infections increase the burden on the health services that can least afford effective intervention strategies.

10.5 SUMMARY

1. A cross-sectional sero-survey was conducted among pregnant women in Western Sierra Leone to determine the sero-prevalence, and examine the sero-epidemiology of past and/or present immunological markers of infection with syphilis, toxoplasmosis, CMV, HBV and HIV.
2. The sero-prevalence of immunological markers was found to be thus: antibodies to *T. pallidum* 7.8%; *T. gondii* IgG 76.0%; CMV IgG 82.7%; antibodies to HIV 0.0%; HBsAg 11.3%; HBeAg 3.9%; anti-HBe 6.1%.

3. The sero-prevalence of *T. gondii* IgG increased significantly with age among peri-urban residents. A higher sero-prevalence of CMV IgG was associated with pregnant women with one or more children. The odds on past or present infection with syphilis was higher among those of low SES. Single women who had used modern methods of contraception were more likely to be seropositive for HBsAg.
4. Maternal iron deficiency and anaemia in the first trimester of pregnancy was not associated with any of the examined markers of past or present microparasitic infection.
5. In the absence of a national antenatal screening programme, pregnant women should be informed of the risks of these maternal and fetal microparasitic infections and the possibilities for preventative and therapeutic measures. Culturally appropriate health education should be integrated into standard antenatal care.
6. The control of maternal anaemia during pregnancy will reduce the number of women requiring parenteral iron or blood transfusions, which are potential sources of these microparasitic infections in settings where unsterilised needles are reused, or where there is inadequate screening of donor blood or recipient prophylaxis.
7. The integration of the HBV vaccine into the EPI in Sierra Leone is recommended.

Table 10.1 Baseline demographic, social and reproductive characteristics of the study sub-population screened for immunological markers of past and/or present infection with syphilis, toxoplasmosis, cytomegalovirus (CMV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

Characteristic			Maternal infection	
			CMV (*n*=139)	Syphilis, toxoplasmosis, HBV and HIV (*n*=179)
Age (years)		mean (SD)	25.1 (5.90)	25.0 (5.77)
Residence	rural	frequency (%)	91 (65.5)	114 (63.7)
	urban	frequency (%)	48 (34.5)	65 (36.3)
Ethnic group	Temne	frequency (%)	83 (59.7)	107 (59.8)
	Susu	frequency (%)	26 (18.7)	31 (17.3)
	Mende	frequency (%)	14 (10.1)	19 (10.6)
	other	frequency (%)	16 (11.5)	22 (12.3)
Marital status	single	frequency (%)	11 (7.9)	15 (8.4)
	married	frequency (%)	125 (89.9)	161 (89.9)
	widowed	frequency (%)	3 (2.2)	3 (1.7)
Education	none	frequency (%)	87 (62.6)	115 (64.2)
	primary	frequency (%)	22 (15.8)	25 (14.0)
	secondary/ tertiary	frequency (%)	30 (21.6)	39 (21.8)
Socio-economic status	low	frequency (%)	68 (48.9)	85 (47.5)
Gravidity		median (QR)	4 (2, 6)	3 (2, 6)
Primigravidae		frequency (%)	22 (15.8)	29 (16.2)
Grandmultigravidae		frequency (%)	39 (28.1)	45 (25.1)
Number of children[‡]		median (QR)	2 (1, 3)	2 (1, 3)
Months since previous delivery[‡]		median (QR)	26 (22.0, 42.5)	27.0 (22.0, 36.5)
Use of modern contraceptives		frequency (%)	16 (11.5)	21 (11.7)
Previous miscarriage[‡]		frequency (%)	32 (23.0)	43 (28.7)
Previous still birth[‡]		frequency (%)	11 (7.9)	13 (8.7)
Previous child death[‡]		frequency (%)	52 (37.4)	64 (42.7)

† Among married subjects.
‡ Among multigravidae only.

Table 10.2 Sero-prevalence of *Toxoplasma gondii* IgG, *Treponema pallidum* TPHA, cytomegalovirus (CMV) IgG, hepatitis B virus surface antigen (HBsAg), hepatitis B virus envelope antigen (HBeAg), antibodies to hepatitis B virus envelope antigen (anti-HBe) and antibodies to human immunodeficiency virus (HIV) among pregnant women in Western Sierra Leone.

Infection	*n*	Sero-prevalence (%) (95% CI)
T. pallidum TPHA	179	7.8 (3.9, 11.8)
T. gondii IgG	179	76.0 (69.7, 82.3)
CMV IgG	139	82.7 (76.3, 89.1)
HBsAg	179	11.2 (6.5, 15.8)
HBeAg		3.9†
anti-HBe		6.1 (2.5, 9.7)
Antibodies to HIV	179	0.0†

† Not reasonable to calculate confidence interval as the assumptions for the approximation of the Binomial distribution to the Normal distribution are not met if the sero-prevalence <5%.

Table 10.3 Risk factors for a sero-positive status for *Toxoplasma gondii* IgG, *Treponema pallidum* TPHA, cytomegalovirus (CMV) IgG and hepatitis B virus surface antigen (HBsAg) among pregnant women in Western Sierra Leone.

Infection	Univariate analysis†						Multivariate analysis‡			
	Risk Factor	*n*	Frequency (%)	Odds ratio§ (95% CI)	χ^2	*p*	Risk Factor	Odds ratio§ (95% CI)	Wald	*p*
T. gondii IgG	Age (years)						Increasing age group			
	15-19	37	25 (67.6)	1.41 (1.03, 1.92)††	4.61‡‡	0.032	peri-urban	3.44 (1.24, 9.56)††	5.61	0.018
	20-24	51	37 (72.6)				rural	1.30 (0.91, 1.86)††	2.11	0.15
	25-29	52	40 (76.9)							
	30-34	26	22 (84.6)							
	35-40	13	12 (92.3)							
	Residence									
	peri-urban	65	56 (86.2)	2.64 (1.18, 5.94)	4.95	0.026				
	rural	114	80 (70.2)							
T. pallidum TPHA	Socio-economic status									
	low	85	12 (14.1)	7.55 (1.64, 34.81)	7.32	0.0068				
	high	94	2 (2.1)							

† Chi-square tests of independence.
‡ Multiple logistic regression.
§ Reference category is category with lowest frequency.
†† Odds increase per unit increase in age group.
‡‡ Chi-square for trends.

Table 10.3 Risk factors for a sero-positive status for *Toxoplasma gondii* IgG, *Treponema pallidum* TPHA, cytomegalovirus (CMV) IgG and hepatitis B virus surface antigen (HBsAg) among pregnant women in Western Sierra Leone (continued).

Infection	Univariate analysis†						Multivariate analysis‡			
	Risk Factor	*n*	Frequency (%)	Odds ratio§ (95% CI)	χ^2	*p*	Risk Factor	Odds ratio§ (95% CI)	Wal d	*p*
CMV	Number of children						Number of children			
IgG	≥1	101	88 (87.1)	2.76 (1.11, 6.86)	3.93	0.047	≥1	2.76 (1.11, 6.86)	4.76	0.029
	0	38	27 (71.1)							
	Gravidity									
	multigravidae	117	100 (85.5)	2.75 (0.98, 7.72)		0.065††				
	primigravidae	22	15 (68.2)							
HBsAg	Marital status									
	single	15	5 (33.3)	4.97 (1.50, 16.45)		0.015††	Marital status*contraception (single, contraceptive users)	27.88 (2.75, 283.13)	7.92	0.0049
	married/widowed	164	15 (9.1)							
	Contraception‡‡									
	used	21	6 (28.6)	4.11 (1.38, 12.29)		0.017††				
	never used	158	14 (8.9)							

† Chi-square tests of independence.
‡ Multiple logistic regression.
§ Reference category is category with lowest frequency.
†† Fisher's Exact Test.
‡‡ Modern methods of contraception, including oral contraceptives, condoms and intra-uterine devices.

Table 10.4 Recent sero-surveys for *Toxoplasma gondii* IgG, *Treponema pallidum* TPHA, cytomegalovirus (CMV) IgG, hepatitis B virus surface antigen (HBsAg), hepatitis B virus envelope antigen (HBeAg) and antibodies to human immunodeficiency virus (HIV) among pregnant women and women of child-bearing age in West Africa.

Infection	Country	*n*	Sero-prevalence (%)	Reference
T. gondii IgG	Benin	211	54.0	Rodier *et al.*, 1995
	Cameroon	192	77.1	Ndumbe *et al.*, 1992
	Nigeria	352	78.0	Onadeko *et al.*, 1995
	Senegal	404	44.4	Diallo *et al.*, 1996
T. pallidum TPHA	Benin	211	2.4	Rodier *et al.*, 1995
	Cameroon	544	15.9	Ndumbe *et al.*, 1992
CMV IgG	Benin	211	97.2	Rodier *et al.*, 1995
	Gambia	178	87.5	Bello & Whittle, 1991
HBV HBsAg/HBeAg	Cameroon	150	25.3/1.3	Ndumbe *et al.*, 1992
	Ghana	692	6.4/1.2	Acquaye *et al.*, 1994
	Nigeria	224	11.6/1.4	Harry *et al.*, 1994a
	Senegal	284	13.7/1.4	Roingeard *et al.*, 1993
Antibodies to HIV	Benin	211	0.0	Rodier *et al.*, 1995
	Burkino Faso	1,294	8.0	Sangare *et al.*, 1997
	Cote d'Ivoire	1,000	13.0	Brattegaard *et al.*, 1993
	Senegal	12,498	0.8	Diouf *et al.*, 1996

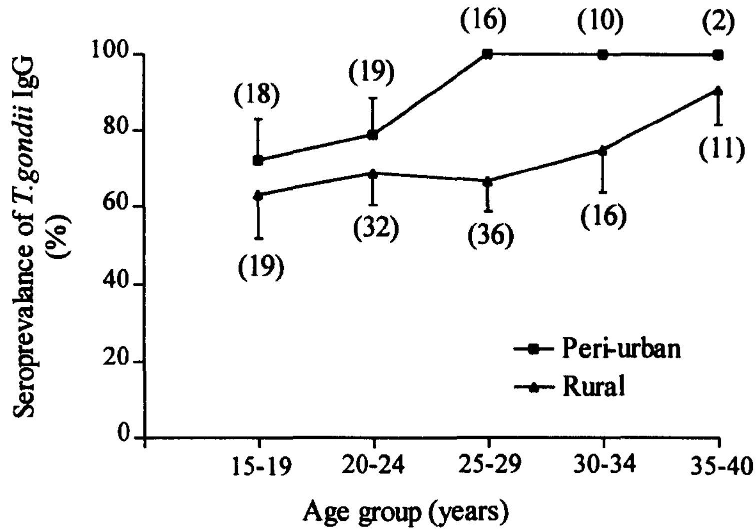

Figure 10.1 Sero-prevalence of *Toxoplasma gondii* IgG in pregnant women by age group in peri-urban and rural study areas in Western Sierra Leone. Sample sizes are indicated in parentheses (n); error bars indicate standard error.

Chapter 11

Traditional beliefs and practices relating to dietary intake, intestinal worm infections and anaemia during pregnancy among the Temne ethnic group in Western Sierra Leone

11.1 INTRODUCTION

11.1.1 Role of ethnographical research in health care delivery

Disease control programmes in developing countries have often failed to achieve their objectives because the interventions were not compatible with traditional perceptions of aetiology, prevention and control in the target population (Gyapong *et al.*, 1996). The belief system of traditional societies, and their perceptions of a disease, its cause, consequences and means of prevention or cure strongly influence their attitudes towards control interventions (Gyapong *et al.*, 1996). In recent years, there has been increasing support for ethnographical research on indigenous concepts of health and disease as a means of improving health care delivery in developing countries (Dunn, 1979; Kroeger, 1983; Heggenhougen & Shore, 1986; Bentley *et al.*, 1988; Weiss, 1988; Holland, 1989; Inhorn, 1995; Rauyajin *et al.*, 1995; Sachs & Krantz, 1997).

A major factor that has contributed to the poor success of many anaemia control programmes is low compliance on the part of the target population (ACC/SCN, 1991; Galloway & McGuire, 1994; Pappagallo & Bull, 1996). Factors causing poor compliance may include misunderstanding instructions, the side effects of treatment, frustration over the number and frequency of tablets taken, fear of bearing large infants and low perception of individual risk (Galloway & McGuire, 1994; Yip, 1996). An understanding of the traditional beliefs and practices relating to anaemia and intestinal worm infections may help identify the cultural factors that aggravate these conditions and create barriers to intervention during pregnancy (WHO, 1993; WHO, 1996b). This information can enable health professionals to develop culturally appropriate interventions and make appropriate use of existing traditional (indigenous) human and material resources (Fosu, 1981; Scrimshaw & Hurtado, 1988; Weiss, 1988; Stapleton, 1989; Kirby, 1993; Opala & Boillot, 1996; Pitts *et al.*, 1996).

At the time of writing, there are no available reports on research that have specifically investigated the traditional beliefs and practices relating to intestinal worm infections and anaemia among pregnant women in Sierra Leone. The ethnographic study described in this chapter focuses on the Temne people, the predominant ethnic group in the study area. As indigenous concepts of illness and healing cannot be studied in isolation from the culture's total system of belief (Fosu, 1981; Opala & Boillot, 1996), the world view of the Temne is briefly described[1]. World view is a culture's perception of how the world and its human inhabitants function, and provides the reasoning for cultural practices (Opala & Boillot, 1996).

[1] Much of the background information relating to the Temne world view can be found in Littlejohn (1960) and Shaw (1982).

11.1.2 Temne world view

The Temne is second only to the Mende as the largest ethnic group in Sierra Leone. Temneland is located in the Northwestern and central parts of the country, although population shifts following colonisation and the civil war of the 1990's has led to a breakdown of geographical boundaries between the ethnic groups. The social organisation of the Temne centres around the 'paramount chief' of the chiefdom, village chiefs, secret societies and the patrilineage. The paramount chief is a political figure who is believed to have access to extraordinary knowledge that is unavailable to ordinary people. He is considered to be chosen by God, and is thus a representative of God. Secret societies are predominantly concerned with the ritualistic transformation from childhood into adulthood. The patrilineage governs residence, inheritance and succession. Marriage is ideally polygynous, and the basic social unit is the extended family compound, which usually consists of the male household head together with his brother or adult son, their spouses and children.

Alongside Islam (the prevailing religion), traditional beliefs and practices remain a powerful influence throughout the Temne region. In Temne cosmology, the distinction is made between the 'visible' world and the 'hidden' worlds. The latter comprises the world of the ancestors, the world of the devils and the world of the witches. The hidden worlds and their inhabitants (ancestors, devils and witches) are described as being spatially 'here', but are invisible to ordinary people.

The ancestors dwell in the towns and houses of their descendants, and can only be seen by their living relatives in dreams. They give protection, fertility and prosperity and uphold the moral behaviour of their descendants, particularly that of children and wives. The descendants offer sacrifices to their ancestors in order to encourage their protection and good-will or to appease offended ancestors when social codes of behaviour are broken.

All devils[2] (*krifi*[3]) can be dangerous towards people, irrespective of whether they give protection and fertility. They are not normally seen by ordinary people, but may appear disguised as an animal, a human, a gust of wind or a whirlpool in a river. The consequences of seeing a devil are usually severe. For example, a personal devil of an enemy may appear to a woman in a dream as her brother or other male relative, and cause infertility, miscarriages and birth complications by making love to her. Devils are broadly categorised as town (family), river or bush devils. The nature of the devil is associated with the environment it inhabits. The town is perceived as a social area and place of order. Town devils may live in the bush, but come to the town on occasion. They are responsible for the fertility and prosperity of the

---

[2] The word 'devil' is used in preference to 'spirit' as this is the usual translation in Sierra Leonean English (Opala & Boillet, 1996).

[3] The Roman alphabet has been used to transcribe local terms; they have been italicised and their English translations, if known, are given in inverted commas.

social group. Conversely, the bush is regarded as asocial and chaotic. Bush devils are less controllable and carry out asocial, unpredictable, malevolent actions such as destroying crops and causing miscarriages. River devils have characteristics or both town and bush devils, reflecting the intermediate position of the river between the social town and asocial bush. They are responsible for the welfare of an individual rather than social group. In return for sacrifices, they may aid the individual to obtain wealth, take revenge on an enemy or help a woman bear children. If the sacrifices to a personal devil are not offered, or if a promise is not fulfilled, the devil may punish the individual by causing misfortune, illness or infertility.

Witches are humans of either gender who possess supernatural powers. They are believed to have two invisible eyes that enable them to see the invisible worlds and those that inhabit them. An individual may be born with 'four eyes', or acquire the trait later in life if a witch drops a special medicine into the eyes of a non-witch. Particular groups in the community who have unusual characteristics or skills such as diviners, secret society officials, twins, herbalists, blacksmiths, white people, powerful chiefs, warriors, hunters and traders are believed to practice witchcraft. Witches have extraordinary knowledge and perform hidden acts, often using their powers of invisibility or ability to metamorphose into animals. Witchcraft is considered a deliberate and destructive activity, which causes direct harm to people or affects the source of their livelihood. Witches may suck the blood and eat the heart of a child, turn into wild animals and kill people or destroy crops.

11.1.3 Aims and objectives

The aim of the work described in this chapter was to examine how the traditional beliefs and practices of the Temne ethnic group, which relate to dietary intake, intestinal worm infections and anaemia, may affect compliance with interventions to control intestinal nematode infections and anaemia during pregnancy.

The specific objectives were:

1. To describe the traditional and Western health care resources available to the Temne people in Western Sierra Leone.
2. To examine the traditional beliefs and practices relating to dietary intake during pregnancy.
3. To examine the knowledge and beliefs concerning the symptoms and aetiology of intestinal worm infections and anaemia, and the appropriate treatment-seeking behaviour during pregnancy.
4. To assess how these traditional beliefs and practices may affect compliance with interventions to control intestinal nematode infections and anaemia during pregnancy.

11.2 METHODS

11.2.1 Data analysis

Field-notes were kept at all interviews and later analysed to identify themes, concepts and explanations. For the analysis of the quantitative results, the responses were categorised, and the response frequencies and proportions calculated. Most respondents gave multiple responses to questions, but were unable to assign a hierarchy of value. The responses of key informants were not included in the quantitative results, as their knowledge was influenced by biomedical training.

11.3 RESULTS

11.3.1 Characteristics of study population

The nine key informants included medical personnel (mother-child health aides, state-enrolled community health nurses and community health officers) and a parasitologist (B.Sc.). In-depth interviews of approximately one hour's duration were conducted for 42 Temne individuals (32 females and 10 males); the sample size for all statistics is thus 42. The mean (SD) age of the subjects was 34.9 (11.0) years. The majority were married (59.5%) or single (38.1%) and over half (57.1%) had not received formal education. Twenty-six (61.9%) resided in rural areas and the remainder in peri-urban areas. The sample included two village head men, six traditional birth attendants, three traditional herbalists, two drug peddlers, five housewives, five farmers, ten petty traders, two cooks, four teachers, a driver, a student and a child minder.

11.3.2 Health care systems available to the Temne

Two health care systems coexist in the Temne community, the traditional and Western.

(i) Traditional health care resources

Traditional medicine among the Temne relies on the cultural assumption that human illness has closely interrelated physical, natural, social, moral and supernatural causes, expressions and consequences. The reason and practice of traditional medicine is grounded within the religious, social and moral conceptions of the body, personal well-being, soul, society and cosmos. Traditional healers are highly recognised members of the community who are often sought to diagnose and treat illnesses of natural or supernatural causation, to advise on social problems and to foretell events. Their methods of treatment are in harmony with the indigenous system of belief pertaining to the causation of illness or misfortune.

There are four main categories of traditional healers: pharmacists, herbalists, diviners and traditional birth attendants (TBAs). In additional to the traditional healers, lay individuals have a limited knowledge of the preparation and use of herbal treatments, particularly in rural areas, where the ingredients for home remedies can be found locally. Treatment of minor sicknesses often takes place in the home.

Most markets have stalls attended by traditional pharmacists where herbs and preparations can be obtained. Traditional herbalists specialise in the use of wild plants gathered in the bush and are highly knowledgeable in the efficacy, dosage and compounding of herbs. They are usually sought for the treatment of illness that are perceived to have a natural origin.

Temne diviners have access to hidden or supernatural knowledge of secret or future events, and are consulted in cases of illness or social problems attributed to supernatural forces such as ancestors, witches, devils and other malevolent individuals. They include the murimen and jujumen. Murimen are sought to reveal the source, course and prognosis of severe or chronic illnesses that fail to respond to herbal treatment or are perceived to be supernatural in origin. They are commonly consulted for reproductive problems such as menstrual irregularities, barrenness, miscarriages or complications at delivery. Such illnesses are often attributed to the violation of social or moral norms, and treatment, which usually involves ritual therapy in addition to herbal remedies, depends on acceptable behaviour by the client. Jujumen are regarded as highly powerful and feared members of the community. They are sometimes referred to as 'bad people', and are sought for supernatural intervention on problems associated with social stress and problematic relationships, protection from enemies, assistance in employment or financial matters and fortune telling. They identify the personal agent responsible for the illness or problem, counteract the work of malevolent persons and can inflict harm on victims designated by their clients.

Traditional birth attendants (TBAs) are highly respected women found in almost every village who assist pregnant women at delivery. They are trained by the women's secret societies and through experience. In recent years, many have also attended formal training programmes sponsored by the World Health Organization and the Sierra Leonean government (Ross, 1991). Many women still rely on TBAs, particularly in rural areas where access to Western medical services is limited.

(ii) Western medicine

Western medicine was imported into Africa during the colonial era. It is available through self-help initiatives from drug peddlers, market stalls, dispensaries and pharmacies, or following consultation with medical personnel at government and non-government organisation (NGO) clinics or hospitals.

The use of modern health care facilities is often deterred by cost, time and a shortage of medicines. Consequently, many people rely on Western pharmaceuticals sold without supervision. Self-treatment is frequently the first and most common form of treatment. Drug peddlers and market stalls sell a limited range of drugs, including analgesics, anthelminthics and antibiotics. Remote villages often depend on traveling drug peddlers as the only source of Western pharmaceuticals. Most moderately sized towns have at least one dispensary. They are a major provider of primary health care, as free consultation is often provided by the dispenser, who has usually attended a three month training course. In Freetown and other major cities, the pharmacies tend to be run by dispensers, who may be supervised by a more highly trained pharmacist.

The government and NGO clinics and hospitals provide consultation, treatment and vaccinations. Mobile health teams visit remote rural and peri-urban areas that have inadequate or no health services within reasonable walking distance. Most clinics hold weekly antenatal clinics, although these were reported to be poorly attended in the study areas. Household-level decisions involving cash are usually made by the male head of a Temne household, and as treatment-seeking behaviour usually involves cash expenditure, it is common for women to seek the permission of the husband before they attend an antenatal clinic. Most pregnant women wait until they are at least five months pregnant before commencing antenatal care. Attendance in early pregnancy is rare, not only for economic reasons, but also because many women prefer to conceal their pregnancy in the early stages in order to protect themselves from the malicious intentions of supernatural beings such as witches and devils. If a woman experiences a normal delivery during her first pregnancy, she will not expect to encounter difficulties in subsequent pregnancies. A multiparous woman who has successfully delivered several children will usually see no need to attend a Western antenatal clinic and her female relatives will often share the same opinion. When birth complications do occur, the pregnant woman and her family typically suspect supernatural causes; as Western medical intervention is perceived to be ineffective in such cases, formal treatment is not be sought, or is sought at a late stage when other traditional and self-medication methods have been exhausted.

11.3.3 Dietary intake during pregnancy

One-third of respondents (33.3%) considered that food intake is, or should be, reduced during pregnancy. This reduction in dietary intake may occur during the first three months of pregnancy or throughout pregnancy, but usually during the last few months of pregnancy. In particular, carbohydrate-rich 'heavy' food such as cassava and cassava dishes tend to be avoided. The primary reason for a reduction dietary intake is to limit the size of the fetus, thus facilitating delivery and avoiding complications that would require a costly hospital delivery. One state-enrolled community health nurse (SECHN) reported that pregnant women may even request medication to suppress their appetite. A reduction in dietary intake was also desirable to avoid the discomfort associated with a large abdomen and to prevent insomnia. Over one-third of respondents (38.1%) reported that food intake is not purposely reduced other than by individual appetite and economic means. Instead, pregnant women are encouraged to eat plenty to meet the extra demands, maintain a healthy pregnancy and encourage fetal growth. The remaining respondents (28.6%) also indicated that there is no cultural restriction on the quantity of food intake, but acknowledged that while some pregnant women increase their intake, others reduce intake, particularly if they experienced complicated deliveries in a previous pregnancy.

The 24-h recall results (Chapter 5) indicated that the group mean intake of energy increased across pregnancy. This does not however contradict the notion that some pregnant women reduce their dietary intake during pregnancy. Further studies with multiple 24-h recalls over the duration of pregnancy are required to identify the extent of dietary intake restriction.

Besides economic limitations, food choice during pregnancy was governed by four factors: individual aversion, religious dietary proscriptions, family-associated dietary proscriptions and cultural (ethnic) dietary proscriptions.

(i) Individual aversion

During pregnancy, Temne women have individual aversions to particular foodstuffs that are distinct from dietary proscriptions. These food aversions, which are frequently observed among pregnant women world-wide (Walker *et al.*, 1985), usually originate from the association of the foodstuff with undesirable symptoms during a previous or the present pregnancy. The mere sight or smell of these food items may be enough to initiate nausea. For example, some pregnant women find that cow's milk, potato leaves and rice aggravate nausea and vomiting, some associate palm oil, roast cassava and roast potatoes with heart-burn, while foods such as beans and oranges are simply not appetising to others.

(ii) Religious dietary proscriptions

Although not specific to pregnant women, the consumption of pork and 'bush meat' (non-domesticated animals such as monkeys and bush boars) is forbidden by the Islamic doctrine, as they are considered impure.

(iii) Family-associated dietary proscriptions

Each Temne family name is associated with a totem, a specific animal that the family members are forbidden to kill or eat. For example, a bearer of the name Kamara must not eat the 'Kamara bird' (*Lapoonistica senegalensis*, Senegal fire finch), or else suffer serious consequences. As with religious dietary proscriptions, totems are not specific to pregnancy, but if a pregnant women disobeys the proscriptions, miscarriages, stillbirths or other complications may occur.

(iv) Cultural dietary proscriptions

The majority of respondents were familiar with the specific dietary proscriptions associated with pregnancy. Many different foodstuffs are linked with deleterious outcomes for the infant, or occasionally mother, if eaten during pregnancy (Table 11.1). The operative word 'may' is used, as these outcomes are not considered inevitable, and in some cases the proscription only applies to excessive consumption of a particular food item, rather than total elimination from the diet. Many of these proscripted foods items are probably not unique to the Temne ethnic group, as population movement and inter-tribal marriage will have introduced influences from other ethnic groups.

The most common deleterious infant outcomes associated with these proscripted foods include skin conditions, gastrointestinal disorders, convulsions, enlarged genitals, behavioural defects and *wanka*. In many cases, the association between the proscripted food and outcome appears to be related to the physical appearance of the food. For example, women fear that if they eat pineapple during the pregnancy, the skin of infants will develop rashes thus resembling the texture of pineapple. In addition, the behavioural characteristics of particular animals are believed to develop in the infant of a woman who eats the meat of these animals during pregnancy. The Temne people regard monkeys as mischievous animals and pregnant women fear that if they eat monkey meat during the pregnancy, the infant will develop mischievous behaviour in later life.

Wanka is a curse that may cause harm to any individual in a community, although fetuses, infants and pregnant women are particularly vulnerable. The curse is often manipulated by witches and jujumen. The Temne may attribute any illness that is not perceived as natural in origin to *wanka*. Often they are acute illnesses that do not respond to treatment, such as convulsions, tetanus, anaemia, or sudden unexplained death in young children. In pregnant women possessed by *wanka*, unfavourable outcomes such as miscarriages, stillbirths and obstructed labour may occur. It is believed that if a mother eats chicken or eggs during pregnancy, both the mother and her fetus will be possessed by *wanka*. The mother of the immature chicken within the egg takes revenge for the death of her unborn offspring by imposing the *wanka* curse on the pregnant woman. The mother may miscarry the fetus, but if the pregnancy reaches term, the infant may be possessed by *wanka*. An infant possessed by *wanka* in this way will be deeply pigmented, have convulsions, persistent diarrhoea and may subsequently die.

Comparatively few foods are associated with detrimental outcomes for the pregnant women. Limes are believed to cause anaemia, and oranges are associated with malaria at delivery.

To determine how rigidly Temne women adhere to these proscriptions during pregnancy, the dietary recall data for Temne pregnant women in the longitudinal study was examined. The number of pregnant women who reported eating proscripted foods is given in Table 11.2. Only a small number of pregnant women and a small proportion of the proscripted food items were involved: nine women reported eating coconut, one chicken, one eggs, and three women ate oranges close to delivery in the third trimester.

11.3.4 Intestinal worm infections

No attempt was made to assess the ability of respondents to distinguish between intestinal infections caused by macroparasites or microparasites.

(i) Knowledge of the symptoms of intestinal worm infection

The Temne term for worms, *ehboke*, is applied to any worm-shaped or snake-shaped creeping organism. Worms are described variously as 'small snakes', 'very small animals', 'long thin animals' and 'germs'. There is a widespread belief that worms are 'not good for the body' and cause sickness. They are known to eat ingested food and suck the blood of humans. Most respondents were able to identify symptoms of worm infection (Table 11.3). Frequently reported symptoms include weight loss, vomiting, nausea, distended abdomen and poor growth in children, abdominal pain and anaemia.

(ii) Vertical transmission of intestinal worm infection

Eight (19.0%) respondents believed that pregnant women can pass worm infections to the fetus. One respondent understood the route of this transmission to be the same as that by which the fetus receives food from the mother:

> 'The baby gets its food from the womb of the mother, so the worm can also get to the baby in the same way.'
> (Female petty trader)

Most of these respondents described severe or fatal consequences of fetal infection:

> 'The pregnant woman passes worms to the fetus and this is why the infant has malformations such as eaten mouth[4]. The worms eat up the fetus and lead to death.'
> (Female petty trader)

Twice as many respondents (38.1%) believed that a breast-feeding mother could pass an infection to her infant. The milk is understood to carry the infection to the infant, and a lactating mother who suspects she is infected may refrain from breast-feeding.

---

[4] Cleft lip.

(iii) Perceived causes of intestinal worm infection

The perceived causes of intestinal worm infections can be broadly categorised as natural or supernatural (Table 11.4).

Over 90% of respondents mentioned 'natural' causes of infection that were related to the diet and/or hygiene. The consumption of raw, undercooked or unwashed meat, fish, fruit and vegetables is regarded as common causes of worm infection. Palm kernel nuts, eggs and cow's milk were also reported as sources of infection. Palm kernel nuts picked from the ground are often infested with small white grubs, which are believed to be the worms that cause human intestinal infections.

The majority of respondents denied that intestinal worm infections are caused by supernatural forces; only two individuals associated infection with witchcraft. However, a key informant explained that the symptoms of an intestinal worm infection may be misdiagnosed by the individual and attributed to witchcraft.

(iv) Treatment of intestinal worms

There was a strong preference for either traditional or Western treatment for intestinal worm infections.

One third of respondents (33.3%) preferred Western medicine, chiefly because they believed it to be more efficacious and fast-acting. None of these respondents described supernatural causes of infection. Most had experience of levamisole and piperazine. A suspected infection is usually self-treated with one of these drugs purchased from a drug peddler or pharmacy, and no respondent reported the use of clinic, hospital or independent laboratories for diagnosis.

The majority of respondents (66.7%) preferred traditional medicine to Western medicine, although some women mentioned that their husbands sometimes force them to take the Western alternatives. This group of individuals had greater confidence in herbal treatments, which they believed to be more efficacious than the Western alternatives. Many of these traditional treatments have been within the communities for generations, and they find it difficult to abandon them for Western drugs of which they have inadequate knowledge and experience. Herbal remedies are also cheaper and more readily available, particularly in rural areas. A number of herbal treatments were described (Table 11.5). They commonly comprise the leaves or seeds of specific plants, which are pounded together with water. The remedies are consumed or tied around the abdomen of the sick individual.

Over half of the respondents (54.8%) associated anthelminthic treatment (traditional or Western) during pregnancy with poor outcomes such as abortion, miscarriage, premature delivery and deformity of the fetus. One SECHN remarked that many pregnant women will not seek treatment for a suspected intestinal worm infection, since they do not believe that there is a safe remedy for use during pregnancy, and fear that the risk of an unfavourable pregnancy outcome is too great. Once they have delivered, they are not averse to taking treatment. Of those who did not believe there to be any ill-effects of treatment during pregnancy (35.7%), only one respondent mentioned that treatment should only be taken after the first three months of pregnancy.

11.3.5 Maternal anaemia

(i) Knowledge of the symptoms of maternal anaemia

The study revealed that community members are well-aware of anaemia during pregnancy. Indeed, most pregnant women enrolled in the study were conscious of their blood level and expected to lose blood at delivery:

> 'Every pregnant women is believed to be anaemic. It is an indisputable fact.'
> (Female cook)

There is no specific Temne word for anaemia. It is usually expressed as 'not enough blood' indicating reduced volume. Some also described the blood as 'pale'. Most respondents could correctly identify the symptoms of anaemia (Table 11.6). Frequently reported symptoms included pale skin ('the body looks white') and/or conjunctiva, dizziness, breathlessness, weakness, tiredness, fatigue and oedema.

(ii) Perceived causes of maternal anaemia

The perceived causes of maternal anaemia included natural and supernatural factors (Table 11.7).

Natural causes of anaemia were mentioned by 78.6% of respondents. The most frequently reported natural cause of anaemia was a diet inadequate in quality and/or quantity. Specific food items, including lime juice and coconut are believed to cause anaemia during pregnancy. The acidity of lime juice is thought to cause anaemia by eroding the bones. Two respondents believed that the fetus is responsible for maternal anaemia, as it takes some of the mother's food. Other natural causes of maternal anaemia include infections (intestinal worm infections and malaria), trauma and failure to take preventative medicine. Over one quarter (26.2%) of respondents associated intestinal worm infections with anaemia, and a similar proportion (21.4%) mentioned intestinal worm infections as a cause of maternal anaemia.

Over half of the respondents (57.1%) identified supernatural causes of anaemia in pregnant women; almost two-thirds of these respondents also attributed anaemia to natural causes. Anaemia is frequently associated with witches. Ancestors may indirectly mediate maternal anaemia by withdrawing their protection from a pregnant women who has committed social and moral taboos (such as adultery), thus leaving her vulnerable to witchcraft. Witches are believed to cause anaemia by drinking the blood of the pregnant woman. They may also drink the blood of the fetus causing fetal anaemia, or eat and thus kill the fetus. The witch may be the pregnant mother herself, her husband, or a close relative, commonly the husband's mother, sister or aunt. When it is the witch's turn to contribute to a communal witch-feast, he/she will turn into his/her witch form at night. The witch 'borrows' the fetus from the mother and offers or sells it to the fellow witches. If the fetus is to be eaten, all the witches (including the pregnant mother if she is the witch) first eat the head, the liver and some fleshy parts of the body. The maternal blood is collected from the blood vessels, and as soon as the witches begin to drink this blood, the pregnant woman becomes anaemic. The heart of the fetus is normally the last organ to be eaten, and when the operation is finished, the fetus dies immediately. The dead fetus is returned to the mother before daybreak.

One of the subjects in our longitudinal study experienced heavy bleeding following delivery due to a retained placenta. She became severely anaemic and so her relatives sought the advice of a muriman. The family were told that the woman had performed witchcraft, and consequently her wrists and ankles had been 'tied' causing the loss of blood. She was told that she and her infant would die unless she confessed to practicing witchcraft. By the time we were alerted, her haemoglobin concentration had fallen from 97 g/l (two weeks previously) to 26 g/l. We commenced treatment, and she made a full recovery, though the infant died because the mother was unable to breast-feed.

Devils may also cause anaemia, as recalled by one respondent;

> 'A pregnant woman I knew began to bleed heavily during her pregnancy. She went to the murimen to seek his advice. It happened that the pregnant woman had done wrong to her co-wife. The co-wife had taken her pants and placed them under a stone in a nearby stream, and had sworn to the river devils to harm the pregnant woman. As the stream ran through the pants, the pregnant woman began to bleed.'
> (Housewife)

(iii) Treatment of maternal anaemia

As with intestinal worm infections, there was a strong preference for either traditional or Western treatment for maternal anaemia and no respondent professed to using both. All but one respondent showed preference for the same health care system (traditional or Western) for the treatment of both intestinal worm infections and anaemia.

Over one third of respondents (35.7%) preferred Western medicines, including iron supplements, vitamin tablets and anthelminthics, or would attend a clinic or hospital. All these respondents attributed anaemia to natural origins, although one-fifth of these respondents (21.4%) described both natural and supernatural origins.

One half of respondents preferred to seek the advice and treatment of traditional herbalists or murimen. The majority of individuals in this group described supernatural causes of maternal anaemia (80.9%), although almost half (47.1%) also attributed illness to natural origins. A person affected by an illness attributed to supernatural causes will not seek Western medicine as only traditional medicine is believed to be effective. Even if the pregnant woman is willing to attend a clinic, family members may discourage or prevent her from doing so. The remaining individuals in this group (19.1%) attributed anaemia to purely natural origins.

The traditional herbalist is sought for treatment of natural causes of anaemia. The herbalist prepares a 'blood medicine' called *rabaenya* from herbs found by the riverside. The medicine is consumed or rubbed onto the stomach. Murimen are usually summoned by the family of the pregnant woman if herbal treatment is ineffective, or if supernatural causes of anaemia are suspected. The muriman uses his unique powers to identify the cause of the anaemia. He may report that the pregnant woman has been bewitched by a family member who is sucking the blood of the mother, or that she has offended the ancestors by committing a taboo. The muriman will prescribe treatment according to the cause. If the anaemia is attributed to *wanka*, the muriman provides medicine to cure the *wanka* before the anaemia is treated. In the case of witchcraft, the treatment usually include charms, amulets and talismen known as *sebeh*, magical potions and rituals. The *sebeh* comprise a piece of Muslim writing wrapped in cloth and animal hide. They are worn around the waist, wrist or upper arm as protection against witches and devils. The magic potions include *mananse* and *maro megben*, which are rubbed over the body after washing: *mananse* is prepared with special herbs, which are ground and mixed with scent to neutralise the smell of the herbs; *maro megben* comprises palm oil, which is burnt until it turns white, mixed with potions and scent. If the ancestors appear to have been offended, the pregnant woman is forced to confess her evil deeds (for example, the name of her lover if she has committed adultery), and the family is advised to perform rituals to appease the ancestors, which usually involve sacrifices. The size of the offering reflects the economic status of the afflicted, but dough and kola nuts form the basis; chicken, palm oil, coins and cooked rice may also be offered.

(iv) Preventing maternal anaemia

One woman implied that maternal anaemia is inevitable and thus unavoidable:

'There is no question about prevention: all pregnant mothers will have anaemia'.
(Petty trader)

However, most respondents were familiar with ways of preventing anaemia during pregnancy (Table 11.8).

Only seven respondents suggested the use of vitamin and iron supplements to prevent anaemia during pregnancy. However, almost all respondents had basic knowledge on the nutritional prevention of anaemia. Most respondents identified that a good quality diet was essential in maintaining healthy blood and preventing anaemia. The quantity of food intake was also deemed important. Green leaves, palm oil, fish, meat and beans were most frequently reported as important for 'making blood'. Red food items such as palm oil are believed to promote the quality of the blood. Other erroneous sources of dietary iron included bananas, plantain, sweet potatoes and peanuts. Lime juice (thought to erode the bones and cause anaemia) and oranges (associated with malaria) were considered detrimental to the quality of the blood.

Almost one third of respondents believed that pregnant women should take precautions against supernatural sources of anaemia. In order to satisfy the ancestors, they must adhere to social and moral codes of behaviour, such as respectfulness, obedience and fidelity towards the husband. They must also refrain from confrontations that would encourage malevolent beings to impose *wanka* on them. As additional protection against witches and devils, the pregnant women should also fortify themselves with medicines and charms from murimen.

11.4 DISCUSSION

Across many African ethnic groups, pregnant women are known to receive differential treatment to ensure a healthy pregnancy and successful delivery. There is evidence that Temne women and men view pregnancy as a special state. Both mother and fetus are considered to be vulnerable to certain health risks, including external forces mediated by ancestors, witches and devils. Pregnant women anticipate problems in advance and seek to prevent illness during the pregnancy and complications at delivery. Thus, the concept of preventive antenatal care is consistent with the Temne traditional health care system. The first defense behaviour is to conceal the pregnancy for as long as possible, so that exposure to malevolent beings is limited. When this is no longer possible, traditional healers are sought for special medicines and charms.

Behavioural proscriptions are considered essential, as it is believed that poor pregnancy outcomes may result from lapses in the social and moral behaviour of the mother. Many of the proscripted social behaviours relate to the woman's relationship with individuals in her community, particularly her husband. These taboos may have social value by strengthening the stability of relationships, but the underlying ideology could also be the cause of potential harm to the mother and fetus. In order that the pregnancy is concealed, attendance at antenatal clinics may be delayed until the latter stages of pregnancy. Furthermore, Western medical intervention may be delayed or denied if an illness or obstetric complication is perceived to have a supernatural aetiology.

11.4.1 Dietary intake during pregnancy

In West Africa, concepts of health and illness are closely linked with diet, and dietary proscriptions are frequently observed among pregnant women (Ogbeide, 1974; Etkin & Ross, 1982; Jackson & Jackson, 1987; Odebiyi, 1989; Adetunji, 1996). Dietary adjustments form an integral part of traditional antenatal care among the Temne. Most respondents acknowledge that some pregnant woman reduce their intake to facilitate delivery and alleviate discomfort, particularly during the last trimester. This is a common behaviour in developing countries (Rosenberg, 1980; Hutter, 1996), and contradicts the internationally recognised dietary requirement standards, which recommend that pregnant women increase their intake during pregnancy (FAO/WHO, 1974; 1985; 1988). Expectant mothers are also obliged to follow numerous dietary proscriptions to ensure that their child is born physically perfect and lacking undesirable behavioural traits. As Temne women are pregnant for a substantial proportion of their child-bearing years, these proscriptions are a frequently enforced. The nature of the feared physical defect in the infant usually resembles the physical appearance of the proscripted vegetable or fruit, and the undesirable behavioural characteristic mimics the behavioural traits of the proscripted animal. Notably few dietary proscriptions applied to the fate of the mother herself. From a Western stand-point, none of the associations between proscripted food item and outcome had a rational explanation.

Dietary proscriptions are often considered to be detrimental to nutritional status as they limit the range of acceptable food items in the diet (McKay, 1971; Bolton, 1972; Wilson, 1973; Ogbeide, 1974). However, there has been some discussion to the contrary, and it is argued that in most cases the contribution of dietary proscriptions to the health of the mother and future infant is minimal, either because the proscripted items are not part of the habitual diet (Ulijaszek & Strickland, 1993) or because the proscriptions are poorly respected (Ogbeide, 1974). The dietary proscriptions applicable to pregnancy among the Temne limit the potential range and nutritional value of the diet. Indeed, it is feasible that they contribute to the inadequacy of the diet relative to the standard daily requirements for food intake during pregnancy. The proscriptions relevant to iron nutrition include animal foods such as chicken, chimpanzee and lizard, which are rich sources of haem-iron. However, it is unlikely that any of these proscripted animal foods significantly limit the intake of dietary iron, as fish is the only habitual animal food in the diet, and these proscripted items are rarely eaten by any members of these Temne communities. Some proscriptions restrict access to foods containing vitamin C, including pineapples, limes and oranges, which could limit non-haem iron absorption. Again, it is unlikely that these proscriptions aggravate iron nutrition, as pineapples are a cash crop and rarely eaten by villagers, limes are only a common ingredient of rice pap (an infant weaning food that is not usually eaten by adults), while oranges are only proscripted close to delivery.

In general, the dietary proscriptions reported by the Temne people involve foods that do not make a sizable contribution to the habitual diet. Where ingredients of staple dishes are involved (for example, food condiments such as hot peppers, which are added to almost all rice dishes), the proscription invariably applied to 'excessive' intake, rather than total elimination from the diet. Furthermore, the Temne usually refer to probable or possible consequences of breaking dietary proscriptions: since the detrimental outcomes are not considered a certainty, this may allow for behavioural flexibility contrary to the cultural food ideology. To understand the significance of dietary proscriptions, it is therefore important to compare the theoretical food ideology, which may be idealised (Bentley *et al.*, 1988), with actual patterns of dietary behaviour. It is ultimately the latter that influences nutritional status (Laderman, 1984), a fact that is frequently overlooked in many discussions of dietary proscriptions (Ogbeide, 1974). The actual intake of Temne pregnant women participating in the core study was in most cases compatible with the beliefs regarding appropriate dietary behaviour during pregnancy. In making this observation, several points should be noted (1) the reported food ideology and dietary intake data relate to different groups of individuals, albeit from the same ethnic group and community, and further studies should attempt to study both aspects in the same group of pregnant women (2) the dietary recall data represents the food intake for just one day each trimester, and it is possible that proscripted foods were eaten at other times during pregnancy (3) data on the pre-pregnancy dietary intake was not collected, and so it is not known whether food intake was altered relative to the pre-pregnant state (4) the pregnant women may have withheld information on breaches of these proscriptions.

11.4.2 Intestinal worm infections and anaemia

Both intestinal worm infections and anaemia are regarded by the Temne community as potentially serious health problems during pregnancy. The signs and symptoms of these illnesses are well recognised, although traditional concepts of worm and anaemia aetiology still persist and have not been rejected, even by those with some knowledge of Western biomedical principles.

Examination of the causes mentioned with reference to intestinal worm infections reveals that a clear notion of the germ theory of disease exists. The Temne appear to view worms in the biomedical view, that is, as foreign elements and hostile invaders of the body. None of the respondents associated infection with faeces, although many understood that infection could be acquired from food items, particularly under unhygienic conditions. Few people recognised skin contact with contaminated surfaces as a mode of transmission.

Nonetheless, it is unusual to observe women without shoes, but this may relate to comfort and aesthetic reasoning rather than an understanding of the protective merits of shoes, since it is common to see barefoot children. Intestinal worm infections are not usually regarded as supernatural in origin, although the symptoms may be misdiagnosed and attributed to supernatural forces.

The biomedical understanding of the aetiology of intestinal worm infection probably originates from Western health care messages. Among the Mende ethnic group of Sierra Leone, intestinal worms are believed to generate spontaneously in the human body if the individual eats too many sweet[5] items (Bledsoe & Goubaud, 1985). Little is known about the perceptions of worm infections in other parts of West Africa. Most studies in this region have focused on the guinea worm, which is considered to be a part of the body in both Nigeria and Ghana (Ogunniyi & Amole, 1990; Brieger & Kendall, 1992; Bierlich, 1995). Elsewhere in Africa, intestinal worms are also regarded as obligatory inhabitants of human bodies (Kamunvi & Ferguson, 1993; Green, 1997; Geissler, 1998).

Maternal anaemia is frequently attributed to supernatural sources, particularly witchcraft, and prevention is related to behavioural proscriptions associated with moral and social conduct. Across African cultures, illnesses that are life-threatening, chronic or severely debilitating such as filariasis (Gyapong *et al.*, 1996) and leprosy (Opala & Boillot, 1996) are commonly attributed to supernatural forces, while minor or acute illnesses such as worms tend to be perceived as natural in origin (Opala & Boillot, 1996). Maternal anaemia may also have a natural explanation, including an inadequate diet, disease, trauma or failure to take preventative medicine. Approximately one quarter of respondents associated intestinal worm infection with anaemia, and a similar proportion mentioned intestinal worm infections as a cause of maternal anaemia. The latter responses may be due to knowledge of the study.

In contemporary Sierra Leone, two types of medical systems coexist, the traditional and Western. The Temne people do not appear to combine therapies from both systems for the treatment of intestinal worm infections or maternal anaemia. Each individual appears to be committed to the philosophical or technical premises of one system, and all but one respondent indicated preference for the same medical system for the treatment of both illnesses. Studies elsewhere in the developing world have found variations in the way in which indigenous peoples choose between these two medical systems (Kroeger, 1983). Some ethnic groups dichotomise between illnesses that respond to either traditional or Western therapies (Gould 1957), while members of other ethnic groups, such as the Cokwe of Zaire and Pokot of Kenya, may consult both traditional and Western healers for the same illness, either

[5] In this context, 'sweet' refers to foods that are sugary, such as fruit or confectionery, or simply good to taste, particularly chicken and meat

simultaneously or consecutively (Yoder, 1982; Nyamwaya, 1987). In many cases, the choice of appropriate therapy for a particular illness is dependent on the ascription of causation (Fosu, 1981; Lasker, 1981; Yoder, 1981; Nyamwaya, 1987). Among the Temne, the most important criteria that determine the differential use of the health care services for the treatment of intestinal worm infections or anaemia include: (1) the perceived efficacy of treatment, as influenced by the individual's understanding of the causation of illness, their knowledge and past experiences (2) the availability in terms of geographical distribution, and (3) the relative cost of treatments.

All those who had a purely biomedical understanding of aetiology of intestinal worm infection or anaemia preferred Western therapies. These individuals were in the minority. Treatment is usually sought from drug peddlers or pharmacists, often without medical consultation. The misuse of medications is a serious problem in developing countries, where drugs are frequently sold without medical supervision (Wolf-Gould *et al.*, 1991). A further problem in Sierra Leone is the distribution of counterfeit or expired drugs (Z.Bahsoon, personal communication[6]).

The majority of the respondents preferred traditional herbal therapies for worm infections. These traditional therapies have remained in the Temne communities for generations, and their therapeutic properties are fully trusted. The Western biomedical system is not compatible with the underlying belief system of illness and the holistic approach to therapy, whereby the whole person is treated with little distinction made between body, mind and spirit. In addition, the poor distribution and higher cost of Western pharmaceuticals relative to traditional therapies discourages many individuals, including those who would otherwise prefer to patronise the Western biomedical system.

Individuals who ascribe maternal anaemia to supernatural causes prefer to seek the advice and treatment of murimen in the event of illness. Western therapies are believed to be ineffectual when the aetiology of the illness involves a personal agent such as an ancestor, witch or devil. The subset of these individuals who also believe that some illness episodes are have a natural aetiology would select traditional herbal treatments to cure a natural illness. However, the preference for iron supplements was not uncommon among these individuals, possibly because the use of red medications is consistent with the Temne belief that red food items (such as palm oil) promote the quality of blood.

Until recently, medical health personnel have advised women to avoid anthelminthics during pregnancy, as the teratogenic effects of treatment were not fully understood. The present study has indicated that over half of respondents believe that this is still the case. Pregnant women who suspect that they are infected will not seek treatment until after delivery.

[6] Pharmacologist, Ph.D. (Bristol University)

It is particularly important that these women do obtain treatment, as it is commonly believed that a lactating woman can pass an infection to her infant, and an infected woman might refrain from breast feeding.

11.4.3 Implications for the control of intestinal nematode infections and anaemia during pregnancy

An understanding of the factors that contribute to non-compliance in a particular area can be used to make anaemia control programmes more culturally appropriate (WHO, 1993). The Temne perceive both intestinal worm infections and anaemia as significant causes of morbidity among pregnant women. The use of traditional therapies for the prevention or treatment of both these conditions suggests not only a cultural awareness of these health problems but also a motivation to address them (Kightlinger *et al.*, 1996). This is encouraging, as intervention programmes rely on the target population's perceptions of the severity of an illness and their understanding that appropriate treatment-seeking behaviour can effect change (Pitts *et al.*, 1996). Nonetheless, there are several traditional beliefs and practices that may contribute to inappropriate preventative and treatment-seeking behaviours among the pregnant women. These may create barriers to the use of Western medicine and could inhibit the prevention and control of intestinal nematode infections and anaemia during pregnancy. In these communities, compliance may be adversely affected by low confidence in the efficacy or safety of interventions and low utilisation or poor access to antenatal facilities.

It is clear that a substantial proportion of illness episodes associated with intestinal worm infections and anaemia are still managed outside the national health care system. Traditional therapies have not been abandoned by the Temne and continue to be the most common source of treatment. Many people have low confidence in Western therapies, which they maintain are less effective, difficult to obtain locally and expensive. The attribution of anaemia in pregnancy to supernatural events mitigates against the use of Western therapies, which are perceived to be ineffectual in such cases. Anthelminthics, both traditional and Western, are not considered safe during pregnancy. This has important implications in these communities, as it is widely held that a lactating mother should refrain from breast-feeding if she has an intestinal worm infection, as she may pass the infection to her suckling infant.

In order for intervention programmes to be successful in these communities, it is important to provide health education on issues related to the aetiology and control of maternal anaemia and intestinal nematode infection during pregnancy. Since most Temne people continue to hold onto their traditional world view, biomedical health messages that are not consistent with their traditional way of thinking are less likely to be effective (Weiss, 1988; Opala & Boillot, 1996). An example of the way in which analogous concepts drawn from the

Temne world view can be exploited to promote positive preventative and treatment-seeking behaviour is provided by the prophylactic measures against maternal anaemia. A significant proportion of respondents believe that the maintenance of healthy blood is controllable through social and moral obedience or protection from witches. Moreover, the knowledge of iron prophylaxis is poor. Nonetheless, it is evident that the Temne understand the principle of prevention, and their preventative behaviours could be modified through culturally sensitive health education (Rauyajin *et al.*, 1995). The use of red iron supplements for anaemia would seem to follow logically from their traditional beliefs, as red food items are believed to promote the quality of the blood. The same viewpoint is held by other ethnic groups in Sierra Leone (Bledsoe & Goubaud, 1985). Thus, health workers can exploit this association in order to promote more widespread use of iron supplements during pregnancy. The emphasis therefore, is in not on altering their world view, which is resistant to change, but on making Western therapy relevant *within* the context of their world view. The means by which the acceptance of other Western pharmaceuticals such as anthelminthics can be promoted through cultural integration or reinterpretation (Bledsoe & Goubaud, 1985) requires further investigation.

Maternal health care providers should encourage positive traditional health care practices. Not only will this ensure that these practices are maintained, but it may also improve the relationship between the patient and health care provider. Traditional pregnancy care among the Temne involves proscriptions related to dietary intake that safeguard the health of the mother and fetus. These proscriptions are unlikely to interfere with strategies to improve the intake of absorbable iron during pregnancy, as the proscripted items do not make a sizable contribution to the habitual diet. The ideology that underlies these dietary adjustments supports the notion that poor pregnancy outcomes can be prevented by modifying the diet. Therefore, the promotion of positive dietary practices to increase the intake of absorbable dietary iron and other haematopoietic nutrients should not conflict with these traditional beliefs and practices if the benefit of these practices on fetal and infant health is emphasised.

Given the high prevalence of anaemia during pregnancy in Western Sierra Leone, the need to reach pregnant women with appropriate interventions is urgent. Temne women often conceal their pregnancy for as long as possible to prevent exposure to malevolent individuals in the community. This practice delays attendance at the antenatal clinics, and thus reduces the time available for the correction or control of anaemia. Some pregnant women, and in particular multigravidae, regard antenatal care with complacency. In addition, Temne women have low autonomy with respect to treatment-seeking decisions. In a male dominant society, antenatal clinic attendance will only be optimised if their husbands have an understanding of the health requirements of their wives, and it is imperative that they are involved in all aspects of control initiatives that target maternal health.

Traditional health workers are trusted by the community, and share the same concepts of health and illness (Nyamwaya, 1987). It is possible that traditional practitioners could improve compliance by distributing anthelminthics and iron-folate supplements to pregnant women in areas poorly serviced by Western medical facilities. Since WHO officially recognised the potential of traditional medicine as a means to improve primary health care (WHO, 1978; WHO & UNICEF, 1978), several African countries have investigated ways to integrate traditional healers into national health care systems (Anyinam, 1987). In terms of both human and material resources, the traditional health care system has the potential to enhance self-sufficiency in resource poor countries like Sierra Leone (Sofowora, 1980; Akerele, 1987; Chi, 1994). The feasibility of utilising TBAs to distribute iron supplements (Menendez *et al.*, 1994) and malaria prophylaxis (Greenwood *et al.*, 1989) to pregnant women has been successfully demonstrated in the Gambia. Traditional birth attendants are found in almost every village in Sierra Leone, and may be the only maternal health care providers available to women in remote areas. The training programmes provided by the government to promote safe and hygienic practices among TBAs in Sierra Leone could be extended to include information on the distribution of iron-folate supplements and anthelminthics to pregnant women.

Finally, increased efforts should be directed towards the evaluation and development of traditional treatments, which are more prevalent, more widely accessible and the most accepted mode of treatment (Sofowora, 1980; Waller, 1993). Numerous herbal treatments for maternal anaemia and intestinal worm infections are available in the Temne community, and although the anthelminthic properties of *Carica papaya* are well established (Etkin & Ross, 1982)[7], the therapeutic value of many of these preparations is unknown. A further 10 traditional treatments for *Ascaris* have been described among the Mende ethnic group in Sierra Leone (Barnish & Samai, 1992). Field trials are currently underway in Sierra Leone to evaluate the efficacy of traditional anthelminthic remedies, but further work should also focus on the psychosocial as well as biomedical aspects of treatment. This work should be complemented with research to improve the quality, safety, and production of efficacious treatments (Barnish & Samai, 1992). Those of proven efficacy could then be considered for promotion in health care programmes (Sofowora, 1980; Kliks, 1985; Stapleton, 1989).

[7] The active constituents include proteolytic enzymes with activity against *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (Etkin & Ross, 1982).

11.5 SUMMARY

1. An ethnographic study was carried out to examine the traditional beliefs and practices relating to dietary intake, intestinal worm infections and anaemia during pregnancy among the Temne ethnic group in Western Sierra Leone. A multi-method approach of predominantly qualitative-based research techniques was employed including key informant interviews, in-depth interviews, participant observation and case study reports.
2. In contemporary Sierra Leone, two types of medical systems coexist, the traditional and Western. The Temne people do not appear to freely combine therapies from both systems and each individual appears to be committed to the philosophical or technical premises of one system. The most important criteria that determine the differential use of the health care services for the treatment of intestinal worm infections or anaemia include the perceived efficacy, the availability in terms of geographical distribution, and the relative cost of treatments.
3. Traditional pregnancy care among the Temne involves proscriptions related to dietary intake that are believed to safeguard the health of the mother and fetus. As the proscripted food items do not make a sizable contribution to the habitual diet, they are unlikely to be a significant determinant of poor iron nutriture during pregnancy or to interfere with strategies to improve the intake of absorbable iron during pregnancy.
4. The Temne regard maternal anaemia and intestinal worm infections as significant causes of morbidity among pregnant women. The signs and symptoms of these illnesses are well recognised.
5. Compliance with maternal anaemia and intestinal nematode control programmes may be compromised by delayed or low utilisation of Western antenatal clinics and low confidence in the efficacy or safety of Western pharmaceuticals. Prevention and treatment for anaemia is actively sought during pregnancy, but many still believe that anthelminthic treatment, whether traditional or Western, is contraindicated due to adverse affects on the fetus. Traditional therapies have not been abandoned by the Temne and continued to be favoured by many, particularly if an illness episode is believed to have a supernatural aetiology. However, the use of iron supplements tablets during pregnancy follows logically from their traditional belief that red food items promote the quality of blood.
6. The employment of traditional birth attendants to provide information on anaemia during pregnancy and to distribute iron-folate supplements and anthelminthic treatment should be further investigated as a means to improve compliance.

Table 11.1 Dietary proscriptions during pregnancy reported by Temne adults in Western Sierra Leone.

Proscripted food item	Outcome
Aubergine	If eaten in excess, the infant develops a white scaly skin with blisters.
Banana	The genital organs of the infant will be abnormally large.
Chicken	The fetus is possessed by *wanka*: it may die within the womb, but if born alive, the infant will develop severe diarrhoea and convulsions and subsequently die.
Chimpanzee	Chimpanzees are ugly animals and pregnant women fear that their infant will also be ugly. However, chimpanzees are believed to have a single bone in the hand which makes them very strong, and the infant will develop the same extraordinary strength.
Coconut	The infant will vomit excessively.
Cow penis	The genital organs of a male infant will be abnormally large.
Cow head	The infant will have persistent headaches.
Eggs	Fetus is possessed by *wanka*: the mother of the immature chicken within the egg takes revenge for the death of her unborn offspring. The fetus will die within the womb of the pregnant woman, but if born alive, the infant will develop severe diarrhoea and convulsions and subsequently die.
Electric fish	Causes repeated miscarriages. Live-born infants are nervous and will develop epilepsy.
Lime	The infant will develop rashes such as prickly heat. The infant will vomit excessively and may later die. Causes anaemia in the mother.
Lizard	The infant will have convulsions
Milk (cow)	The infant will vomit excessively.
Monkeys	Monkeys are believed to be mischievous and the infant will develop similar characteristics.
Pepper (hot)	If eaten in excess, the infant will develop skin rashes.
Orange	If eaten prior to delivery, pregnant woman will experience malaria during the delivery. May also cause excessive maternal haemorrhaging following delivery and malaria in the newborn.
Pineapple	Infant will develop scaly skin with red swellings.
Plantain	The genital organs of the infant will be abnormally large.

Table 11.1 Number of Temne pregnant women who reported eating proscripted food items during pregnancy.

Proscripted food item	Number of Temne women†		
	Trimester		
	First (*n*=109)	Second (*n*=88)	Third (*n*=80)
Fu-fu	12	6	4
Orange	26	5	3‡
Coconut		2	7
Chicken	1		
Egg			1

† Total number of Temne pregnant women for whom food intake data available; data from a single 24-h dietary recall in each trimester.
‡ Proscription only applies to fruit eaten close to delivery.

Table 11.3 Perceived symptoms of intestinal worm infections during pregnancy reported by Temne adults in Western Sierra Leone (*n*=42).

Reported symptoms	Number of respondents (%)
General symptoms	
Weight loss, poor growth, underweight	21 (50.0)
Vomiting, nausea	21 (50.0)
Distended abdomen (in children).	16 (38.1)
Abdominal pain	15 (35.7)
Pale skin and/or conjunctiva	15 (35.7)
Anaemia	11 (26.2)
Coughing	8 (19.0)
Spitting	8 (19.0)
Increased appetite, overeating without satiation	6 (14.3)
Anorexia	5 (11.9)
Fever	5 (11.9)
Weakness, tiredness, fatigue	5 (11.9)
Oedema	3 (7.1)
Blood-stained stool	2 (4.8)
Dizziness, breathlessness	2 (4.8)
Passing worms	2 (4.8)
Pregnancy outcome	
Miscarriage	1 (2.4)
Premature delivery	1 (2.4)
Obstructed delivery	1 (2.4)
Maternal death	1 (2.4)

Table 11.4 Lay aetiology of intestinal worm infections during pregnancy reported by Temne adults in Western Sierra Leone (n=42).

Reported causes of infection	Number of respondents (%)
Natural	38 (90.4)
Dietary	30 (71.4)
Fish	28 (66.7)
Beef	22 (52.4)
Palm kernel nut	13 (31.0)
Eggs	2 (4.8)
Coconut	1 (2.4)
Cow's milk	1 (2.4)
Leftover rice stuck to the cooking pot	1 (2.4)
Poor hygiene	25 (59.5)
Consumption of unwashed fruit or vegetables	16 (38.1)
Consumption of raw or undercooked fish or meat	8 (19.0)
Playing with dirt or soil	1 (2.4)
Walking barefoot	1 (2.4)
Failing to wash hands before eating	1 (2.4)
Supernatural	2 (4.8)
Witchcraft	2 (4.8)

Table 11.5 Traditional herbal remedies for intestinal worm infections reported by Temne adults in Western Sierra Leone.

Botanical Name	Common names†		Preparation
Afraomomum melegueta	Alligator pepper Aligeta-pepe Ta-sena-ta-rek-rek	E K T	Preparation details unknown.
Annona muricata	Soursop	K	Leaves are pounded in a mortar and left overnight. The juice is squeezed out and taken in the morning and night. Alternatively, the leaves are heated in a saucepan and tied around the abdomen overnight.
Annona squamosa	Sweetsop	K	Leaves are pounded with lime. Some of the preparation is diluted in water to drink. The remainder is tied around the abdomen with a piece of cloth.
Bafodeya bena	Seko	T	The hard nut contains fibrous hairs which are extracted and eaten with fu-fu or banana.
Carica papaya	Pawpaw Popo Am-papai	E K T	Leaves are pounded. Some of the preparation is smoked, and the juice squeezed out to drink. The remainder is tied around the stomach with a piece of cloth.
Hyptis suaveolens	Tea-bush Bush-ti-bush Gbintho	E K T	Leaves are pounded with lime and water. The preparation is taken in the morning on an empty stomach.
Sesamum indicum	Sesame Beni Ma-yenteh	E K T	Seeds are beaten with sugar in a mortar and taken.
Zingiber officinale	Ginger Jinja Ta-sena	E K T	Preparation details unknown.

† E=English, K=Krio, T=Temne

Table 11.6 Perceived symptoms of anaemia during pregnancy reported by Temne adults in Western Sierra Leone (*n*=42).

Reported symptoms	Number of respondents (%)
Pale skin and/or conjunctiva	38 (90.5)
Dizziness, faintness	25 (59.5)
Weakness, tiredness, fatigue	19 (45.2)
Swollen body especially legs and feet	11 (26.2)
Puffy face	6 (14.3)
Anorexia	6 (14.3)
Vomiting	4 (9.5)
Persistent headache	4 (9.5)
Insomnia	3 (7.1)
Loss of weight, thin	3 (7.1)
Glutinous	1 (2.4)
Salivation	1 (2.4)
Fever	1 (2.4)

Table 11.7 Lay aetiology of anaemia during pregnancy reported by Temne adults in Western Sierra Leone (*n*=42).

Causes of anaemia	Number of respondents (%)
Natural	33 (78.6)
Poor diet	31 (73.8)
Disease	10 (23.8)
Intestinal worms	9 (21.4)
Malaria	1 (2.4)
Trauma	2 (4.8)
Failure to take preventative medicine	3 (7.1)
Supernatural	24 (57.1)
Breaking cultural taboos (ancestors)	4 (9.5)
Witchcraft	20 (47.6)
Bush devil	1 (2.4)
Wanka	14 (33.3)

Table 11.8 Preventative measures against anaemia during pregnancy reported by Temne adults in Western Sierra Leone (n=42).

Prevention of anaemia	Number of respondents (%)
Prophylactic medicine	10 (23.8)
Iron tablets	5 (11.9)
Vitamin tables	2 (4.8)
Herbal/native medicine	3 (7.1)
Quality diet	35 (83.3)
Green leaves, especially potato leaves	24 (57.1)
Palm oil	20 (47.6)
Fish	16 (38.1)
Meat (chicken and beef)	13 (31.0)
Beans	13 (31.0)
Bananas, plantain, potatoes, peanuts	7 (16.7)
Positive health behaviours	3 (7.1)
Taking sufficient rest, not carrying heavy loads	3 (7.1)
Protective measures against wanka	13 (31.0)

Chapter 12

General discussion

12.1 INTRODUCTION

In developing countries, the health of pregnant women is frequently affected by both parasitic infections and anaemia. Intestinal nematode infections are ubiquitous in tropical and sub-tropical regions where there is faecal contamination of the environment. These infections may contribute to anaemia by affecting the supply of nutrients for erythropoiesis. Hookworm infections are the leading cause of pathological blood loss in these regions (Fleming, 1989) and may precipitate iron deficiency, which is the most common human nutritional deficiency and the predominant cause of anaemia in pregnant women (ACC/SCN, 1997). As anaemia poses a serious threat to the health of both mother and fetus, hookworms are perceived to present a major public health problem for pregnant women (WHO, 1996b). This perception persists in spite of the lack of data on the contribution of hookworms to anaemia during pregnancy.

In 1994, the World Health Organization put forward a recommendation that anthelminthic treatment should be included in strategies to improve the nutritional status of pregnant women after the first trimester of pregnancy in areas where hookworms are endemic and anaemia is prevalent (WHO, 1996b). Few countries have yet to integrate anthelminthic treatment into maternal anaemia control programmes. It is possible that this situation might be improved if the adverse effects of intestinal nematode infections during pregnancy were better documented and if the efficacy of anthelminthic intervention for the control of maternal anaemia was adequately demonstrated.

The central aim of the research presented in this thesis was to examine the contribution of intestinal nematode infections and iron-folate deficiency to maternal nutritional status and neonatal outcomes in an area endemic for these infections. The impact of intervention with daily iron-folate supplements (36 g iron and 5 mg folate) and a single dose of albendazole (400 mg) on parasitological and nutritional status in pregnant women was investigated. A randomised controlled factorial design was applied in order that the two interventions, daily iron-folate supplements (Fe) and a single dose anthelminthic drug (A), be simultaneously compared with each other and with the placebo alternatives (P_{Fe} and P_A). The intervention groups thus comprised FeA, FeP_A, P_{Fe}A and $P_{Fe}P_A$.

Pregnant women in peri-urban areas of Freetown (*n*=67) and rural areas in Kaffu-Bollom and Lokomasama Chiefdoms (*n*=117), Western Sierra Leone, were invited to participate in the study. A baseline assessment was performed in the first trimester of pregnancy. Intervention began during the first two weeks of the second trimester. Follow-up assessments were conducted in the second and third trimester. Further follow-up assessments were performed at or closely after delivery and at one month postpartum, but interpretation of this data was hampered by intervention-related drop-outs. The assessments were aimed at assessing the effects of intervention on intestinal nematode infections, anthropometric measures of body size and composition, haemoglobin (Hb) concentration and serum ferritin (SF) concentration. The birth outcomes of the women were followed-up in terms of neonatal weight, length, gestational age, Hb concentration and infection with intestinal nematodes. The adequacy of the dietary intake during pregnancy was examined, with particular focus on iron. A cross-sectional sero-survey of immunological markers to several microparasitic infections was performed for the same group of women, with a view to assessing the impact of these infections on the control of maternal anaemia. Finally, an ethnographic study was carried out among the Temne ethnic group to assess how compliance with interventions to control intestinal nematode infections and anaemia during pregnancy may be influenced by traditional beliefs and practices relating to dietary intake, intestinal worm infections and anaemia.

12.2 SUMMARY OF THE FINDINGS

Living conditions in the study areas were modest, although the communities appeared to be fairly well supplied with pit latrines and safe drinking water. However, access to sanitary facilities was controlled by social rules and access to water facilities was limited by restrictions on the opening times of the wells and the seasonality of the water supply. Most of the communities were within walking distance of a government or non-government clinic, although these medical services were reported to be underutilised. The Temne was the predominant ethnic group, followed by the Susu and Mende. Most women were married, two-thirds had no formal education and three-quarters were engaged in petty trading or farming. The reproductive histories of the women were characterised by high gravidity and high pregnancy wastage. Few women had used modern methods of contraception. Nonetheless, pregnancies were fairly well-spaced unless a pregnancy resulted in miscarriage or stillbirth or if a child died.

Pre-pregnancy data was not obtained from the subjects, and the nutritional status of the study population was characterised using anthropometric and biochemical data collected in the first trimester. Chronic undernutrition (height <150 cm) and chronic energy deficiency (body mass index <18.5 kg/m^2) was found in 5.4% and 8.2% of women respectively. The anthropometric findings indicate a mild degree of undernutrition, although the prevalence of chronic energy deficiency is likely to be underestimated due to weight gain during the first trimester. In contrast, the prevalence of anaemia was severe according to current criteria set by the World Health Organization and constitutes a risk factor for women in this region

(ACC/SCN, 1997). Mild or moderate anaemia (70 g/l ≤ Hb < 110 g/l) was diagnosed in 58.2% of women. Severe anaemia (Hb <70 g/l) was only found in one pregnant woman, and is therefore not a common feature of early pregnancy in these communities. The prevalence of anaemia was even higher among 145 non-pregnant women matched for household with the pregnant subjects (Hb <120g/l: 80.7%). This finding is unusual given that the nutritional demands of pregnancy are normally associated with an increased risk of anaemia. However, the cut-off value that is recommended by the World Health Organization to diagnose anaemia in pregnant women is 10 g/l lower than in non-pregnant women. The use of a lower cut-off point for anaemia is perhaps questionable during the first trimester of pregnancy, when the extent of haemodilution is low (Bentley, 1985).

Iron deficiency was clearly an important aetiological factor of anaemia among the pregnant women. Iron deficiency (SF <20 μg/l) was diagnosed in 37.5% of women, and associated with anaemia in 21.2% of women. It is likely that these values underestimate the true extent of iron deficiency and iron-deficiency anaemia in these tropical communities, where infectious and inflammatory conditions are common, as SF concentration is elevated independently of iron status by infectious and inflammatory processes (Lipschitz *et al.*, 1974; Cook & Finch, 1979; Bradley-Moore *et al*, 1985; Kent *et al.*, 1994).

The dietary intake data, estimated from a single 24-h dietary recall each trimester, indicated that iron deficiency is likely to have a dietary basis in this group of women. The dietary intake was simple and monotonous. Most women had two meals a day, which typically comprised rice with a vegetable-based soup or sauce containing small quantities of fish. The estimated mean dietary iron intake did not exceed 8 mg at any of assessments during pregnancy, which is below the basal daily iron requirements of non-pregnant women (FAO/WHO, 1988). The Food and Agriculture Organization/World Health Organization do not stipulate a recommended daily iron intake for pregnant women, as the requirements in the second and third trimester are not expected to be met by dietary intake, regardless of the quality of the diet (FAO/WHO, 1988). Due to the low intake of animal foods, most of the dietary iron intake was non-haem iron. The intake of vitamin C, which is known to enhance non-haem iron absorption (Bothwell *et al.*, 1989), was high relative to the reference daily intake standard (FAO/WHO, 1977). However, most of the vitamin C intake comprised fruits that were usually eaten as snacks between meals. The percentage iron absorption for the typical Sierra Leonean diet is unknown, but if a conservative estimate of 10% absorption is assumed, the average diet of these pregnant women supplied only 0.6 mg of absorbable iron in the first trimester, rising to 0.8 mg in the third trimester. This amount is far below the iron requirement during pregnancy, which is estimated to rise to 5-6 mg during the second and third trimesters (Bothwell *et al.*, 1989).

High fertility and closely spaced pregnancies placed additional stress on the iron stores of women in these communities. Iron stores, as reflected by SF concentration, were lower among grandmultiparae (parity >6), women who had delivered ≤12 months previously and women who had never used modern methods of contraceptives. Without effective contraceptive methods, there is poor opportunity to avert the substantial iron cost associated with each pregnancy (Hallberg, 1988) and the concomitant increase in menstrual blood loss with parity (Andrade *et al.*, 1991).

It is against this background of poor dietary iron intake and continuous child-bearing that the iron status and Hb concentration of pregnant women is further deteriorated by parasitic infections. Intestinal nematode infections were endemic in both rural and peri-urban communities. Many pregnant women were found to be infected with *Trichuris trichiura* (71.9%) and *Necator americanus* (66.5%), infections which cause intestinal blood loss (Roche & Layrisse, 1966; Layrisse *et al.*, 1967) and are likely to elevate group iron requirements. A smaller proportion were infected with *Ascaris lumbricoides* (21.1%) and the trematode *Schistosoma mansoni* (9.7%). Faecal egg counts were highly overdispersed, with 75% of the total egg count of *A. lumbricoides*, *N. americanus* and *T. trichiura* excreted by 12.8%, 22.8% and 25.0% of subjects respectively.

The Hb concentration of the women in the first trimester of pregnancy was found to be inversely related to the intensity of *N. americanus* infection. The majority of women harboured light *N. americanus* infections and only 10.3% of women had egg counts exceeding 1,000 egg per gram. However, a substantial proportion of these women were clearly in precarious iron balance. Those with low or depleted iron stores were presumably absorbing iron at a maximal rate and therefore had little capacity to compensate for any additional iron losses. In this context, there is limited capacity to buffer the blood losses associated with infection, and low intensity infections are not meaningless.

Even though the effect of *N. americanus* infection on anaemia is believed to be primarily mediated through iron deficiency, there was no significant relationship between SF concentration and the intensity of *N. americanus* infection after controlling for other maternal factors. This finding may indicate that the association between intensity and SF concentration is concealed by some other mechanism that is unrelated to iron metabolism. For example, the intake of folate may be reduced due to anorexia induced by infection or SF concentration may be elevated independently of iron status due to concomitant infections and inflammatory disorders. The relationship between SF concentration and *N. americanus* infection may also be confounded by variable dietary iron intake by the subjects. It is a limitation of the present study that dietary iron intake could not be accurately quantified, or that the role of other important determinants of anaemia, such as malaria, sickle cell disease and other nutritional deficiencies, was not examined. Over one-third of women had anaemia without severely depleted iron stores (37.5%), which clearly indicates that other aetiological factors besides iron deficiency contribute to maternal anaemia in these communities. Many infections such as urinary tract infections are associated with a degree of anaemia, and these infections may elevate serum ferritin levels independently of iron status. Anaemia without depleted iron stores could therefore be an artifact of high serum ferritin levels due to infection.

There were marked seasonal differences in the parasitological and nutritional status of the women in the first trimester of pregnancy. Women assessed in the early dry season had a higher prevalence of *N. americanus* infection, lower fat stores (as indicated by upper arm fat area) and lower iron stores than women assessed in the late dry or early wet season. No comparison can be made with the late wet season, as no women were enrolled during this season. The early dry season immediately follows the previous wet season, when the relative high humidity and lower temperatures are most conducive to the survival and development of *N. americanus* larvae (Smith, 1990). The incidence of new infections is likely to rise during the rains as the population density of infective larvae in the soil increases (Chandiwana, 1990). The low fat and iron stores among women in the early dry season may result from mobilisation of these stores during the wet season. Seasonal alterations in energy balance are probably not profound in Sierra Leone because cassava and yams are grown as reserve crops, replacing rice during the wet season, and imported rice is widely available in the Western Area. However, there is increased physical activity related to agriculture (Aitken, 1990; personal observations), which may result in negative energy balance. The availability of citrus fruits and mangoes declines rapidly during the rains, and therefore non-haem iron absorption may be reduced by the lower intake of vitamin C. The intake of fish (and thus haem iron) may also be reduced, as less attention is given to fishing activities due to the increased demand for manpower in the fields. The incidence of malaria also increases during the wet season (Aitken, 1990; Blacklock & Gordon, 1925). It is therefore is not surprising that women who were enrolled in the early wet season, and had a large proportion of their pregnancy in the wet season, experienced a significantly greater decline in Hb concentration during pregnancy than women enrolled in the dry season.

A causal relationship between intestinal nematode infection and maternal anaemia cannot be affirmed using cross-sectional analyses, as the association may be coincidental or due to some unknown confounding factor. Randomised placebo-controlled intervention studies provide a robust means of confirming a causal pathway, and at the same time produce information on the efficacy of the alternative interventions. The longitudinal findings reported from this study may be biased by the loss to follow-up of 59 subjects, including eight women who were withdrawn at the second trimester assessment due to anaemia (Hb <80 g/l).

Nonetheless, the intervention groups were similar at baseline with respect to their social, demographic and reproductive characteristics, the prevalence and intensity of intestinal nematode infections, anthropometric measures of body size and composition (with the exception of upper arm fat area), Hb concentration, SF concentration and the prevalence of iron deficiency, anaemia and iron-deficiency anaemia. One other exception was the season at baseline, and the effects of intervention were controlled for this variable in the multivariate analyses.

Albendazole, administered as a single dose at the beginning of the second trimester of pregnancy, was highly effective in eliminating infection and reducing the baseline egg counts of *A. lumbricoides* and *N. americanus*. Albendazole was less effective in clearing *T. trichiura* infections but reduced the geometric mean egg counts considerably. The protection afforded by a single dose of albendazole, in terms of reducing the intensities below the levels associated with morbidity, extended for the duration of pregnancy for all infections in these communities. While this finding suggests that re-treatment with anthelminthics is unnecessary during pregnancy, it should be noted that the full yearly cycle of seasons was not covered in the study, as women were recruited over a six month period from December to June. Iron-folate supplements did not appear to induce any effect on the prevalence or intensity of infection.

Intervention with daily iron-folate supplements and/or albendazole treatment was not effective in improving Hb or SF concentration or in decreasing the prevalence of iron deficiency, anaemia or iron-deficiency anaemia between baseline and the third trimester. However, these values did not change significantly in the group of women who received both iron-folate supplements and albendazole (intervention group FeA). In addition, the prevalence of iron deficiency and baseline SF concentration did not change significantly in the group of women who received iron-folate supplements and the albendazole placebo (intervention group FeP_A).

After controlling for baseline Hb concentration and season, the mean benefit of iron-folate supplements and albendazole treatment, relative to their respective controls, on the change Hb concentration between baseline and the third trimester was 13.7 g/l Hb and 6.6 g/l Hb respectively. The effects of iron-folate supplements and albendazole treatment were additive, and thus the benefit associated with intervention group FeA was 20.2 g/l. The effect of albendazole treatment may be even greater in the rural provinces of Sierra Leone, where higher intensities of *N.americanus* infection have been reported (see Appendix 2). Women who received iron-folate supplements (intervention groups FeA and FeP_A) also experienced less decline in baseline SF concentration. Albendazole treatment did not appear to influence the change in SF concentration, which may indicate that iron savings associated with treatment were not sufficient to allow net storage of iron. Alternatively, the effects of albendazole treatment may have been confounded by concomitant infections, inflammatory processes or heterogeneous dietary iron intake across the intervention groups.

These findings confirm that intestinal nematode infections and iron-folate deficiency are important aetiological factors of low Hb concentration in these women. They support an earlier study conducted in Sri Lanka, which reported that a course of mebendazole administered after the first trimester of pregnancy resulted in a marked increase in Hb concentration compared with iron-folate supplements alone (Atukorala *et al.*, 1994). The same study, which also found a significant improvement in maternal iron status, has frequently been quoted in support of hookworm control as a strategy to control maternal anaemia during pregnancy. However, the prevalence and particularly the intensity of intestinal nematode infections were not measured in these women. As the results cannot be related to the severity of infection, it is difficult to extrapolate the results of this study to other communities where the prevalence and intensity of intestinal nematode infections are known. The findings from the present study indicate that in the context of increased nutritional requirements due to pregnancy and underlying iron deficiency due to poor dietary iron intake, menstruation and high fertility, the removal of light intestinal nematode infections can result in a marked Hb benefit that is independent of the effects of iron-folate supplementation.

Although the iron-folate supplements effected a greater improvement in Hb and SF concentration than the albendazole treatment, there are other advantages associated with albendazole. The Hb benefit induced by albendazole was achieved by a single dose of treatment, which was easily administered under supervision at the antenatal clinic. This benefit was therefore not dependent on further follow-up and re-treatment. In contrast, the Hb benefit induced by iron-folate supplements relied upon the daily intake of tablets by the pregnant women. In developing countries, many women take inadequate iron-folate supplements during pregnancy, either due to poor supply of these supplements or due to poor compliance (ACC/SCN, 1991; Galloway & McGuire, 1994). Once iron-folate supplementation is discontinued, the Hb levels may decline if the causes of anaemia (including gastrointestinal blood loss) are not addressed. In this respect, hookworm control may be more effective than iron-folate supplementation, albeit less efficacious under controlled conditions. Albendazole may also improve food intake by correcting anorexia, by reducing nutrient loss due to malabsorption, diarrhoea and vomiting (Stephenson, 1987a), and may reduce the risk of complications due to ectopic migration of nematode larvae and worms (Pinus, 1985; MacLeod, 1988). The benefits of albendazole treatment may even persist postnatally, although this could not examined in the present study due to the large number of treatment-related drop-outs. Nonetheless, it is clear that the poor iron and Hb status of women in this community cannot be corrected with albendazole alone. A substantial number of pregnant women with no evidence of *N. americanus* infection were iron deficient and had iron-deficiency anaemia, indicating profound underlying iron deficiency. Iron-folate supplementation is therefore an essential component of anaemia control in these pregnant women.

While intervention with FeA minimised the decline in Hb or SF concentration, it did not normalise these levels or even elicit a convincing improvement in group mean concentrations. Further studies are needed to determine whether the haematological response in these women was limited by some other factor or whether the frequency and/or dose of the iron supplements were insufficient. The dose of folate was probably adequate, as 5 mg/day folate is considered sufficient to replenish any subject's body stores (Fleming, 1989), and re-treatment with albendazole was not indicated during pregnancy.

Both the baseline analyses and longitudinal analyses of the effects of intervention suggest that intestinal nematode infections do not pose a threat to maternal body size and growth during pregnancy. Given the mild degree of underlying chronic undernutrition and chronic energy deficiency in these women, the relatively low grade intestinal nematode infections may have been insufficient to effect an impact on maternal growth. It is feasible, however, that the impact of infection on anthropometric measures of nutritional status may have been concealed by physiological or behavioural adaptations to infection. For example, the symptoms associated with intestinal nematode infection may have reduced physical activity, thus conserving energy and compensating for nutrient imbalances due to infection (Durnin, 1993).

Measures of weight, length, gestational age at delivery and Hb concentration were obtained from the 105 neonates of mothers who remained in the study until delivery. Unfortunately, the impact of maternal iron-folate supplementation and albendazole treatment on these outcomes could not be evaluated, as the effects of intervention may have been distorted by the large number of intervention-related drop-outs at the third trimester assessment and by the variable time interval between delivery and the neonatal assessment. However, exploratory analyses were performed to identify maternal determinants of the neonatal outcomes. These analyses indicated that the weight of the neonates was inversely related to the decline in maternal Hb concentration between baseline and the third trimester, after controlling for various maternal and background factors including gestational age at delivery, maternal weight gain between baseline and the third trimester assessment, neonate sex and primigravidity. For each gram per litre decline in baseline maternal Hb concentration, mean neonatal weight decreased by 7 g. It is feasible that iron-folate supplementation and/or albendazole treatment may have mediated these findings, given that women who received these interventions experienced less decline in baseline Hb concentration and these nutrients have an important role in erythropoiesis, cellular growth, metabolism and immune function (Wagner, 1995; Prasad & Prasad, 1991). However, this suggestion is speculative, and the findings do not confirm a causal pathway between maternal Hb concentration (or intervention) and neonatal weight. Nonetheless, the findings justify further investigations to establish the role of maternal anaemia in the aetiology of low birth-weight in these communities, and the possible merits of intervention with iron-folate supplements and albendazole treatment. Arguments for these interventions during pregnancy will be greatly supported if the advantages for the fetus as well as mother are more clearly defined.

No significant relationship was found between maternal Hb or SF concentration and neonatal length, Hb concentration or gestational length. None of the stool samples obtained from 101 neonates were found to contain intestinal nematode eggs. The latter findings do not preclude vertical transmission of *A. lumbricoides* infection in this setting as prepatent infections are not detectable using the Kato-Katz method of diagnosis for intestinal nematode infections.

Adequate control of maternal iron deficiency and anaemia during pregnancy is essential in Western Sierra Leone, given that this setting is highly infectious for several deleterious microparasitic infections. If the iron or anaemia status of pregnant women is neglected during pregnancy, severe anaemia in the third trimester may be treated with parenteral iron or blood transfusion. Both forms of treatment carry a risk of several deleterious microparasitic infections that are transmitted by blood, either from contaminated syringes or via transfusion of blood products from an infected donor. Obviously, this applies only to settings where unsterilised needles are reused among patients, or where there is inadequate screening of donor blood or recipient prophylaxis (Fleming, 1989; Fleming, 1991; WHO, 1993). The risks are substantial in this setting, given the high sero-prevalence of markers of past or present microparasitic infection in the pregnant women. None of the pregnant women was found to be infected with human immunodeficiency virus, but the relatively high prevalence of past or present syphilis infection (7.8%) suggests a high incidence of risky sexual behaviour. The high carrier rate for hepatitis B virus (11.3%) indicates that this infection is highly endemic among the antenatal population, and integration of the hepatitis B virus vaccine into the Expanded Programme on Immunisation in Sierra Leone is recommended. Most women were seropositive to past markers of cytomegalovirus (82.7%), but these women were not fully immune as a secondary infection may result from reactivation of a latent infection or reinfection (Huang *et al.*, 1980). Prior toxoplasmosis infection was indicated in 76.0% of women. None of the examined markers of past or present microparasitic infection was found to be related to maternal Hb concentration, SF concentration or the prevalence of anaemia, iron deficiency or iron-deficiency anaemia in the first trimester of pregnancy. These findings may reflect the time interval since infection in the case of toxoplasmosis and cytomegalovirus infection, and the relatively small number of women infected with syphilis and hepatitis B virus infections.

Ethnographic research indicated that traditional pregnancy care among the Temne ethnic group of Western Sierra Leone involves proscriptions related to dietary intake that safeguard the health of the mother and fetus. As the proscripted food items do not make a sizable contribution to the habitual diet, they are unlikely to be a significant determinant of poor iron nutriture during pregnancy. The ideology that underlies these dietary adjustments supports the notion that poor pregnancy outcomes can be prevented by modifying the diet. Therefore, health education to improve the intake of absorbable iron and other haematopoietic nutrients should not conflict with these traditional beliefs and practices if the benefit to fetal and infant health is emphasised.

Both intestinal worm infections and anaemia and are perceived by the Temne to cause significant morbidity among pregnant women. The signs and symptoms of these illnesses are well recognised, although traditional concepts of worm and anaemia aetiology still persist, even among those with some knowledge of Western biomedical principles. The Temne appear to view worms in the biomedical view, that is, as foreign elements and hostile invaders of the body. Most people understand that intestinal worm infection may be acquired from food items, particularly under unhygienic conditions, although few appeared to recognise the pericutaneous route of transmission, and more importantly, the central role of faecal pollution. These infections are not usually regarded as supernatural in origin, in contrast to maternal anaemia, which is frequently attributed to witchcraft. Maternal anaemia may also have a natural explanation, such as poor dietary intake, disease, trauma or failure to take preventative medicine. Approximately one quarter of respondents recognised the link between intestinal worm infection with anaemia, perhaps in response to knowledge of the study.

Many Temne people still believe that anthelminthic treatment, whether traditional or Western, is contraindicated during pregnancy due to adverse affects on the fetus. Prevention and treatment for anaemia is actively sought during pregnancy, but knowledge of iron prophylaxis is poor. The Temne have not abandoned traditional therapies for intestinal worm infections and anaemia. Those who favour traditional therapies do so because they are more efficacious than Western treatments (particularly if an illness episode is believed to have a supernatural aetiology), more widely available and less expensive.

The low confidence in the safety and efficacy of Western pharmaceuticals could present a major obstacle to an anaemia control programme. However, the experiences of the field trial indicated that the pregnant women were amenable to the assurances given by the field assistants. These pregnant women were receptive to their own health requirements, and the field assistants were able to promote the interventions by highlighting the connection between maternal nutritional status and pregnancy outcome. None of the women expressed concern over albendazole treatment and they clearly valued the red iron supplements, not surprisingly since the use of red medications to prevent anaemia follows logically from the traditional belief that red food items promote the quality of the blood. Only two women complained of mild gastro-intestinal side-effects due to these supplements. It is necessary to point out, however, that the side-effects of iron are likely to be greater at higher doses, which are probably necessary in this population, and these side-effects might have a negative impact on compliance. The possibility that side-effects could be minimised at higher doses by administering the supplements at weekly or biweekly intervals rather than daily has been reported (Lui *et al.*, 1994; Chew *et al.*, 1996; Viteri *et al.*, 1996; Yip, 1996), and needs to be further examined in this population.

The inadequate and unreliable supply of iron-folate supplements and low utilisation of public health services are major constraints in most anaemia control programmes (ACC/SCN, 1991; Galloway & McGuire, 1994). Many people, particularly those in rural areas, remarked that Western drugs are poorly available. Most antenatal clinics in the study areas distribute iron and vitamin supplements to pregnant women, but the supply to the clinics is sometimes intermittent. In any case, these clinics are underutilised by pregnant women, who usually attend late in pregnancy, if at all. Pregnant women believe that they should conceal their pregnancy for as long as possible in order to protect themselves and their fetuses from the malicious intentions of malevolent beings in the community. This practice delays attendance at antenatal clinics and reduces the time for interventions to correct or control anaemia. Multigravidae are often complacent about their requirements for antenatal services, particularly if they have not experienced major problems during previous pregnancies. Other women may not attend the antenatal clinics due to the long distances to the clinics, other more pressing commitments or due to the lack of support from their partners.

The feasibility of addressing parasitic infections and anaemia through the traditional health sector could be considered as a means to improve compliance. Traditional birth attendants (TBAs) are highly regarded members of the community who share the same concepts of health and illness as the pregnant women and have detailed knowledge of the traditional beliefs and practices. They are found in almost every village throughout the country, and may be the only maternal health care providers available to women in remote areas. Many TBAs in Sierra Leone have attended formal training programmes to promote safe and hygienic practices (Ross, 1991). These individuals could be trained to provide information on anaemia during pregnancy and supplied with iron-folate supplements and anthelminthics to distribute among pregnant women.

12.3 IMPLICATIONS FOR THE CONTROL OF PARASITIC INFECTION AND ANAEMIA DURING PREGNANCY IN SIERRA LEONE

The findings of the present study indicate that intestinal nematode infections contribute to poor Hb levels in pregnant women in Western Sierra Leone. Anthelminthic treatment should therefore be included in strategies to control maternal anaemia in this setting. It is recommended that pregnant women routinely receive a single course of anthelminthics after the first trimester, alongside daily iron-folate supplements, to minimise the decline in maternal Hb concentration during pregnancy. Mass treatment of pregnant women is recommended as (1) the prevalence of intestinal nematode infections and anaemia is high (2) routine screening is not practical due to economic and logistic constraints and the loss to follow-up that these investigations would entail (3) there were no well defined groups of pregnant women at increased risk of high intensity infections or anaemia, and therefore all pregnant women should be regarded as high risks subjects.

Given the high prevalence of parasitic infections and anaemia during pregnancy in Western Sierra Leone, the need to reach pregnant women with appropriate interventions is urgent. Both iron-folate supplements and anthelminthic treatment are relatively simple and low cost interventions (ACC/SCN, 1991; Stoltzfus *et al.*, 1996), and are clearly efficacious. However, the effectiveness of these interventions as strategies to reduce the prevalence of maternal anaemia is dependent upon an effective distribution infrastructure (WHO, 1993). A feasibility study should be carried out in Sierra Leone to investigate the effectiveness of iron-folate supplements and anthelminthic treatment during pregnancy under realistic field conditions. Such a study should investigate the use of Western and traditional health structures as distribution routes. The World Health Organization recommends that actions to control maternal anaemia and intestinal nematodes be integrated into existing primary health care structures to minimise the cost of drug delivery and optimise access to treatment (WHO, 1996b). In both Sri Lanka and the Seychelles, hookworm control is already included in the national strategies for the prevention and control maternal anaemia during pregnancy (Ministry of Health and Women's Affairs, 1994; WHO, 1996b). Such national strategies, which include free treatment, are still beyond the limited health care budget of many African countries. In these countries, control interventions can be made available to pregnant women at a cost recovery price through the Bamako Initiative Programme. This programme has been implemented in many sub-Saharan countries, including Sierra Leone, as a means of improving the sustainability of primary health care and thus increasing the availability of essential drugs (Habiyambere & Wertheimer, 1993). In Sierra Leone, the government antenatal clinics have an existing infrastructure to distribute iron and vitamin supplements to pregnant women, and the integration of intestinal nematode control is unlikely to incur any significant additional cost. The availability of anthelminthic treatment may encourage earlier and more widespread attendance at the antenatal clinics. However, pregnant women residing far from antenatal clinics are likely to be disadvantaged. Furthermore, many pregnant women are reluctant to attend antenatal clinics until late in pregnancy, when the time left for interventions is reduced, or are unable to attend due to due poor support from their partners. The possibility of supplying TBAs with iron-folate supplements and anthelminthics to distribute among pregnant women could be investigated as a means of improving coverage and compliance.

The study also highlights the need to incorporate other forms of public health interventions that are potentially effective in preventing and controlling maternal parasitic infection and anaemia during pregnancy. The ultimate goal for the control of intestinal nematode infections is the prevention of infection through improvements in environmental health, including the provision of safe drinking water and adequate disposal of sewage. These remain distant goals for many resource poor countries like Sierra Leone. In the meantime, culturally appropriate health education should form an essential component of a control programme. Health education is an integral part of government antenatal services in Sierra Leone, and information on soil-transmitted helminths could be integrated into the existing format. Maternal health care providers can encourage improved personal hygiene and sanitation in order to break the transmission cycle of intestinal parasites and prevent reinfection among their patients. These health care messages should be combined with health education on ways to improve the intake and bioavailability of dietary iron and on fertility regulation to reduce the number of unplanned pregnancies. Pregnant women should also be informed of the risks of maternal and fetal microparasitic infections, which have largely been neglected in the context of antenatal care.

To effect these measures, it is essential that maternal health care providers at all levels receive training to increase their awareness of the risks of intestinal nematode infections and microparasitic infections to maternal and fetal health so that they can inform and advise their patients on the possibilities for preventative and therapeutic measures. They should also be made aware that any health care interventions must be responsive to the traditional beliefs and practices. Since many people in Sierra Leone, including the Temne people, continue to hold onto their traditional world view, biomedical health messages that are not consistent with their traditional way of thinking are less likely to be effective (Weiss, 1988; Opala & Boillot, 1996). For example, if maternal health care providers explain that women need 'strong' blood for a healthy pregnancy, the red iron supplements are likely to be considered desirable among Sierra Leonean women. However, if too much emphasis is placed on the connection between iron supplements and improved birth-weight, women who attempt to limit the size of the fetus to prevent complicated deliveries may be less enthusiastic about these supplements.

In developing countries, women rarely have contact with Western health services outside pregnancy, and therefore pregnancy is an opportune time to implement interventions to improve the health of women and their fetuses. However, it is increasingly recognised that the iron status of non-pregnant women should also be improved so that women enter pregnancy with adequate iron stores (Viteri, 1997). Several iron supplementation programmes have indicated that the dose, frequency or duration of iron supplementation is less important than the initial Hb concentration in determining Hb concentration at term (Lui *et al.*, 1995; Chew *et al.*, 1996). It has also been suggested that women should take anthelminthic treatment throughout their lives and not only during pregnancy (Brabin & Brabin, 1982; Stoltzfus, 1996b). The latter recommendations must to be approached with caution, at least in Sierra Leone. Mass anthelminthic treatment of non-pregnant women in Sierra Leone is not recommended unless pregnant women in the first trimester can be accurately identified and excluded. Pregnant women from a number of ethnic groups in Sierra Leone, including the Temne, conceal their pregnancy for the first few months. Women in their first trimester of pregnancy may therefore inadvertently receive anthelminthic treatment if this practice, which is deep-rooted in the women's belief system and may be resilient to change, is not appropriately addressed.

12.4 CONCLUSIONS

The findings from Western Sierra Leone lend support to the recommendations made by the World Health Organization regarding the use of anthelminthic treatment to control maternal anaemia in areas endemic for intestinal nematode infections. In this setting, pregnant women should take a single course of anthelminthic treatment at their first contact with antenatal health services after the first trimester. Pregnant women should also take iron-folate supplements to replace iron and folate stores and minimise the decline in Hb concentration.

The implications of the findings for public health policy in other antenatal populations will depend on the local epidemiology of intestinal nematode infections and on the extent of underlying maternal iron deficiency and anaemia. Therefore, the findings of the present study should not be blindly extrapolated to other communities, and each antenatal control program should be designed to reflect the local epidemiological situation. For example, in areas where reinfection with intestinal nematodes is rapid, re-treatment with anthelminthics may be indicated during the pregnancy, while in populations with high dietary intake of iron, these infections may have little impact on maternal nutritional status. Notwithstanding, one of the most revealing findings from the present study is that in settings where there is high fertility, widespread anaemia and profound underlying iron deficiency, low intensity intestinal nematode infections are not meaningless in pregnancy and should be addressed.

Appendices

Appendix 1 Glossary of terms and abbreviations

AFA
Arm fat area: the estimated cross-sectional area of the fat in the mid-upper arm, calculated from the triceps and biceps skinfolds and the mid-upper arm circumference.

ΔAFA
Change in arm fat area between baseline (first trimester assessment) and the third trimester assessment.

AIDS
Acquired immune deficiency syndrome.

AMA
Arm muscle area: the estimated cross-sectional area of the muscle in the mid-upper arm, calculated from the triceps and biceps skinfolds and the mid-upper arm circumference.

ΔAMA
Change in arm muscle area between baseline (first trimester assessment) and the third trimester assessment.

Anaemia
A disorder in which the blood haemoglobin concentration is lower than normal (Hb <110 g/l in pregnant women and Hb <120 g/l in non-pregnant women).

BMI
Body mass index: a measure of body mass relative to height calculated as weight (kg)/height^2 (m^2).

ΔBMI
Change in body mass index between baseline (first trimester assessment) and the third trimester assessment.

BMR
Basal metabolic rate.

CMV
Cytomegalovirus.

EPI
Expanded programme on immunisation.

FAO
Food and Agriculture Organization

FeA
See intervention groups.

FeP_A
See intervention groups.

Gestational age
Duration of pregnancy, expressed in weeks or months.

Grandmultigravidae
Gravidity ≥ six.

Gravidity
The number of all previous pregnancies regardless of the gestation at which they ended, including the current pregnancy.

Hb
Haemoglobin

ΔHb
Change in haemoglobin concentration between baseline (first trimester assessment) and the third trimester assessment.

HBV
Hepatitis B virus.

HIV
Human immunodeficiency virus.

Incidence of infection
Number of new cases of infection appearing in a population within a given period of time divided by number of uninfected individuals in the population at the beginning of the time period.

Intensity
Number of individuals of a particular parasite species in an infected host. In the present study, the intensity of intestinal nematode infection was estimated indirectly as eggs per gram faeces.

Iron deficiency
Insufficient supply of iron for optimal physiological production and function of iron dependent cellular components, as reflected by SF <20 μg/l in pregnant women in the present study.

Iron-deficiency anaemia
Anaemia caused by inadequate supply of iron for the synthesis of haemoglobin, as reflected by Hb <110 g/l and SF <20 μg/l in the same pregnant woman in the present study.

Intervention groups
The intervention groups are defined as follows:
FeA = iron-folate supplements and anthelminthic
FeP_A = iron-folate supplements and anthelminthic placebo
$P_{Fe}A$ = iron-folate placebo and anthelminthic
$P_{Fe}P_A$ = iron-folate placebo and anthelminthic placebo

IUGR
Intrauterine growth retardation: defined as the 10th percentile of birth-weight-for-gestational age.

LBW
Low birth-weight, defined as birth weight <2500 g.

Mean intensity of infection
The mean number of individuals of a particular parasite species per infected host in a sample.

Miscarriage
Delivery before 28 weeks gestation.

MUAC
Mid-upper arm circumference: the circumference of the upper arm measured at the mid-point between the tip of the acromion process and the tip of the olecranon process.

Neonatal
The period of life extending for 28 days following delivery.

NGO
Non-government organisation.

Parity
The number of previous pregnancies that have ended after 28 completed weeks.

$P_{Fe}A$
See intervention groups.

$P_{Fe}P_A$
See intervention groups.

Post-term
Birth at >42 weeks gestation.

Preterm delivery
Birth at <37 weeks gestation.

Prevalence
The proportion of a population with an infection, disease or condition.

Recommended daily intake (RDI)
Also known as safe level of intake: the level of intake that will maintain health and appropriate nutrient reserves in almost all healthy people, with a very low risk of nutrient depletion.

Safe level of intake
See recommended daily intake.

Seasons in Sierra Leone
Early dry: November to February
Late dry: March to April
Early wet: May to July
Late wet: August to October

Sero-prevalence
The proportion of individuals in a population whose serum is positive for a specific immunological marker of past or present infection.

SES
Socio-economic status.

SF
Serum ferritin

ΔSF
Change in serum ferritin concentration between baseline (first trimester assessment) and the third trimester assessment.

Stillbirth
Delivery of a dead fetus at or after 28 weeks gestation.

TPHA
Treponema pallidum haemagglutination assay.

Trimesters of pregnancy
The trimesters of pregnancy are defined as follows:
First <14 weeks gestation
Second 15-28 weeks gestation
Third >28 weeks gestation.

WHO
World Health Organization

WT
Body weight.

ΔWT
Change in body weight between baseline (first trimester assessment) and the third trimester assessment.

Appendix 2 Results of previous surveys for intestinal nematode infections in Sierra Leone.

Site	Sample size	Age (years)	Prevalence % (intensity: eggs per gram faeces)				Reference
			Hookworm	*A. lumbricoides*	*T. trichiura*	*Strongyloides*[†]	
Connaught Hospital, Freetown	9,203	Mixed	13.1	18.5	12.3	9.7 (SS)	Williams (1974)
Njala	38 villages	Mixed	24.8	37.6	13.1	-	White (1977)
Freetown	111	3-14	5.4	34.2	18.0	3.6 (SS)	Awosika-Sekoni (1987)
Freetown	70	0-5	4.3	10.0	5.7	-	Williams (1988)
Masanga Leprosy Hospital	5,550	Mixed	31.6	6.0	3.5	4.5 (SS)	Hodges (1988)
Freetown	148	0-5	16.2	17.6	8.1	1.4 (SS)	Grant (1989)
Moyamba District	1,200	5-40	26.0	77.0	15.0	3.3 (S?)	Alghali *et al.* (1990).
Freetown	343	5-9	20.7 (108)	43.4 (2,659)	80.5 (292)	4.7 (SS)	Webster *et al.* (1990)
Tabe River	379	>5	85.0	56.0	23.0	6.0 (S?)	Whitworth *et al.* (1991)
Freetown	129	Mothers	11.5	38.5	46.9	-	Wilson *et al.* (1991)
Alikalia	60	Mothers	59.0	40.6	10.0	-	Wilson *et al.* (1991)

† SF = *Strongyloides fuelleborni*; SS = *Strongyloides stercoralis*; S? = *Strongyloides*, species not specified

Results of previous surveys for intestinal nematode infections in Sierra Leone (continued)

Site	Sample size	Age (years)	Prevalence % (intensity: eggs per gram faeces)				Reference
			Hookworm	*A. lumbricoides*	*T. trichiura*	*Strongyloides*[†]	
Freetown and Alikalia	191	<1	0.0	1.6	1.6	-	Wilson *et al.* (1991)
Moyamba (rural)	305	0-5	19.3	28.2	10.8	8.9 (SF) 4.3 (SS)	Crompton *et al.* (1992)
Rowollon	465	Mixed	67.1 (1,149)	20.9 (4,277)	48.6 (162)	0.4 (SS) (271)	Ewald *et al.* (1993)
Kroo Bay	343	Children	1.5	23.6	33.2	-	Rogers (1992)
Freetown	148	0-5	4.1	11.5	12.2	-	Smithie (1992).
Foria	451	Mixed	61.6 (867)	32.8 (6,022)	1.1 (30)	2.9 (SS) (459)	Ewald *et al.* (1993)
Kroo Bay	429	Mixed	20.8 (632)	25.6 (4,950)	59.2 (453)	0.9 (SS) (582)	Ewald *et al.* (1993)
Blama	334	Mixed	48.2 (950)	42.5 (1,890)	0.0	-	Bayoh & Hodges (1994)
York	320	Mixed	60.3 (650)	37.8 (1,987)	33.8 (362)	-	Bayoh & Hodges (1994)

[†] SF = *Strongyloides fuelleborni*; SS = *Strongyloides stercoralis*; S? = *Strongyloides*, species not specified

Results of previous surveys for intestinal nematode infections in Sierra Leone (continued)

Site	Sample size	Age (years)	Prevalence % (intensity: eggs per gram faeces)				Reference
			Hookworm	*A. lumbricoides*	*T. trichiura*	*Strongyloides*†	
Magbil	266	Mixed	49.2 (1,247)	43.2 (3,650)	21.8 (1,076)	-	Bayoh & Hodges (1994)
Bo	202	Mixed	90.0	39.0	15.0	-	Behnke *et al.*, (1994a).
Njala	1,820	6-17	33.3	10.4	14.6	-	Gbakima *et al.* (1995)
Gloucester	99	6-9	10.0 (203)	32.0 (1,327)	65.0 (413)	6.0 (SS)	Koroma (1995)
Alicalia, Koinadugu District	98	6-9	25.0 (220)	46.0 (1,579)	1.0 (100)	-	Koroma (1995)
Kissy displaced camp	182	6-9	45.1	22.5	19.8	-	Yarjah (1996)
Bo displaced camp	230	Pregnant women	52.2	28.7	11.3	-	Yarjah (1996)
Moyamba	207	1-5	26.5 (171)	18.8 (2,333)	30.4 (163)	0.5 (SS)	Alcorn *et al.* (1999)

† SF = *Strongyloides fuelleborni*; SS = *Strongyloides stercoralis*; S? = *Strongyloides*, species not specified

Appendix 3 Pilot studies

The pilot studies were conducted in peri-urban Freetown (Allentown, Wellington, Portee and Regent) in July 1995[1] and rural Kaffu-Bollom Chiefdom (Konakridee, Yongoro and Tagrin) in November 1995. Pregnant women attending government and non-government organisation antenatal clinics in the study sites were invited to participate.

A preliminary version of the demographic, social and reproductive questionnaire was piloted. Information on maternal age, marital status, maternal education, maternal and paternal occupation, sanitary facilities, source of drinking water, number of children, previous pregnancies and current gestational age was obtained from all pregnant women. A fresh stool sample was collected from 110 pregnant women in peri-urban Freetown and 124 pregnant women from Kaffu-Bollom Chiefdom. The samples were fixed in 10% formalin within 60 min of collection, and examined for intestinal nematode eggs using a modified version of the Kato-Katz technique (Robertson *et al.*, 1989). A capillary blood sample was taken from the left index finger of 86 pregnant women in peri-urban Freetown. The haemoglobin concentration was measured using the QBC Centrifugal Hematology System (Becton Dickinson, New Jersey, USA).

The demographic, social and reproductive characteristics of the pregnant women in the pilot studies are summarised in Table A3.1. The prevalence and intensity of intestinal nematode infections and *Schistosoma mansoni* infection among these women is shown in Table A3.2. The mean haemoglobin concentration of 86 pregnant women in peri-urban Freetown was 90 (SD 12.7) g/l. The prevalence of anaemia (Hb <110 g/l) and severe anaemia (Hb <70 g/l) was 94.2% and 7.0% respectively.

The results indicate a high prevalence of intestinal nematode infections and anaemia. On the basis of these results, the decision was made to base the study in rural Kaffu-Bollom Chiefdom where a high prevalence of hookworm were expected, with an additional smaller sample from peri-urban Freetown. The intensities of intestinal nematode infections among the pilot sample were rather low, but the security situation prohibited investigations further afield, where higher intensities were anticipated.

---

[1] Conducted by M.M. Koroma prior to the arrival of the principal investigator in Sierra Leone.

Table A3.1 Demographic, social and reproductive characteristics of pregnant women participating in pilot studies in peri-urban Freetown and Kaffu-Bullom Chiefdom, Western Sierra Leone.

Characteristic			Peri-urban Freetown (*n*=110)	Kaffu-Bullom Chiefdom (*n*=124)
Age (years)		mean (SD)	24 (5.8)	26 (7.5)
Education	none	frequency (%)	56 (50.9)	95 (76.6)
	primary	frequency (%)	31 (28.2)	13 (10.5)
	secondary	frequency (%)	23 (20.9)	16 (12.9)
Marital status	single	frequency (%)	11 (10.0)	3 (2.4)
	married	frequency (%)	98 (89.1)	121 (97.6)
	widowed	frequency (%)	1 (0.9)	0 (0.0)
Occupation	housewife	frequency (%)	34 (30.9)	16 (12.9)
	farmer	frequency (%)	16 (14.5)	12 (9.7)
	trader	frequency (%)	57 (51.8)	96 (77.4)
	other	frequency (%)	3 (2.7)	
Occupation of partner	farmer	frequency (%)	20 (18.5)	15 (12.1)
	trader	frequency (%)	25 (22.7)	28 (22.6)
	fisherman	frequency (%)	3 (2.7)	51 (33.1)
Sanitation	none	frequency (%)	6 (3.5)	23 (19.8)
	pit latrine	frequency (%)	101 (91.8)	101 (81.5)
	flush latrine	frequency (%)	3 (2.7)	0 (0.0)
Water	stream or swamp	frequency (%)	1 (0.9)	0 (0.0)
	well	frequency (%)	31 (28.2)	114 (91.9)
	pump	frequency (%)	78 (70.9)	10 (8.1)
Gestational age (mo)		median (QR)	7 (5, 8)	7 (4, 8)
Gravidity		median (QR)	3 (2, 5)	3 (2, 5)
Number of children		median (QR)	2 (0, 3)	1 (0, 3)

Table A3.2 Prevalence and intensity of intestinal nematode infections and *Schistosoma mansoni* in pregnant women participating in pilot studies in peri-urban Freetown and Kaffu-Bullom Chiefdom, Western Sierra Leone.

Infection	Peri-urban Freetown (*n*=110)		Kaffu-Bullom Chiefdom (*n*=124)	
	Prevalence (%)	Intensity† geometric mean (±SD)	Prevalence (%)	Intensity† geometric mean (±SD)
Ascaris lumbricoides	12.7	732 (-421, +989)	16.9	194 (-163, +1026)
Hookworm	32.7	2960 (-1672, +3841)	62.1	260 (-191, +717)
Strongyloides stercoralis	2.7	-	0.8	-
Trichuris trichiura	15.5	406 (-244, +613)	50.8	88 (-62, +218)
Schistosoma mansoni	0.9	-	0.8	-

† Intensity estimated indirectly as eggs per gram faeces.

Appendix 4 Sample size determination

In order to determine the approximate sample size necessary to detect a statistically significant difference of given magnitude for a particular hypothesis, an *a priori* test is required before field-work commences. The primary research objective, on which the determination of sample size was based, was the effect of iron-folate supplements and/or anthelminthic treatment on the change in haemoglobin concentration between baseline (first trimester assessment) and the third trimester assessment.

As it was not possible to estimate the size of the population variance and the sum of the squared population intervention effects and interaction effects from previous research, the required sample size was determined by specifying the number of levels of iron-folate supplements (p=2), the number of levels of albendazole treatment (q=2), the significance level (α=0.05), the power (β=0.8) and the measure of the strength of association (ϖ). The choice of values for α and β was based on the widely accepted conventions that the probability of making a type I error should be less than or equal to 0.05 and the minimum acceptable power should be greater or equal to 0.80 (Kirk, 1995). The strength of association is defined as the proportion of population variation in the dependent variable that is accounted for by intervention group. Guideline ϖ values are arbitrarily set at ϖ=0.059 and ϖ=0.138 for medium and large associations respectively (Cohen, 1988). Small associations were not considered because the corresponding sample sizes required are very large, and the effect of a small association is unlikely to be of major public health significant compared to other environmental factors than influence iron status. According to the sample-size tables developed by Foster (1993), the required sample size n for iron-folate supplements is n=16 for β=0.8 and ϖ=0.138 (large association) and n=39 for β=0.8 and ϖ=0.059 (medium association). The required sample size for anthelminthic treatment are the same, because iron-folate supplements and anthelminthic treatment have the same degrees of freedom. Assuming a drop-out rate of 20%, the study requires 1.25(npq) subjects. Thus the number of subjects required in each intervention group for a large and medium association is 20 and 49 respectively. The target sample size in each intervention group was set at 49. It should be noted that the power of the test of the intervention interaction is less than 0.80 because the interaction effects are computed from n observations, whereas intervention effects are computed from np or nq observations.

Appendix 5 Ethical permission

Research and Ethics Committe
Department of Health
4th Floor, Youyi Building
Freetown

January 11, 1996

Dr Mary Hodges
St. Andrews Clinic
Bathurst Street
Freetown.

Dear Dr. Hodges,

RESEARCH PROPOSAL: THE CONTRIBUTION OF HOOKWORM INfECTION TO IRON-DEFICIENCY IN PREGNANCY AND PREGNANCY OUTCOME

Please refer to your application, to the Research and Ethics Committee, for clearance to undertake the above investigation; the proposal has been reviewed. It is noted that Dr. Mike Kamara, specialist Obstetrician and Gynaecologist, PCM Hospital, is a member of the Research Team. This obviates the need for the committee's assignment of a counterpart with that speciality.

I confirm that your proposal satisfies the requirements of the Research and Ethics Committee. I congratulate your team and wish you success with the investigations.

With best regards.

Sincerely,

....

Dr. Haruna Rashid Sesay
Chairman, Research and Ethics Committee.

CC: Chief Secretary of State
& Secretary of State
Health and Social Services

Director General Medical Services.

Appendix 6 Baseline questionnaire: demographic, social and reproductive characteristics

1. Name
2. Address
3. Age in years at last birthday
4. Ethnic group
5. Marital status (single; married; divorced; widowed)
6. Occupation
7. Husband or partner's occupation
8. Education level (primary; secondary; tertiary)
9. Toilet facilities on compound (none; pit latrine; flush latrine)
10. Source of drinking water (swamp or river; well; stand-pump or tap)
11. Radio ownership (yes; no)
12. Sickle cell disease (yes; no)
13. Smoke (number of cigarettes smoked per week)
14. Number of previous pregnancies
15. Number of previous miscarriages
16. Number of previous stillbirths
17. Number of live children
18. Time interval between previous delivery and present pregnancy
19. Use of modern contraceptive methods (yes; no)
20. Duration of menstrual bleeding during menstruation (days)

Appendix 7 Ethnographic field guide: traditional beliefs and practices

Section 1: socio-demographic background

1. Name
2. Address
3. Age
4. Education level
5. Occupation

Section 2: dietary adjustments during pregnancy

1. Do women eat more or less when they are pregnant and why?
2. What foods do women avoid during pregnancy and why?

Section 3: intestinal worm infections

1. What are worms?
2. How do you get worms?
3. How can you tell if some-one has worms in their body?
4. What kind of medicine do you take when you get worms? Do you prefer traditional medicine or Western medicine and why?
5. Can pregnant women take worm medicine?
6. Can a pregnant woman pass worms to her fetus?
7. Can a lactating woman pass worms to her infant?

Section 4: anaemia

1. How can you tell if some-one has anaemia?
2. What causes anaemia in a pregnant women?
3. If a pregnant woman becomes anaemic, what treatment should she take?
4. How do women avoid anaemia during pregnancy?

Appendix 8 Daily requirements of energy and nutrients during pregnancy

A8.1 FAO/WHO standard daily requirements of energy and nutrients during pregnancy

The most recently published values of the daily requirements of energy, protein, vitamin A, vitamin C, folic acid, calcium and iron for pregnant women as proposed by the FAO/WHO expert committees are summarised in Table A8.1. Unless otherwise indicated, the value given for each micronutrient is the recommended daily intake (also known as the safe level of intake), which corresponds to the mean requirement plus two standard deviations for a normal distribution or the 95th percentile of a skewed distribution. When sustained, this level of intake will maintain health and appropriate nutrient reserves in almost all healthy people, with a very low risk of nutrient depletion (FAO/WHO, 1988). A joint FAO/WHO expert consultation was held in September 1998 to update the vitamin and mineral requirements, but the revised values have not yet been published.

Table A8.1 Daily requirements of energy protein, vitamin A, vitamin C, folic acid, calcium and iron during pregnancy

Nutrient	Daily requirement	Notes	Reference
Energy (MJ)	10.50 (10.13)	†	FAO/WHO (1985)
Protein (g)	51.7	‡	FAO/WHO (1985)
Vitamin A (RE) (μg)	600	§	FAO/WHO (1988)
Vitamin C (mg)	30 - 50		FAO/WHO (1974)
Calcium (mg)	450 - 1100	††,‡‡	FAO/WHO (1974)
Folic acid (mg)	420	‡‡	FAO/WHO (1988)
Iron (mg)	See notes	§§	FAO/WHO (1988)

† The standard value for energy represents the recommended population mean requirement. The energy requirement is based on a pre-pregnant weight of 55 kg and moderate activity level amounting to 1.6 x BMR (basal metabolic rate). The values were increased by 5% to account for the lower digestibility of a diet moderately high in fibre. The upper value applies if pre-pregnant activity level is sustained throughout pregnancy; the lower value applies if activity level is reduced during pregnancy.

‡ The protein requirement is based on a pre-pregnant weight of 55 kg. The standard values for protein were increased by 10% to account for the lower digestibility of proteins in cereal- and vegetable-based diets. This correction factor is intermediate between that suggested for a diet based on course whole grain cereals and vegetables (85% digestibility) and a diet based on

refined cereals (95% digestibility). No correction was necessary for the amino acid pattern of the diet, as most diets in developing countries appear to meet the amino acid requirements of adults (FAO/WHO/UNO, 1985).

§ RE = retinol equivalents, and is the sum of vitamin A provided by preformed retinol and carotenoids. Recommendations for requirements of vitamin A are based on the assumption that 1 RE equals 6 μg of β-carotene or 12 μg of other provitamin A carotenoids such as α-carotene. This assumption may not always be accurate, as the conversion of carotenoids to retinol depends on a number of factors including the matrix in which the carotenoid is incorporated, the nutrient status of the host, and host-related factors such as gastrointestinal infections and parasites (Mann & Truswell, 1998).

†† The lower value applies to the first half of pregnancy and the upper value to the second half of pregnancy.

‡‡ The value(s) given is (are) the mid-point of the range of values presented in the reference.

§§ There are no recommended daily intake values for pregnant women as iron requirements in the second and third trimester of pregnancy are not expected to be met by dietary intake alone, regardless of the quality of the diet. The median basal requirement for non-pregnant women is 10.5 mg (13 mg including variability), and the median amount required to prevent anaemia in non-pregnant women is 7 mg (9 mg including variability) (FAO/WHO, 1988).

A8.2 Limitations of the standard daily requirements

There are a number of limitations associated with use of RDI standards as indicators of dietary sufficiency, which include the following (Gibson, 1990): (1) they should not be confused with actual requirements, as they are augmented by a factor that takes into account inter-individual variability; for this reason, they cannot be used to quantify the risk of deficiency or malnutrition in an individual or group (2) they are only applicable to groups of healthy people and may not be appropriate for those with pathological disorders such as infections or those with high physical activity levels (3) they are calculated ignoring possible interactions involving nutrients and other dietary components, and assume that the requirements for energy and other nutrients are met.

References

ACC/SCN (1991). *Controlling Iron Deficiency. A Report Based on an ACC/SCN Workshop.* State-of-the-Art Series on Nutrition Policy Discussion Paper No.9. Geneva: Administrative Committee on Coordination/Subcommittee on Nutrition.

ACC/SCN (1997). *Third Report on the World Nutrition Situation.* Geneva: Administrative Committee on Coordination/Subcommittee on Nutrition.

Acquaye, J.K. & Mingle, J.A. (1994). Hepatitis B viral markers in Ghanaian pregnant women. *West African Journal of Medicine* **13**, 134-137.

Adair, L.S. & Bisgrove, E.Z. (1991). Maternal anthropometry during pregnancy, pregnancy weight gain, and pregnancy outcome. In: *Anthropometric Assessment of Nutritional Status*, Himes, J.H. (editor). New York: Wiley-Liss Inc., pp. 233-257.

Adetunji, J.A. (1996). Preserving the pot and water: a traditional concept of reproductive health in Yoruba community, Nigeria. *Social Science and Medicine* **43**, 1561-1567.

Adler, S.P., Finney, J.W., Manganello, A.M. & Best, A.M. (1996). Prevention of child-to mother transmission of cytomegalovirus by changing behaviors: a randomized controlled trial. *Pediatric Infectious Diseases Journal* **15,** 240-246.

Ahlfors, K., Harris, S., Ivarson, S-A. & Svanberg, L. (1981). Secondary maternal cytomegalovirus infection causing symptomatic genital infection. *New England Journal of Medicine* **305**, 284.

Aitken, I.W. (1990). Determinants of low birth-weight among the Mendi of Sierra Leone: implications for medical and socio-economic strategies. *International Journal of Gynecology and Obstetrics* **33**, 103-109.

Ajuwon, A.J., Brieger, W.R., Oladepo, O. & Adeniyi, J.D. (1995). Indigenous surgical practices in rural southwestern Nigeria: implications for disease prevention. *Health Education Research* **10**, 379-384.

Akerele, O. (1987). The best of both worlds: bringing traditional medicine up to date. *Social Science and Medicine* **24**, 177-181.

Alberman, E. (1984). Low birthweight. In: *Perinatal Epidemiology*, Bracken, M.B. (editor). Oxford: Oxford University Press, pp. 86-98.

Albonico, M., Smith, P.G., Hall, A., Chwaya, H.M., Alawi, K.S. & Savioli, L. (1994). A randomized controlled trial comparing mebendazole and albendazole against *Ascaris*, *Trichuris* and hookworm infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 585-589.

Alcorn, N., Pearson, D., Crompton, D.W.T. & Hodges, M. (1999). The association of intestinal helminthiasis, socioeconomic conditions and nutritional status of children 1-4 years old in the Moyamba District. *Journal of the Sierra Leone Medical and Dental Association* (in press).

Alghali, S.T.O., Gage, G.N., Blockarie, A., Collier, H.E., Terry, B.C. & Bangura, S. (1990). *Schistosomiasis in Sierra Leone. A Longitudinal Study of its Epidemiology in Moyamba District, Southern Province. Final Project Report.* Department of Community Health, University of Sierra Leone.

Allen, L.H. (1982). Calcium bioavailability and absorption: a review. *American Journal of Clinical Nutrition* **35**, 783-808.

Allen, L.H. (1993). Iron-deficiency anemia increases risk of preterm delivery. *Nutrition Reviews* **51**, 49-52.

Allen, LH. (1997). Pregnancy and iron deficiency: unresolved issues. *Nutrition reviews* **55**, 91-101.

Altman, D.G. (1982). Misuse of statistics is unethical. In: *Statistics in Practice*, Gore, S.M. & Altman, D.G. (editors). London: British Biomedical Association, pp. 1-2.

Altman, D.G. (1985). Comparability of randomised groups. *Statistician* **34**, 125-136.

Altman, D.G. (1991). *Practical Statistics for Medical Research*. London: Chapman & Hall.

Anderson, R.M. (1986). The population dynamics and epidemiology of intestinal nematode infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 686-696.

Anderson, R.M. & May, R.M. (1979). Population biology of infectious diseases: Part 1. *Nature* **280**, 361-367.

Anderson, R.M. & May, R.M. (1982). Population dynamics of human helminth infections: control by chemotherapy. *Nature* **297**, 557-563.

Anderson, R.M. & May, R.M. (1985). Helminth infections of humans: mathematical models, population dynamics, and control. *Advances in Parasitology* **24**, 1-101.

Anderson, R.M. & May, R.M. (1991a). *Infectious Diseases of Humans: Dynamics and Control.* Oxford: Oxford University Press.

Anderson, R.M. & May, R.M. (1991b). Biology of host - macroparasite associations. In: *Infectious Diseases of Humans: Dynamics and Control.* Oxford: Oxford University Press, pp. 433- 466.

Anderson, R.M. & May, R.M. (1991c). Biology of host - microparasite associations. In: *Infectious Diseases of Humans: Dynamics and Control.* Oxford: Oxford University Press, pp. 27-65.

Anderson, R.M. & Medley, G.F. (1985). Community control of helminth infections of man by mass and selective chemotherapy. *Parasitology* **60**, 629-660.

Andiman, W.A. (1998). Medical management of the pregnant woman infected with human immunodeficiency virus type 1 and her child. *Seminars in Perinatology* **22**, 72-86.

Andrade, A.T., Souza, J.P., Shaw, S.T., Belsey, E.M. & Rowe, P.J. (1991). Menstrual blood loss and body iron stores in Brazilian women. *Contraception* **43**, 241-249.

Annegars, J.F. (1973). Seasonal food shortages in West Africa. *Ecology of Food and Nutrition* **2**, 251-257.

Anyinam, C. (1987). Availability, accessibility, acceptability and adaptability: four attributes of African ethno-medicine. *Social Science and Medicine* **25**, 803-811.

Areekul, S. (1979). The relationship between anaemia and hookworm infection. *Journal of the Medical Association of Thailand* **62**, 378-382.

Areekul, S., Devakul, K., Viravan, C. & Harinasuta, C. (1970). Studies on blood loss, iron absorption and reabsorption in hookworm patients in Thailand. *Southeast Asian Journal of Tropical and Geographical Medicine* **1**, 519-523.

Arthur, C.K. & Isbister, J.P. (1987). Iron deficiency: misunderstood, misdiagnosed and mistreated. *Drugs* **33**, 171-182.

Ashworth, A. (1998). Effects of intrauterine growth retardation on mortality and morbidity in infants and young children. *European Journal of Clinical Nutrition* **52** (supplement), S34-S42.

Atukorala, T.M.S., de Silva, L.D.R., Dechering, W.H.J.C., Dassenaeike, T.S. de C. & Perera, R.S. (1994). Evaluation of effectiveness of iron-folate supplementation and anthelminthic therapy against anemia in pregnancy - a study in the plantation sector of Sri Lanka. *American Journal of Clinical Nutrition* **60**, 286-292.

Aukett, M.A., Parks, Y.A. & Scott, P.H. (1986). Treatment with iron increases weight gain and psychomotor development. *Archives of Diseases in Childhood* **61**, 849-854.

Ault, K.A. & Faro, S. (1993). Viruses, bacteria and protozoans in pregnancy: a sample of each. *Clinical Obstetrics and Gynecology* **36**, 878-885.

Awosika-Sekoni, O.G. (1987). *The Prevalence of Intestinal Helminthiasis Among Children of Fourah Bay College, Freetown*. Dissertation for Diploma in Community Medicine and Health, University of Sierra Leone.

Bailey, K.V. & Ferro-Luzzi, A. (1995). Use of body mass index of adults in assessing individual and community nutritional status. *Bulletin of the World Health Organization* **73**, 673-680.

Bailey, M. (1989). Determinants of fertility in a rural society: some evidence from Sierra Leone. *Social Science and Medicine* **28**, 285-292.

Baker, S.J. & DeMaeyer, E.M. (1979). Nutritional anaemia: its understanding and control with reference to the work of the World Health Organization. *American Journal of Clinical Nutrition* **32**, 368-417.

Bakta, I.M., Widjana, D.P. & Sutisna, P. (1993a). Some epidemiological aspects of hookworm infection among the rural population of Bali, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* **24**, 87-93.

Bakta, I.M., Widjana, D.P. & Sutisna, P. (1993b). Hookworm infection and iron stores: a survey in a rural community in Bali, Indonesia. *Southeast Asian Journal of Tropical Medicine and Public Health* **24**, 501-504.

Balogh, M., Kahn, H.A. & Medalie, J.H. (1971). Random repeat 24-hour dietary recalls. *American Journal of Clinical Nutrition* **24**, 304-310.

Barnish, G. & Samai, S.K. (1992). *Some Medicinal Plant Recipes of the Mende, Sierra Leone*. Freetown: SLADEA Publication.

Barreau, N.Z. (1993). *Food Composition Table for Use in Ghana (November 1992 to January 1993)*. Regional Office for Africa: Food and Agriculture Organization.

Barrett, J.F.R., Whittaker, P.G., Williams, J.G. & Lind, T. (1994). Absorption of non-haem iron from food during normal pregnancy. *British Medical Journal* **309**, 79-92.

Barrett-Connor, E. (1972). Anemia and infection. *American Journal of Medicine* **52**, 242-253.

Bates, C.J., Fuller, N.J. & Prentice, A.M. (1986). Folate status during pregnancy and lactation in a West African rural community. *Human Nutrition: Clinical Nutrition* **40C**, 3-13.

Bates, C.J., Prentice, A.M. & Paul, A.A. (1994). Seasonal variations in vitamins A, C, riboflavin and folate intakes and status in pregnant and lactating women in a rural Gambian community: some possible implications. *European Journal of Clinical Nutrition* **49**, 660-668.

Bates, C.J., Prentice, A.M., Prentice, A., Paul, A.A. & Whitehead, R.G. (1982). Seasonal variations in ascorbic acid status and breast milk ascorbic acid levels in rural Gambian women in relation to dietary intake. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**, 341-347.

Bauerfeind, P., Allemann, A. & Gyr, N. (1992). Differential diagnostic value of occult blood in stool in hookworm infection in the Third World. Study from Papua New Guinea. *Zeitschrift fuer Gastroenterologie* **30**, 791-794.

Bayoh, M.N. & Hodges, M. (1994). Soil-transmitted helminth infections in three isolated communities, Sierra Leone. *Journal of the Sierra Leone Medical and Dental Association* **8**, 1-7.

Bayoh, M.N., Crompton, D.W.T., Stoddart, R.C. & Hodges, M.H. (1992). Identification of *Necator americanus* from a community in Bo, Sierra Leone. *Journal of the Sierra Leone Medical and Dental Association* **6**, 44-48.

Beaman, M.H., McCabe, R.E., Wong, S-Y. & Remington, J.S. (1995). *Toxoplasma gondii*. In: *Principles and Practice of Infectious Diseases*, Mandell, G.L., Douglas, R.G. & Bennett, J.E. (editors). New York: Livingston Churchill, pp. 2455-2475.

Beasley, R.P. & Hwang, I.Y. (1983). Postnatal infectivity of hepatitis B surface antigen-carrier mothers. *Journal of Infectious Diseases* **147**, 185-190.

Beasley, R.P., Trepo, C., Stevens, C.E. & Szmuness, W. (1977). The e antigen and vertical transmission of hepatitis B surface antigen. *American Journal of Epidemiology* **105**, 94-98.

Behets, F.M.T., Desormeaux, J., Joseph, D., Adrien, M., Coicou, G., Dallabetta, G., Hamilton, H.A., Moeng, S., Davis, H., Cohen, M.S. & Boulos, R. (1995). Control of sexually transmitted diseases in Haiti: results and implications of a baseline study among pregnant women living in Cité Soleil shantytowns. *Journal of Infectious Diseases* **172**, 764-771.

Behnke, J.M. (1987). Do hookworms elicit protective immunity in man? *Parasitology Today* **3**, 200-206.

Behnke, J.M., Pritchard, D.I., Wakelin, D., Park, J.R., McNicholas, A.M. & Gilbert, F.S. (1994a). Effect of ivermectin on infection with gastro-intestinal nematodes in Sierra Leone. *Journal of Helminthology* **68**, 187-195.

Behnke, J.M., Rose, R. & Little, J. (1994b). Resistance of the hookworms *Ancylostoma ceylanicum* and *Necator americanus* to intestinal inflammatory responses induced by heterologous infection. *International Journal for Parasitology* **24**, 91-101.

Bello, C. & Whittle, H. (1991). Cytomegalovirus infection in Gambian mothers and their babies. *Journal of Clinical Pathology* **44**, 366-369.

Bentley, D.P. (1985). Iron metabolism and anaemia in pregnancy. *Clinical Haematology* **14**, 613-628.

Bentley, M.E., Pelto, G.H., Straus, W.L., Schumann, D.A., Adegbola, C., de la Pena, E., Oni, G.A., Brown, K.A. & Huffman, S.L. (1988). Rapid ethnographic assessment applications in a diarrhea management program. *Social Science and Medicine* **27**, 107-116.

Bhargava, M., Iyer, P.U., Kumar, R., Ramji, S., Kapani, V. & Bhargava, S.K. (1991). Relationship of maternal serum ferritin with foetal serum ferritin, birth weight and gestation. *Journal of Tropical Pediatrics* **37**, 149-152.

Bierlich, B. (1995). Notions and treatment of guinea worm in Northern Ghana. *Social Science and Medicine* **41**, 501-509.

Blacklock, D.B. & Gordon, R.M. (1925). Malaria infection as it occurs in late pregnancy; its relationship to labour and early infancy. *Annals of Tropical Medicine and Parasitology* **19**, 327-364.

Blaumslag, N., Edelstein, T. & Metz, J. (1970). Reduction of incidence of prematurity by folic acid supplementation in pregnancy. *British Medical Journal* **1**, 16-17.

Bledsoe, C.H. & Goubaud, M.F. (1985). The reinterpretation of Western pharmaceuticals among the Mende of Sierra Leone. *Social Science and Medicine* **21**, 275-282.

Blot, L., Papiernik, E., Kaltwasser, J.P., Werner, E. & Tchernia, G. (1981). Influence of routine administration of folic acid and iron during pregnancy. *Gynecologic and Obstetric Investigation* **12**, 294-304.

Bolton, J.M. (1972). Food taboos among the Orang Asli in West Malaysia: a potential nutritional hazard. *American Journal of Clinical Nutrition* **25**, 789-799.

Booth, M. & Bundy, D.A.P. (1992). Comparative prevalences of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections and the prospects for combined control. *Parasitology* **105**, 151-157.

Bothwell, T.H., Baynes, R.D., MacFarlane, B.J. & MacPhail, A.P. (1989). Nutritional iron requirements and food iron absorption. *Journal of Internal Medicine* **226**, 357-365.

Bothwell, T.H., Charlton, R.W., Cook, J.D. & Finch, C.A. (1979). Iron nutrition. In: *Iron Metabolism in Man*. London: Blackwell, pp. 7-42.

Bourne L.T., Langenhoven, M.L., Steyn, K., Jooste, P.L., Laubscher, J.A. & Van der Vyver, E. (1993). Nutrient intake in the African population of the Cape Peninsula, South Africa: the BRISK study. *Central African Journal of Medicine* **39**, 238-247.

Bouvier, P., Doumba, O., Breslow, N., Robert, C-F., Mauris, A., Picquet, M., Kouriba, B., Dembele, H.K., Delley, V. & Rougemont, A. (1997). Seasonality, malaria, and impact of prophylaxis in a West African village. I. Effect of anemia on pregnancy. *American Journal of Tropical Medicine and Hygiene* **56**, 378-383.

Brabin, B.J. (1983). An analysis of malaria in pregnancy in Africa. *Bulletin of the World Health Organization* **616**, 1005-1016.

Brabin, B.J. (1985). Epidemiology of infection in pregnancy. *Reviews of Infectious Diseases* **7**, 579-603.

Brabin, L. & Brabin, B.J. (1992). Parasitic infections in women and their consequences. *Advances in Parasitology* **31**, 1-81.

Bradley, M. & Chandiwana, S.K. (1990). Age-dependency in predisposition to hookworm infection in the Burma valley area of Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 826-828.

Bradley, M., Chandiwana, S.K., Bundy, D.A.P. & Medley, G.F. (1992). The epidemiology and population biology of *Necator americanus* in a rural community in Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 73-76.

Bradley-Moore, A.M., Greenwood, B.M., Bradley, AK., Akintunde, A., Attai, E.D.E., Fleming, A.F., Flynn, F.V., Kirkwood, B.R. & Gilles, H.M. (1985). Malaria chemoprophylaxis with chloroquine in young Nigerian children. IV. Its effect on haematological measurements. *Annals of Tropical Medicine and Parasitology* **79**, 585-595.

Brattegaard, K., Kouadio, J., Adom, M.L., Doorly, R., George, J.R. & De Cock, K.M. (1993). Rapid and simple screening and supplemental testing for HIV-1 and HIV-2 infections in West Africa. *AIDS* **7**, 883-885.

Brieger, W.R. & Kendall, C. (1992). Learning from local knowledge to improve disease surveillance: perceptions of the guinea worm illness experience. *Health Education Research* **7**, 471-485.

Bruce-Chwatt, L.J. (1983). Malaria and pregnancy. *British Medical Journal* **286**, 1457-1458.

Bundy, D.A.P. (1986). Epidemiological aspects of *Trichuris* and trichuriasis in Caribbean communities. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 706-718.

Bundy, D.A.P. (1988). Population ecology of intestinal helminth infections in human communities. *Philosophical Transactions of the Royal Society of London B* **321**, 405-420.

Bundy, D.A.P (1990). Is hookworm just another geohelminth? In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 147-164.

Bundy, D.A.P., Chan, M.S. & Savioli, L. (1995). Hookworm infection in pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 521-522.

Bundy, D.A.P., Chandiwana, S.K., Homeida, M.M.A., Yoon, S. & Mott, K.E. (1991a). The epidemiological implications of a multi-infection approach to the control of human helminth infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 274-276.

Bundy, D.A.P. & Cooper, E.S. (1989). *Trichuris* and trichuriasis in humans. *Advances in Parasitology* **28**, 108-173.

Bundy, D.A.P., Cooper, E.S., Thompson, D.E., Didier, J.M., Anderson, R.M. & Simmons, I. (1987a). Predisposition to *Trichuris trichiura* infection in humans. *Epidemiology and Infection* **98**, 65-71.

Bundy, D.A.P., Cooper, E.S., Thompson, D.E., Didier, J.M. & Simmons, I. (1987b). Epidemiology and population dynamics of *Ascaris lumbricoides* and *Trichuris trichiura* infection in the same community. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 987-993.

Bundy, D.A.P., Cooper, E.S., Thompson, D.E., Didier, J.M. & Simmons, I. (1988). Effect of age and initial infection status on the rate of reinfection with *Trichuris trichiura* after treatment. *Parasitology* **97**, 469-476.

Bundy, D.A.P. & Golden, M.H.N. (1987). The impact of host nutrition on gastrointestinal helminth populations. *Parasitology* **95**, 623-635.

Bundy, D.A.P. & Medley, G.F. (1992). Immuno-epidemiology of human geohelminthiasis: ecological and immunological determinants of worm burden. *Parasitology* **104** (supplement), S105-S119.

Bundy, D.A.P., Lillywhite, J.E., Didier, J.M., Simmons, I. & Bianco, A.E. (1991b). Age-dependency of infection status and serum antibody levels in human whipworm (*Trichuris trichiura*) infection. *Parasite Immunology* **13**, 629-638.

Bundy, D.A.P., Thompson, D.E., Cooper, E.S., Golden, M.H.N. & Anderson, R.M. (1985a). Population dynamics and chemotherapeutic control of *Trichuris trichiura* infection of children in Jamaica and St. Lucia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 759-764.

Bundy, D.A.P., Thompson, D.E., Golden, M.H.N., Cooper, E.S., Anderson, R.M. & Harland, P.S.E. (1985b). Population distribution of *Trichuris trichiura* in a community of Jamaican children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 232-237.

Burke, T.M. & Robertson, E.L. (1985). Prenatal and lactational transmission of *Toxocara canis* and *Ancyclostoma canium*: experimental infection of the bitch at midpregnancy and at parturition. *International Journal for Parasitology* **15**, 485-490.

Campbell, W.C. & Benz, G.W. (1984). Ivermectin: a review of efficacy and safety. *Journal Veterinary Pharmacology and Therapeutics* **7**, 1-16.

Carretti, N.G., Eremita, A.G., Paternoster, D., Pellizzari, P. & Grella, P. (1992). Iron balance in pregnancy in relation to anemia. *Clinical and Experimental Obstetrics and Gynecology* **19**, 218-221.

Carriaga, M.T., Skikne, B.S., Finley, B., Cutler, B. & Cook, J.D. (1991). Serum transferrin receptor for the detection of iron deficiency in pregnancy. *American Journal of Clinical Nutrition* **54**, 1077-1081.

Carroll, S.M. (1990). Immunity: hookworm in animal model systems. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 391-403.

Cassidy, C.M. (1994). Walk a mile in my shoes: culturally sensitive food-habit research. *American Journal of Clinical Nutrition* **59** (supplement), S190-S197.

Castro, G.A., Behnke, J.M. & Weisbrodt, N.W. (1990). Hookworm infection and malabsorption: a critical review. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S (editors). London: Taylor & Francis, pp. 265-278.

Caulfield, L.E. (1991). Birth-weight as an indicator of fetal nutritional status. In: *Anthropometrical Assessment of Nutritional Status*, Himes, J.H. (editor). New York: Wiley-Liss, Inc., pp. 259-271.

Ceesay, S.M., Prentice, A.M., Cole, T.J., Foord, F., Weaver, L.T., Poskitt, E.M. & Whitehead, R.G. (1997). Effects on birth weight and perinatal mortality of maternal dietary supplements in rural Gambia: 5 year randomised controlled trial. *British Medical Journal* **315**, 786-790.

Chamberlain, G. (1984). Epidemiology and etiology of the preterm baby. *Clinics in Obstetrics and Gynecology* **11**, 297-314.

Chan, M.S., Bundy, D.A.P. & Kan, S.P. (1994a). Aggregation and predisposition to *Ascaris lumbricoides* and *Trichuris trichiura* at the familial level. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 46-48.

Chan, M.S., Bundy, D.A.P. & Kan, S.P. (1994b). Genetic relatedness as a determinant of predisposition to *Ascaris lumbricoides* and *Trichuris trichiura* infection. *Parasitology* **108**, 77-80.

Chan, M.S., Medley, G.F., Jamison, D. & Bundy, D.A.P. (1994c). The evaluation of potential global morbidity attributable to intestinal nematode infections. *Parasitology* **109**, 373-387.

Chanarin, I. & Rothman, D. (1971). Further observation on the relation between iron and folate status in pregnancy. *British Medical Journal* **2**, 81-84.

Chandiwana, S.K. (1990). Hookworm population ecology in Zimbabwe. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 165-176.

Chandler, S.H., Alexander, E.R. & Holmes, K.K. (1985). Epidemiology of cytomegalovirus infection in a heterogeneous population of pregnant women. *Journal of Infectious Diseases* **152**, 249-256.

Chandra, R.K. (1981). Iron, immunity and infection: is there a causal link? *Food and Nutrition Bulletin* **26**, 952-957.

Charoenlarp, P., Dhanamitta, S., Kaewvichit, R., Silprasert, A., Suwanaradd, C., Na-Nakorn, S., Prawatmuang, P., Vatanavicharn, S., Nutcharas, U., Pootrakul, P., Tanphaichitr, V., Thanangkul, O., Vaniyapong, T., Thane Toe, Valyasevi, A. Baker, S., Cook, J., DeMaeyer, E.M., Garby, L. & Hallberg, L. (1988). A WHO collaborative study on iron supplementation in Burma and in Thailand. *American Journal of Clinical Nutrition* **47**, 280-297.

Chew, F., Torun, B. & Viteri, F.E. (1996). Comparison of weekly and daily iron supplementation to pregnant women in Guatemala (supervised and unsupervised). *Federation of American Societies for Experimental Biology Journal* **10**, A4221.

Chi, C. (1994). Integrating traditional medicine into modern health care systems: examining the role of Chinese medicine in Taiwan. *Social Science and Medicine* **39**, 307-321.

Chu, W-G., Chen, P-M., Huang, C-C. & Hsu, C-T. (1972). Neonatal ascariasis. *Journal of Pediatrics* **87**, 783-785.

Chwang, L.C., Soemantri, A.G. & Pollitt, E. (1988). Iron supplementation and physical growth of rural Indonesian children. *American Journal of Clinical Nutrition* **47**, 496-501.

Cline, B.L., Little, M.D., Bartholomew, R.K. & Halsey, N.A. (1984). Larvicidal activity of albendazole against *Necator americanus* in human volunteers. *American Journal of Tropical Medicine and Hygiene* **33**, 387-394.

Cohen, J. (1998). *Statistical Power Analysis for the Behavioral sciences*. Hillsdale: Erlbaum.

Colomer, J., Colomer, C., Gutierrez, D., Jubert, A., Nolasco, A., Donat, J., Fernandez-Delgado, R., Donat, F. & Alvarez-Dardet, C. (1990). Anaemia during pregnancy as a risk factor for infant iron deficiency: report from the Valencia Infant Anaemia Cohort (VIAC) study. *Paediatric and Perinatal Epidemiology* **4**, 196-204.

Conder, G.A. (1995). Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. *Advances in Parasitology* **35**, 1-84.

Cook, G.C. (1990). Use of benzimidazole chemotherapy in human helminthiases: indications and efficacy. *Parasitology Today* **6**, 133-136.

Cook, G.C. (1993). Problems associated with antiprotozoan and anthelminthic chemoprophylaxis/chemotherapy during pregnancy. *Journal of Infection* **26**, 333-351.

Cook, J.D. & Finch, C.A. (1979). Assessing iron status of a population. *American Journal of Clinical Nutrition* **32**, 2115-2119.

Cook, J.D., Lipschitz, D.A., Miles, L.E.M. & Finch, C.E. (1974). Serum ferritin as a measure of iron stores in normal subjects. *American Journal of Clinical Nutrition* **27**, 681-687.

Cook, J.D, & Skikne, B.S. (1982). Serum ferritin: a possible model for the assessment of nutrient stores. *American Journal of Clinical Nutrition* **35**, 1180-1185.

Cook, J.D. & Skikne, B.S. (1989). Iron deficiency: definition and diagnosis. *Journal of Internal Medicine* **226**, 349-355.

Cook, J.D., Skikne, B.S., Lynch, S.R. & Reussner, M. (1986). Estimates of iron sufficiency in the US population. *Blood* **68**, 726-731.

Coombs, I. & Crompton, D.W.T. (1991). *A Guide to Human Helminths*. London: Taylor and Francis.

Cooper, E.S., Bundy, D.A.P., MacDonald, T.T. & Golden, M.H.N. (1990). Growth suppression in the *Trichuris* dysentery syndrome. *European Journal of Clinical Nutrition* **44**, 285-291.

Cossa, H.A., Gloyd, S., Vaz, R.G., Folgosa, E., Simbine, E., Diniz, M. & Kreiss, J.K. (1994). Syphilis and HIV infection among displaced pregnant women in rural Mozambique. *International Journal of STD and AIDS* **5**, 117-123.

Coursaget P., Yvonnet, B. & Chotard, J. (1987). Age and sex-related study of hepatitis B virus chronic carrier state in infants from an endemic area (Senegal). *Journal of Medical Virology* **22**, 1-5.

Crandall, R.B., Crandall, C.A. & Jones, J.F. (1978). Analysis of immunosuppression during early acute infection of mice with *Ascaris suum*. *Clinical Experimental Immunology* **33**, 30-37.

Croll, N.A., Anderson, R.M., Gyorkos, T.W. & Ghadirian, E. (1982). The population biology and control of *Ascaris lumbricoides* in a rural community in Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76**, 187-197.

Crompton, D.W.T. (1984). Influence of parasitic infection on food intake. *Federation Proceedings* **43**, 239-245.

Crompton, D.W.T. (1986). Nutritional aspects of infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 697-705.

Crompton, D.W.T., Kamara, C., Ferret, W., Hodges, M.H. & Stoddart, R.C. (1992). Intestinal helminth infections in young children from Moyamba District, Southern Province, Sierra Leone. *Journal of the Sierra Leone Medical and Dental Association* **6**, 24-31.

Crompton, D.W.T., Nesheim, M.S. & Pawlowski, Z.S. (1989). *Ascariasis and its public health significance*. London: Taylor & Francis.

Crompton, D.W.T. & Whitehead, R.R. (1993). Hookworm infections and human iron metabolism. *Parasitology* **107** (supplement), S137-S145.

da Costa-Macedo, L.M. & Ray, L. (1990). *Ascaris lumbricoides* in neonate: evidence of congenital transmission of intestinal nematodes. *Revista da Instituto de Medicina Tropical de Sao Paulo* **32**, 351-354.

D'Alauro, F., Lee, R.V., Pao-in, K. & Khairallah, M. (1985). Intestinal parasites and pregnancy. *American Journal of Obstetrics and Gynecology* **66**, 639-643.

Dallman, P.R. (1989). Iron deficiency: does it matter? *Journal of Internal Medicine* **226**, 367-372.

Daoh, K.S. (1995). *Symposium on Prevention of Maternal Mortality: the Socio-Cultural and Economic Aspects of Prevention*. Paper presented at the Annual Conference of the Sierra Leone Association of Gynaecologists and Obstetricians, 18th November, 1995.

Daouda, H., Galan, P., Prual, A., Sekou, H. & Hercberg, S. (1991). Iron status in Nigerian mothers and their newborns. *International Journal for Vitamin and Nutrition Research* **61**, 46-50.

Davidson, G.P. & Townley, R.R.W. (1977). Structural and functional abnormalities of the small intestine due to nutritional folic acid deficiency in infancy. *Journal of Pediatrics* **90**, 590-594.

Davis, A.D. (1994). *Clinical and Pharmacological Considerations in Population-Based Chemotherapy of Hookworm Infections in Women*. WHO Informal Consultation on Hookworm Infection in Women, 5-7 December 1994. Working Paper No. 17. Geneva: World Health Organization.

Dawson, E.B. & McGanity, W.J. (1987). Protection of maternal iron stores in pregnancy. *Journal of Reproductive Medicine* **32** (supplement), 478-487.

D'Costa, L.J., Plummer, F.A., Bowmer, I., Fransem L., Piot., R., Ronald, A.R. & Nsanze, H. (1985). Prostitutes are a major reservoir of sexually transmitted diseases in Nairobi, Kenya. *Sexually Transmitted Diseases* **12**, 64-67.

De Cock, K.M., Zadi, F., Adjorlolo, G., Diallo, M.O., Sassan-Morokro, M., Ekpini, E., Sibailly, T., Doorly, R., Batter, V., Brattegaard, K. & Gayle, H. (1994). Retrospective study of maternal HIV-1 and HIV-2 infections and child survival in Abidjan, Cote d'Ivoire. *British Medical Journal* **308**, 441-443.

de Onis, M., Blossner, M. & Villar, J. (1998a). Levels of patterns of intrauterine growth retardation in developing countries. *European Journal of Clinical Nutrition* **52** (supplement), S5-S15.

de Onis, M., Villar, J. & Gulmezoglu, M. (1998b). Nutritional interventions to prevent intrauterine growth retardation: evidence from randomized controlled trials. *European Journal of Clinical Nutrition* **52** (supplement), S83-S93.

de Silva, L.D.R., Guyatt, H. & Bundy, D. (1997). Anthelminthics. A comparative review of their clinical pharmacology. *Drugs* **53**, 769-788.

de Silva, L.D.R., Dassenaeike, T.S. de C. & Perera, R.S. (1995). Anthelmintic therapy and iron supplementation of pregnant women. Reply to Z.S. Pawlowski. *American Journal of Clinical Nutrition* **62**, 1023-1024.

Demmler, G.J., O'Neil, G.W., O'Neil, J.H., Spector, S.A., Brady, M.T. & Yow, M.D. (1986). Transmission of cytomegalovirus from husband to wife. *Journal of Infectious Diseases* **154**, 545-546.

DHSS (1993). *National Health Policy. Republic of Sierra Leone*. Department of Health and Social Sciences. Freetown, Sierra Leone.

Diallo, S., Ndir, O., Dieng, Y., Leye, A., Dieng, T., Bah, I.B., Diop, B.M., Gaye, O. & Faye, O. (1996). Sero-prevalence de la toxoplasmose à Dakae (Senegal) en 1993: etude chez des femmes en periode de procreation. *Cahiers Sante* **6**, 102-106.

Dickinson, J. & Gonick, B. (1990). Teratogenic viral infections. *Clinical Obstetrics and Gynecology* **33**, 242-252.

Diouf, A., Kebe, F., Faye, E.O., Diallo, D., Sarr, A.N., Mboup, S. & Diadhiou, F. (1996). L'infection par le vih chez la femme enceinte a Dakar (Senegal). *Journal de Gynecologie Obstetrique et Biologie de la Reproduction* **25**, 283-287.

Dollery, C. (1992). Albendazole. In: *Therapeutic Drugs*. Edinburgh: Churchill Livingstone, pp. A31-A34.

Dunn, F.L. (1972). Intestinal parasitism in Malayan aborigines. *Bulletin of the World Health Organization* **46**, 99-113.

Dunn, F.L. (1979). Behavioural aspects of the control of parasitic diseases. *Bulletin of the World Health Organization* **57**, 499-512.

Durnin, J.V.G.A. (1993). Energy requirements, in human pregnancy and parasitic infection. *Parasitology* **107** (supplement), S169-S175.

Durnin, J.V.G.A., McKillop, R.M., Grant, S. & Fitzgerald, G. (1985). Is nutritional status endangered by virtually no extra intake during pregnancy? *Lancet* **2**, 823-825.

Durnin, J.V.G.A., McKillop, R.M., Grant, S. & Fitzgerald, G. (1987). Energy requirements of pregnancy in Scotland. *Lancet* **2**, 897-900.

Dwyer, J.T. (1991). Concept of nutritional status and its measurement. In: *Anthropometric Assessment of Nutritional Status*, Himes, J.H. (editor). New York: Wiley-Liss, pp. 5-28.

Edward, N.C. (1989). Payment for deliveries in Sierra Leone. *Bulletin of the World Health Organization* **67**, 163-169.

Elkins, D.B., Haswell-Elkins, M. & Anderson, R.M. (1986). The epidemiology and control of intestinal helminths in the Pulicat region of Southern India. I. Study design and pre- and post-treatment observation on *Ascaris lumbricoides* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 774-792.

Etkin, N.L. & Ross, R.J. (1982). Food as medicine and medicine as food: an adaptive framework for the interpretation of plant utilization among the Hausea of Northern Nigeria. *Social Science and Medicine* **16**, 1559-1573.

Evans, E.E. (1992). Human immunodeficiency virus (HIV) infection. In: *Nelson Textbook of Pediatrics*, 14th Edition, Bralow, L. (editor). Philadelphia: W.B. Saunders Company, pp. 835-842.

Ewald, J.A. (1994). *Factors Influencing the Distribution and Abundance of Helminth Infections: Model Approaches and Community Studies*. Doctoral thesis. Department of Zoology, University of Glasgow.

Ewald, J.A., Bayoh, M.N., Crompton, D.W.T. & Hodges, M.H. (1993). Prevalence and intensity of intestinal helminth infections from three communities in Sierra Leone. *Journal of the Sierra Leone Medical and Dental Association* **7**, 39-44.

FAO (1986). *Some Medicinal Forest plants of Africa and Latin America*. Forest Resources Division, FAO Forestry Paper 67. Rome: Food and Agriculture Organization.

FAO (1994). *1994 Country Tables. Basic Data on the Agricultural Sector*. Economic and Social Policy Document. Rome: Food and Agriculture Organization.

FAO/WHO (1974). *Handbook of Nutritional Requirements*. Food and Nutrition Series No. 4. Rome: Food and Agriculture Organization and World Health Organization.

FAO/WHO (1985). *Energy and Protein Requirements. Report of a joint FAO/WHO expert consultation*. Technical Report Series No. 724. Rome: Food and Agriculture Organization and World Health Organization.

FAO/WHO (1988). *Requirements of Vitamin A, Iron, Folate and Vitamin B-12*. Food and Nutrition Series No. 23. Rome: Food and Agriculture Organization and World Health Organization.

Ferguson, B.J., Skikne, B.S., Simpson, K.M., Baynes, R.D. & Cook, J.D. (1992). Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anaemia. *Journal of Laboratory and Clinical Medicine* **119**, 385-390.

Ferro-Luzzi, A., Ashworth, A., Martorell, R. & Scrimshaw, N. (1998). Report of the IDECG Working Group on effects of IUGR on infants, children and adolescents: immunocompetence, mortality, morbidity, body size, body composition and physical performance. *European Journal of Clinical Nutrition* **52** (supplement), S97-S99.

Ferro-Luzzi, A. & Branca, F. (1993). Nutritional seasonality: the dimensions of the problem. In: *Seasonality and Human Ecology: 35th Symposium Volume of the Society of the Study of Human Biology*, Ulijaszek, S.J. & Strickland, S.S. (editors). Cambridge: Cambridge University press, pp. 149-165.

Flanagan, P.R. (1989). Mechanisms and regulation of intestinal uptake and transfer of iron. *Acta Pediatrica Scandinavica* (Supplement) **361**, 21-30.

Fleming, A.F. (1989). Tropical obstetrics and gynaecology. 1. Anemia in pregnancy in tropical Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 441-448.

Fleming, A.F. (1991). Hematologic diseases. Anemia. In: *Hunter's Tropical Medicine*, Strickland, G.T. (editor). London: W.B. Saunders, pp. 36-40.

Folgossa, E., Bique Osman, N., Gonzalez, C., Hagerstran, I., Bergstrom, S. & Ljungh, A. (1996). Syphilis sero-prevalence among pregnant women and its role as a risk factor for stillbirth in Maputo, Mozambique. *Genitourinary Medicine* **72**, 339-342.

Forrester, J.E., Scott, M.E., Bundy, D.A.P. & Golden, M.H.N. (1988). Clustering of *Ascaris lumbricoides* and *Trichuris trichiura* infections with households. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 282-288.

Forrester, J.E., Scott, M.E., Bundy, D.A.P. & Golden, M.H.N. (1990). Predisposition of subjects and families in Mexico to heavy infection with *Ascaris lumbricoides* and *Trichuris trichiura*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 272-276.

Fortuin, M, Chotard, J., Jack, A.D., Maine, N.P., Mendy, M., Hall, J., Inskip, H.M., George, M.O. & Whittle, H.C. (1993). The efficacy of hepatitis B vaccine in the Gambian Expanded Programme on Immunisation. *Lancet* **341**, 1129-1131.

Foster, B.M. (1993). *Estimation of Sample Size and Power for Commonly used Analysis of Variance Designs: a Simulation-Based Study*. Doctoral dissertation. Baylor University, Waco. Cited in Kirk (1995).

Fosu, G.B. (1981). Disease classification in rural Ghana: framework and implications for health behaviour. *Social Science and Medicine* **15B**, 471-482.

Foulon, W. (1992). Congenital toxoplasmosis: is screening desirable? *Scandinavian Journal of Infectious Diseases* **84** (supplement), 11-17.

Frenkel, J.K. (1991). Toxoplasmosis. In: *Hunter's Tropical Medicine*, 7th Edition, Strickland, G.T. (editor). Philadelphia: W.B. Saunders Company, pp. 658-669.

Frenkel, J.K. & Dubey, J.P. (1972). Toxoplasmosis and its prevention in cats and man. *Journal of Infectious Diseases* **126**, 664-673.

Frisancho, A.R. (1990). *Anthropometrical Standards for the Assessment of Growth and Nutritional Status.* Michigan: University of Michigan Press.

Fyle, C.A. (1976). The use of mother-tongue in education in Sierra Leone. In: *Mother Tongue Education*, Bamgbose, A (editor). UNESCO Press, p. 46.

Galan, P., Cherouvrier, F., Zohoun, I., Zohoun, T., Chauliac, M. & Hercberg, S. (1990). Iron absorption from typical West African meals containing contamination Fe. *British Journal of Nutrition* **64**, 541-546.

Galloway, R. & McGuire, J. (1994). Determinants of compliance with iron supplementation: supplies, side effects or psychology? *Social Science and Medicine* **39**, 381-390.

Garn, S.M. (1991). Pre-pregnancy weight. In: *Maternal Nutrition and Pregnancy Outcomes. Anthropometric Assessment*, Krasovec, K. & Anderson, M.A. (editors). Washington: Pan American Health Organization, pp. 69-85.

Gbakima, A.A. & Nyang, G.A. (1995). Prevalence and risk factors of *Giardia lambia* infection among school children in Njala, Sierra Leone. *Journal of Pure and Applied Science* **4**, 7-11.

Geissler, P.W. (1998). 'Worms are our life', part I: understanding of worms and the body among the Luo of western Kenya. *Anthropology and Medicine* **5**, 63-79.

Ghendon, Y. (1987). Perinatal transmission of hepatitis B in high incidence countries. *Journal of Virology Methods* **17**, 67-79.

Ghendon, Y. (1990). WHO strategy for the global elimination of new cases of hepatitis B. *Vaccine* **9** (supplement), S129-S133.

Gibson, R.S. (1990a). Anthropometric assessment. In: *Principles of Nutritional Assessment.* Oxford: Oxford University Press, pp. 155-162.

Gibson, R.S. (1990b). Anthropometric assessment of body fat. In: *Principles of Nutritional Assessment.* Oxford: Oxford University Press, pp. 187-208.

Gibson, R.S. (1990c). *Principles of Nutritional Assessment.* Oxford: Oxford University Press.

Gilbert, G.L. (1993). Routine antenatal screening and prenatal diagnosis of vertically transmissible infection. *Bailliere's Clinical Obstetrics and Gynecology* **7**, 1-23.

Gilles, H.M. (1975). Hookworm infection and anaemia. *Tropical Doctor* **5**, 51-55.

Gilles, H.M., Watson-Williams, E.J. & Ball, P. (1964). Hookworm infection and anaemia. *Quarterly Journal of Medicine* **33**, 1-24.

Gilman, R.H., Chong, Y.H., Davis, C., Greenberg, B., Virik, H.K. & Dixon, H.B. (1983). The adverse consequences of heavy *Trichuris* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **77**, 432-438.

Godel, J.C., Pabst, H.F., Hodges, P.E. & Johnson, K.E. (1992). Iron status and pregnancy in a northern Canadian population: relationship to diet and supplementation. *Canadian Journal of Public Health* **83**, 339-343.

Goepel, E., Ulmer, R.D. & Neth, R.D. (1988). Premature labor contractions and the value of serum ferritin during pregnancy. *Gynecologic Obstetric Investigation* **26**, 265-273.

Gotoff, S.P. (1992). Hepatitis. In: *Nelson Textbook of Pediatrics*. 14th Edition Bralow, L. (editor). W.B. Saunders Company. Philadelphia, London, pp. 505-506.

Gould, H.A. (1957). The implications of technological change for folk and scientific medicine. *American Anthropologist* **59**, 507-516.

Grant, J.O. (1989). *A General Survey of Some of the Factors Influencing the Health Status of Children aged 0 - 5 Years in Freetown, Sierra Leone*. Dissertation for B.Sc. Department of Zoology, University of Sierra Leone.

Green, E.C. (1997). Purity, pollution and the invisible snake in southern Africa. *Medical Anthropology* **17**, 83-100.

Greenblatt, A.M., Lukehart, S.A., Plummer, F.A., Quinn, T.C., Critchlow, C.W., Ashley, R.L., D'Costa, L.J., Ndinya-Achola, J.O., Corey, L., Ronald. A.R. & Holmes, K.K. (1988). Genital ulceration as a risk factor for human immunodeficiency virus infection. *AIDS* **2**, 47-50.

Greene, P. (1978). *Favourite Sierra Leone recipes*. Freetown: Commercial Printers Company Limited.

Greenwood, B.M., Greenwood, A.M., Snow, R.W., Byass, P., Bennett, S. & Hatib-N'Jie, A.B. (1989). The effects of malaria chemoprophylaxis given by traditional birth attendants on the course and outcome of pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 589-594.

Griffiths, P., Baboonian, C. & Ashley, D. (1985). The demographic characteristics of pregnant women infected with cytomegalovirus. *International Journal of Epidemiology* **14**, 447-452.

Guiro, A.T., Galan, P., Cherouvrier, F., Sall, M.G. & Hercberg, S. (1991). Iron absorption from African pearl millet and rice meals. *Nutrition Research* **11**, 885-893.

Gupta, M.C. (1990). Effect of ascariasis upon nutritional status of children. *Journal of Tropical Paediatrics* **36**, 189-191.

Gupta, M.C., Mithal, S., Arara, K.L. & Tandon, B.N. (1977). Effects of periodic deworming on nutritional status of *Ascaris*-infested preschool children receiving supplementary food. *Lancet* 1, 108-109.

Gyapong, M., Gyapong, J.O., Adjei, S., Vlassoff, C. & Weiss, M. (1996). Filariasis in Northern Ghana: some cultural beliefs and practices and their implications for disease control. *Social Science and Medicine* **43**, 235-242.

Haas, J. & Fairchild, M.W. (1989). Summary and conclusions of the International Conference on Iron Deficiency and Behavioural Development, October 10-12, 1988. *American Journal of Clinical Nutrition* **50**, 703-705.

Habiyambere, V. & Wertheimer, A.I. (1993). Essential drugs should be accessible to all people. *World Health Forum* **14**, 140-144.

Hahn, P.F., Carothers, E.L., Darby, W.J., Martin, M., Sheppard, C.W., Cannon, R.O., Beam, A.S., Densen, P.M., Peterson, J.C. & McClellan, G.S. (1951). Iron metabolism in early pregnancy as studied with the radioactive isotope 59Fe. *American Journal of Obstetrics and Gynecology* **61**, 477-486.

Hall, A. (1981). Quantitative variability of nematode egg counts in faeces: a study among rural Kenyans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **75**, 682-687.

Hall, A. (1982). Intestinal helminths of man: interpretation of egg counts. *Parasitology* **85**, 605-613.

Hall, A. (1994). *Giardia* infections: epidemiology and nutritional consequences. In: *Giardia: From Molecules to Disease*, Thompson, R.C.A., Reynoldson, J.A. & Lymbery, A.J. (editors). Wallington: CAB International, pp. 251-280.

Hall, A., Anwar, K.S. & Tomkins, A.M. (1992). Intensity of reinfection with *Ascaris lumbricoides* and its implication for parasite control. *Lancet* **339**, 1253-1257.

Hall, A.J., Inskip, H.M., Loik, F., Chotard, J., Jawara, M., Vall Mayans, M., Greenwood, B.M., Whittle, H., Njie, A.B.H., Cham, K., Bosch, F.X. & Muir, C.S. (1989). Hepatitis B vaccine in the Expanded Programme of Immunization: the Gambian experience. *Lancet* **1**, 1057-1060.

Hallberg, L. (1988). Iron balance in pregnancy. In: *Vitamins and Minerals in Pregnancy and Lactation*, Berger, H. (editor). Nestle Nutrition Workshop Series No. 16. New York: Raven Press Ltd.

Hallberg, L., Bengtsson, C., Lapidus, L., Lindstedt, G., Lundberg, P.A. & Hulten, L. (1993). Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. *British Journal of Haematology* **85**, 787-798.

Hallberg, L. & Bjorn-Rasmussen, E. (1981). Measurement of iron absorption from meals contaminated with iron. *American Journal of Clinical Nutrition* **34**, 2808-2815.

Hallberg, L., Bjorn-Rasmussen, E., Rossander, L., Suwanik, R., Pleehachinda, R. & Tuutawiroon, M. (1983). Iron absorption from some Asian meals containing contamination iron. *American Journal of Clinical Nutrition* **37**, 272-277.

Hallberg, L., Brune, M., Erlandsson, M., Sandberg, A.S. & Rossander-Hulten, L. (1991). Calcium: effect of different amounts on non-heme and heme iron absorption in humans. *American Journal of Clinical Nutrition* **53**, 112-119.

Hallberg, L. & Rossander-Hulten, L. (1991). Iron requirements in menstruating women. *American Journal of Clinical Nutrition* **54**, 1047-1058.

Hambridge, K.M., Krebs, N.F., Sibley, L. & English, J. (1987). Acute effects of iron therapy on zinc status during pregnancy. *Obstetrics and Gynecology* **70**, 593-596.

Handsfield, H.H., Chandler, S.H., Caine, V.A., Meyers, J.D., Corey, L., Medeiros, E. & McDougall, J.K. (1985). Cytomegalovirus infection in sex partners: evidence for sexual transmission. *Journal of Infectious Diseases* **151**, 344-354.

Harju, E. (1989). Clinical pharmacokinetics of iron preparations. *Clinical Pharmacokinetics* **17**, 69-89.

Harrison, K.A. & Ibeziako, P.A. (1973). Maternal anaemia and fetal birthweight. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **80**, 798-804.

Harry, T.O., Bajani, M.D. & Moses, A.E. (1994a). Hepatitis B virus infection among blood donors and pregnant women in Maiduguri, Nigeria. *East African Medical Journal* **71**, 596-597.

Harry, T.O., Bukbuk, D.N., Idrisa, A. & Akoma, M.B. (1994b). HIV infection among pregnant women. A worsening situation in Maiduguri, Nigeria. *Tropical and Geographical Medicine* **46**, 46-47.

Haswell-Elkins, M.R., Elkins, D.B. & Anderson, R.M. (1987). Evidence for predisposition in humans to infection with *Ascaris*, hookworm, *Enterobius* and *Trichuris* in a South Indian Fishing community. *Parasitology* **95**, 323-337.

Haswell-Elkins, M.R., Elkins, M.R., Manjula, K., Michael, E. & Anderson, R.M. (1988). An investigation of hookworm infection and reinfection following mass anthelminthic treatment in the South Indian fishing community of Vairavankuppam. *Parasitology* **96**, 565-577.

Haswell-Elkins, M.R., Kennedy, M.W., Maizels, R.M., Elkins, D.B. & Anderson, R.M. (1989). The antibody recognition profiles of humans naturally infected with *Ascaris lumbricoides*. *Parasite Immunology* **11**, 615-627.

Haswell-Elkins, M.R., Leonard, H., Kennedy, M.W., Elkins, D.B. & Maizels, R.M. (1992). Immunoepidemiology of *Ascaris lumbricoides*: relationships between antibody specificities, exposure and infection in a human community. *Parasitology* **104**, 153-159.

Heggenhougen, H.K. & Shore, L. (1986). Cultural components of behavioural epidemiology: implications for primary health care. *Social Science and Medicine* **22**, 1235-1245.

Hemminki, E. & Rimpela, U. (1991). A randomized comparison of routine versus selective iron supplementation during pregnancy. *Journal of the American College of Nutrition* **10**, 3-10.

Hemminki, R. & Starfield, B. (1978). Routine administration of iron and vitamins during pregnancy: review of controlled clinical trials. *British Journal of Obstetrics and Gynaecology* **85**, 404-410.

Hercberg, S. & Galan, P. (1989). Biochemical effects of iron deprivation. *Acta Paediatrica Scandinavica Supplement* **361** (Supplement), 63-70.

Hercberg, S., Galan, P., Chauliac, M., Masse-Raimbault, A-M., Devanlay, M., Bileoma, S., Alihonou, E., Zohoun, I., Christides, J-P. & Potier de Courcy, G. (1987). Nutritional anaemia in pregnant Beninese women: consequences on the haematological profile of the newborn. *British Journal of Nutrition* **57**, 185-193.

Herman, E. & Bentley, M.E. (1992). Manuals for ethnographic data collection: experience and issues. *Social Science and Medicine* **35**, 1369-1378.

Heymsfield, S.B. & Casper, K. (1987). Anthropometric assessment of the adult hospitalized patient. *Journal of Parenteral and Enteral Nutrition* **11** (supplement), S36-S41.

Hibbard, B.M. (1988). Iron and folate supplements during pregnancy: supplementation is valuable only in selected patients. *British Medical Journal* **297**, 1324-1326.

Himes, J.H., Roche A.F. & Webb, P. (1980). Fat areas as estimates of total body fat. *American Journal of Clinical Nutrition* **33**, 2093-2100.

Hira, S.K., Chat, G.J., Chikamata, D.M., Nkowane, B., Tembo, G., Perine, P.L. & Meheus, A. (1990). Syphilis intervention in pregnancy: Zambian demonstration project. *Genitourinary Medicine* **66**, 159-164.

Hoagland, K.E. & Schad, G.A. (1978). *Necator americanus* and *Ancylostoma duodenale*: life history parameters and epidemiological implications of two sympatric hookworms of humans. *Experimental Parasitology* **44**, 36-39.

Hodges, M.H. (1988). Unpublished data abstracted with permission from hospital records.

Hodges, M., Sanders, E. & Aitken, C. (1998). Sero-prevalence of hepatitis markers; HAV, HBV, HCV and HEV amongst primary school children in Freetown, Sierra Leone. *West African Journal of Medicine* **17**, 36-37.

Holland, C. (1987a). Hookworm infection. In: *Impact of Helminth Infections on Human Nutrition*, Stephenson, L.S. & Holland, C. London: Taylor & Francis, pp. 128-160.

Holland, C. (1987b). Neglected infections - trichuriasis and strongyloidiasis. In: *Impact of Helminth Infections on Human Nutrition*, Stephenson, L.S. & Holland, C. London: Taylor & Francis, pp. 161-201.

Holland, C.V. (1989). Man and his parasites: integration of biomedical and social approaches to transmission and control. *Social Science and Medicine* **29**, 403-411.

Holland, C.V., Asaolu, S.O., Crompton, D.W.T., Stoddart, R.C., MacDonald, R. & Torimiro, S.E.A. (1989). The epidemiology of *Ascaris lumbricoides* and other soil-transmitted helminths in primary schoolchildren from Ife-Ife, Nigeria. *Parasitology* **99**, 275-285.

Holland, C.V., Taren, D.L., Crompton, D.W.T., Nesheim, M.C., Sanjur, D., Barbeau, I., Tucker, K., Tiffany, J. & Rivera, G. (1988). Intestinal helminthiases in relation to the socioeconomic environment of Panamanian children. *Social Science and Medicine* **26**, 209-213.

Hominick, W.M., Dean, C.G. & Schad, G.A. (1987). Population biology of hookworms in West Bengal: analysis of numbers of infective larvae recovered from damp pads applied to soil surface at defaecation sites. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 978-986.

Horton, J. (1993). The use of antiprotozoan and anthelmintic drugs during pregnancy and contraindications. *Journal of Infection* **26**, 104-105.

Hsieh, C-C., Tzonou, A., Zavitsanos, X., Kaklamani, E., Lan, S-L. & Trichopoulos, D. (1992). Age at first establishment of chronic hepatitis B virus infection and hepatocellular carcinoma risk. A birth order study. *American Journal of Epidemiology* **136**, 1115-1121.

Hsieh, H.C. (1972). Distribution of *Necator americanus* and *Ancylostoma duodenale* in Liberia. *Bulletin of the World Health Organization* **47**, 317-324.

Huang, E-S., Alford, C.A., Reynolds, D.W., Stagno, S. & Pass, R.F. (1980). Molecular epidemiology of cytomegalovirus infections in women and their infants. *New England Journal of Medicine* **303**, 958-962.

Hutter, I. (1996). Reduction of food intake during pregnancy in rural South India. *Tropical Medicine and International Health* **3**, 339-405.

Hwang, L-Y., Roggendorf, M., Beasley, R.P. & Deinhardt, F. (1985). Perinatal transmission of hepatitis B virus: role of maternal HBeAg and anti-HBc IgM. *Journal of Medical Virology* **15**, 265-269.

Hytten, F.E. (1985). Blood volume changes in normal pregnancy. *Clinics in Haematology* **14**, 601-612.

Hytten, F.E. & Leitch, I. (1971). *The Physiology of Human Pregnancy*. Oxford: Blackwell Scientific Publications.

Ingall, D. & Musher, D. (1983). Syphilis. In: *Infectious Diseases of the Fetus and Newborn Infant*, Remington, J.S. & Klein, J.O. (editors). Philadelphia: W.B. Saunders, p. 345.

Inhorn, M.C. (1995). Medical anthropology and epidemiology: divergences or convergences? *Social Science and Medicine* **40**, 285-290.

Iwarson, S.A. (1985). Chronic hepatitis B. In: *Hepatitis B*, Gerety, R.J. (editor). Orlando: Academic Press Inc., pp. 119-153.

Jackson, R.T. & Jackson, L.C. (1987). Biological and behavioural contributors to anemia during pregnancy in Liberia, West Africa. *Human Biology* **59**, 585-597.

Jackson, R.T. & Latham, M.C. (1982). Anemias of pregnancy in Liberia, West Africa: a therapeutic trial. *American Journal of Clinical Nutrition* **35**, 710-714.

James, W.P.T., Ferro-Luzzi, A. & Waterlow, J.C. (1988). Definition of chronic energy deficiency in adults: Report of a Working party of the International Dietary Energy Consultative Group. *European Journal of Clinical Nutrition* **42**, 969-981.

James, W.P.T., Mascie-Taylor, G.C.N., Norgan, N.G., Bistrian, B.R., Shetty, P.S. & Ferro-Luzzi, A. (1994). The value of arm circumference in assessing chronic energy deficiency in Third World adults. *European Journal of Clinical Nutrition* **48**, 883-894.

Jansen, A.A.J., Kusin, J.A., Thiuri, B., Lakhani, S.A. & Mannetje, W. (1984). Machakos project studies No. XXIV. Anthropometric changes during pregnancy in rural African women. *Tropical and Geographical Medicine* **36**, 91-97.

Jenkins, S.N. & Behnke, J.M. (1977). Impairment of primary expulsion of *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius*. *Parasitology* **75**, 71-78.

Jenniskens, F., Obwaka, E., Kirisuah, S., Moses, S., Mohamedali Yusufali, F., Ndinya, Achola, J.O., Fransen, L., Laga, M. & Temmerman, M. (1995). Syphilis control in pregnancy: decentralization of screening facilities to primary health care level, a demonstration project in Nairobi, Kenya. *International Journal of Gynecology and Obstetrics* **48** (supplement), S121-S128.

Jerome, N.W. (1997). Culture-specific strategies for capturing local dietary intake patterns. *American Journal of Clinical Nutrition* **65** (supplement), S1166-S1167.

John-Spear, M.A. & Yip, R. (1994). Hemoglobin differences between black and white women with comparable iron status: justification for race-specific anemia criteria. *American Journal of Clinical Nutrition* **60**, 117-121.

Johnson, A.M. (1992). Epidemiology of HIV infection in women. *Bailliere's Clinical Obstetrics and Gynaecology* **6**, 12-31.

Judisch, J.M., Naiman, J.L. & Oski, F.A. (1986). The fallacy of the fat iron deficiency child. *Paediatrics* **37**, 987-992.

Junge, U. & Deinhardt, F. (1985). The acute manifestations of hepatitis B virus infection. In: *Hepatitis B*, Gerety, R.J. (editors). Orland: Academic Press Inc., pp. 93-118.

Kalderon, A.E., Kikkawa, Y. & Berstein, J. (1964). Chronic toxoplasmosis associated with severe hemolytic anemia. *Archives of Internal Medicine (Chicago)* **114**, 95-101.

Kamunvi, F. & Ferguson, A.G. (1993). Knowledge, attitudes and practices (KAP) of human intestinal helminths (worms) in two rural communities in Nyanza Province, Western Kenya. *East African Medical Journal* **70**, 482-490.

Kaneshige, E. (1981). Serum ferritin as an assessment of iron stores and other hematologic parameters during pregnancy. *Obstetrics and Gynecology* **57**, 238-342.

Kennedy, M.W. (1989). Genetic control of the immune repertoire in nematode infections. *Parasitology Today* **5**, 316-324.

Kent, S., Weinberg, E.D. & Stuart-Macadam, P. (1994). The etiology of the anemia of chronic disease and infection. *Journal of Clinical Epidemiology* **47**, 23-33.

Kew, M.C. (1996). Progress towards the comprehensive control of hepatitis B in Africa: a view from South Africa. *Gut* **38** (supplement 2), S31-S36.

Key, T.C., Horger, E.O. & Miller, J.M. (1981). Geophagia as a cause of maternal death. *Obstetrics and Gynecology* **60**, 525-526.

Keymer, A. & Pagel, M. (1990). Predisposition to helminth infection. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 177-209.

Kightlinger, L.K., Seed, J.R. & Kightlinger, M.B. (1996). *Ascaris lumbricoides* aggregation in relation to child growth status, delayed cutaneous hypersenstivity and plant anthelminthic use in Madagascar. *Journal of Parasitology* **82**, 25-33.

Kiire, C.F. (1990). Hepatitis B infection in sub-Saharan Africa. *Vaccine* **8** (supplement), S107-S112.

Kiire, C.F. (1996). The epidemiology and prophylaxis of hepatitis B in sub-Saharan Africa: a view from tropical and subtropical Africa. *Gut* **38** (supplement), S5-S12.

Kirby, J.P. (1993). The Islamic dialogue with African traditional religion: divination and health care. *Social Science and Medicine* **36**, 237-247.

Kirk, R.E. (1995). *Experimental Design. Procedures for Behavioural sciences*. Washington: Brooks/Cole Publishing Company.

Kliks, M.M. (1985). Studies on the traditional herbal anthelminthic *Chenopodium ambrosioides* L: ethnopharmacological evaluation and clinical field trials. *Social Science and Medicine* **21**, 879-886.

Kloos, H., DeSole, G. & Lemma, A. (1981). Intestinal parasitism in semi-nomadic pastoralists and subsistence farmers in and around irrigation schemes in the Awash Valley, Ethiopia, with special emphasis on ecological and cultural associations. *Social Science and Medicine* **15B**, 457-469.

Kochar, V.K., Schad, G.A., Chowdhury, A.B., Dea, C.G. & Nawalinski, T. (1976). Human factors in the regulation of parasitic infections: cultural ecology of hookworm populations in West Bengal. In: *Medical Anthropology*, Grolling, F.X. & Haley, H.B. (editors). The Hague: Paris Mouton, pp. 287-312.

Koroma, M.M. (1995). *The Effects of Albendazole on the Prevalence and Intensity of Soil-Transmitted Helminths in Primary-School Children in Alikalia and Gloucester (Sierra Leone)*. Dissertation for B.Sc. Department of Zoology, University of Sierra Leone.

Koroma, M.M., Williams, R.A., de la Haye, R.R. & Hodges, M. (1996). Effects of albendazole on growth of primary school children and the prevalence and intensity of soil-transmitted helminths in Sierra Leone. *Journal of Tropical Pediatrics* **42**, 371-372.

Koziel, M.J. (1996). Immunology of viral hepatitis. *American Journal of Medicine* **100**, 98-109.

Kramer, M.S. (1987). Determinants of low birth weight: methodological assessment and meta-analysis. *Bulletin of the World Health Organization* **65**, 663-737.

Krasovec, K. (1991a). Arm circumference. Background issues. In: *Maternal Nutrition and Pregnancy Outcomes. Anthropometric Assessment*, Krasovec, K. & Anderson, M.A. (editors). Washington: Pan American Health Organization, pp. 119-131.

Krasovec, K. (1991b). Maternal height. Background issues. In: *Maternal Nutrition and Pregnancy Outcomes. Anthropometric Assessment*, Krasovec, K. & Anderson, M.A. (editors). Washington: Pan American Health Organization, pp. 93-103.

Krasovec, K. & Anderson, M.A. (1991a). Maternal anthropometry for prediction of pregnancy outcomes: Memorandum from a USAID/WHO/PAHO/Mothercare meeting. *Bulletin of the World Health Organization* **69**, 523-532.

Krasovec, K. & Anderson, M.A. (1991b). *Maternal nutrition and pregnancy outcomes. Anthropometric assessment.* Washington: Pan American Health Organization.

Krech, U. & Tobin, J.A. (1981). Collaborative study of cytomegalovirus antibodies in mothers and young children in 19 countries. *Bulletin of the World Health Organization* **59**, 605-610.

Kroeger, A. (1983). Anthropological and socio-medical health care research in developing countries. *Social Science and Medicine* **17**, 147-161.

Kuvibidila, S., Yu, L., Warrier, R.P., Ode, D. & Mbele, V. (1994). Usefulness of serum ferritin levels in the assessment of iron status in non-pregnant Zairean women of childbearing age. *Journal of Tropical Medicine and Hygiene* **97**, 171-179.

Laderman, C. (1984). Food ideology and eating behavior: contributions from Malay studies. *Social Science and Medicine* **19**, 547-559.

Lasker, J.N. (1981). Choosing among therapies: illness behavior in the Ivory Coast. *Social Science and Medicine* **15A**, 157-168.

Latham, M.C., Lawless, J.W., Stephenson, L.S., Kinoti, S.N., Pertet, A. & Adams, E. (1991). Iron supplementation improves appetite in Kenyan children. *Federation of American Societies for Experimental Biology Journal* **5**, A720

Latham, M.C., Stephenson, L.S., Kinoti, S.N., Zaman, M.S. & Kurz, K.M. (1990). Improvements in growth following iron supplementation in young Kenyan school children. *Nutrition* **6**, 159-165.

Launer, L.J., Kardjati, S., Kusin, J.A. & Reed, G.F. (1991). Patterns of variability in the nutrient intake of nutritionally vulnerable pregnant women. *European Journal of Clinical Nutrition* **45**, 131-138.

Lawless, J.W., Latham, M.C., Stephenson, L.S., Pertet, A., Kinoti, S.N. & Adams, E. (1991). Iron supplementation and physical growth in Kenyan children. *Federation of American Societies for Experimental Biology Journal* **5**, A720.

Lawrence, M., Coward, W.A., Lawrence, F., Cole, T.J. & Whitehead, R.G. (1987a). Fat gain during pregnancy in rural African women: the effect of season and dietary status. *American Journal of Clinical Nutrition* **45**, 1422-1450.

Lawrence, M., Lawrence, F., Coward, W.A., Cole, T.J. & Whitehead, R.G. (1987b). Energy requirements of pregnancy in the Gambia. *Lancet* **2**, 1072-1076.

Lawrence, M., Lawrence, F., Lamb, W.H. & Whitehead, R.G. (1984). Maintenance of the energy cost of pregnancy in rural Gambian women and influence of dietary status. *Lancet* **1**, 363-365.

Lawrence, M. & Whitehead, R.G. (1988). Physical activity and total energy expenditure of child-bearing Gambian village women. *European Journal of Clinical Nutrition* **42**, 145-160.

Layrisse, M., Aparcedo, L., Martinez-Torres, C. & Roche, M. (1967). Blood loss due to infection with *Trichuris trichiura. American Journal of Tropical Medicine and Hygiene* **16**, 613-619.

Leading article (1989). Screening for congenital CMV. *Lancet* **2**, 599.

Leroy, V., De Clercq, A., Ladner, J., Bogaerts, J., Van de Perre, P. & Dabis, F. (1995). Should screening of genital infections be part of antenatal care in areas of high HIV prevalence? A prospective cohort study from Kigali, Rwanda, 1992-1993. *Genitourinary Medicine* **71**, 207-211.

Letsky, E. (1995). Erythropoiesis in pregnancy. *Journal of Perinatal Medicine* **23**, 39-45.

Lindenbaum, J. & Klipstein, F.A. (1963). Folic acid deficiency in sickle-cell anemia. *New England Journal of Medicine* **269**, 875-882.

Lipschitz, D.A., Cook, J.D. & Finch, C.A. (1974). A clinical evaluation of serum ferritin as an index of iron stores. *New England Journal of Medicine* **290**, 1213-1216.

Littlejohn, J. (1960). The Temne house. *Sierra Leone Studies* **14**, 63-79.

Loke, Y.W. (1982). Transmission of parasites across the placenta. *Advances in Parasitology* **21**, 155-228.

Lui, X.N., Yang, W., Zhang, J., Ying, H., Gen, Y., Xie, J. & Viteri, F.E. (1995). Weekly iron supplementation is effective and safe in pregnant women. *Federation of American Societies for Experimental Biology Journal* **9**, A5658.

Lurie, P., Fernandes, M.E.L., Hughes, V., Arevalo, E.I., Hudes, E.S., Reingold, A. & Hearst, N. (1995). Socio-economic status and risk of HIV-1, syphilis and hepatitis B infection among sex workers in Sao Paulo State, Brazil. *AIDS* **9** (supplement), S31-S37.

Lwambo, N.J.S., Bundy, D.A.P. & Medley, G.F.H. (1992). A new approach to morbidity risk assessment in hookworm endemic communities. *Epidemiology and Infection* **108**, 469-481.

MacLeod, C.L. (1988). Intestinal nematode infections. In: *Parasitic Infections in Pregnancy and the Newborn*, MacLeod, C.L. (editor). Oxford: Oxford University Press, pp. 192-215.

MacLeod, C.L. & Lee, R.V. (1988). Parasitic infections. In: *Medical Complications During Pregnancy*, Burrow, G.N. & Ferris, T.F. (editors). Philadelphia: W.B. Saunders, pp. 425-447.

Maisonneuve, H., Rossignol, J.F., Addo, A. & Mojon, M. (1985). Ovicidal effects of albendazole in human ascariasis, ancylostomiasis and trichuriasis. *Annals of Tropical Medicine and Parasitology* **79**, 79-82.

Mambwe Kaoma, F. & Scott, G.B. (1992). Clinical manifestations, management and therapy of HIV infection in children. *Bailliere's Clinical Obstetrics and Gynecology* **6**, 149-164.

Manderson, L. & Aaby, P. (1992). An epidemic in the field? Rapid assessment procedures and health research. *Social Science and Medicine* **35**, 839-850.

Mann, J. & Truswell, S. (1998). Vitamin A and carotenoids. In: *Essentials of Human Nutrition*, Mann, J. & Truswell, S. (editors). Oxford: Oxford Medical Publications, pp. 179-196.

Marinier, E., Barrois, W., Larouze, B., London, T.W., Cofer, A., Diakhate, L. & Blumberg, B.S. (1985). Lack of perinatal transmission of hepatitis B virus infection in Senegal, West Africa. *Journal of Pediatrics* **106**, 843-849.

Martinez-Torres, C., Owed, A., Roche, M. & Layrisse, M. (1967). Hookworm infection and intestinal blood loss. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **64**, 881-884.

Martinson, F.E.A., Weigle, K.A., Royce, R.A., Weber, D.J., Suchindran, C.M. & Lemon, S.M. (1998). Risk factors for horizontal transmission of hepatitis B virus in a rural district in Ghana. *American Journal of Epidemiology* **147**, 478-487.

Martorell, R. (1991). Maternal height as an indictor of risk. In: *Maternal Nutrition and Pregnancy Outcomes. Anthropometric Assessment*, Krasovec, K. & Anderson, M.A. (editors). Washington: Pan American Health Organization, pp. 104-111.

Masawe, A.E.J. (1981). Nutritional anaemias. Part 1: tropical Africa. *Clinics in Haematology* **10**, 815-842.

Massey, A.C. (1992). Microcytic anemia: differential diagnosis and management of iron deficiency anemia. *Medical Clinics of North America* **76**, 549-566.

Maynard, J.E., Kane, M.A. & Hadler, S.C. (1989). Global control of hepatitis B through vaccination: role of hepatitis B vaccine in the Expanded Program on Immunization. *Review of Infectious Diseases* **11** (supplement 3), S574-S578.

McCormick, M.C. (1985). The contribution of low birth weight to infant mortality and childhood morbidity. *New England Journal of Medicine* **312**, 82-90.

McDermott, J., Steketee, R. & Wirima, J. (1996). Perinatal mortality in rural Malawi. *Bulletin of the World Health Organization* **74**, 165-171.

McFee, J.G. (1973). Anaemia: a high risk complication of pregnancy. *Clinical Obstetrics and Gynecology* **16**, 153-171.

McGregor, I.A. (1984). Epidemiology, malaria and pregnancy. *American Journal of Tropical Medicine and Hygiene* **33**, 517-525

McKay, D.A. (1971). Food illness and folk medicine. *Ecology of Food and Nutrition* **1**, 67-72.

Medhat, A., El-Sharkawy, M.M., Shaaban, M.M., Makhlouf, M.M. & Ghaneima, S.E. (1993). Acute viral hepatitis in pregnancy. *International Journal of Gynecology and Obstetrics* **40**, 25-31.

Medley, G.F. (1994). Chemotherapy. In: *Parasite and Infectious Diseases*, Scott, M.E. & Smith, M.E. (editors). Academic Press: New York, pp. 141-157.

Mejia, L.A. & Chew, F. (1988). Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. *American Journal of Clinical Nutrition* **48**, 595-600.

Menendez, C., Todd, J., Alonso, P.L., Francis, N., Lulat, S., Ceesay, S., M'Boge, B. & Greenwood, B.M. (1994). The effects of iron supplementation during pregnancy, given by traditional birth attendants, on the prevalence of anaemia and malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 590-593.

Merchant, K. & Martorell, R. (1990). Consequences for maternal nutrition of reproductive stress across consecutive pregnancies. *American Journal of Clinical Nutrition* **52**, 616-620.

Mertens, T., Hayes, R. & Smith, P. (1990). Epidemiological methods to study the interaction between HIV infection and other STDs. *AIDS* **4**, 57-65.

Meyer, M.B., Bruce, S.J. & Tonascia, J.A. (1976). Perinatal events associated with maternal smoking during pregnancy. *American Journal of Epidemiology* **103**, 464-476.

Miller, G.C. (1981). Helminths and the transmammary route of infection. *Parasitology* **82**, 335-342.

Milman, N., Agger, A.O. & Nielson, O.J. (1991). Iron supplementation during pregnancy. *Danish Medical Bulletin* **38**, 471-476.

Milman, N., Agger, A.O. & Nielson, O.J. (1994). Iron status markers and serum erythropoietin in 120 mothers and newborn infants. *Acta Obstetrica Gynecologia Scandinavica* **73**, 200-204.

Milman, N., Ibsen, K.K. & Christensen, J.M. (1987). Serum ferritin and iron status in mothers and newborn infants. *Acta Obstetrica et Gynecologica Scandinavica* **66**, 205-211.

Ministry of Health and Women's Affairs (1994). *A National Strategy and Broad Plan of Action for the Prevention and Control of Anaemia in Pregnancy*. Sri Lanka.

Minkoff, H.L. (1998). The relationship of pregnancy to HIV disease. *Immunology and Allergy Clinics of North America* **18**, 329-344.

Miotti, P.G, Chiphangwi, J.D. & Dallabetta, G.A. (1992). The situation in Africa. *Bailliere's Clinical Obstetrics and Gynaecology* **6**, 165-186.

MNDEP/UNICEF (1989). *The Children and Women of Sierra Leone. An Analysis of their Situation*. Volume One. Freetown: MEF Enterprises.

Mok, J.Y.Q. (1992). Vertical Transmission. *Bailliere's Clinical Obstetrics and Gynecology* **6**, 85-100.

Monsen, E.R., Hallberg, L., Layrisse, M., Hegsted, D.M., Cook, J.D., Mertz, W. & Finch, C.A. (1978). Estimation of available dietary iron. *American Journal of Clinical Nutrition* **31**, 134-141.

Montresor, A., Crompton, D.W.T., Bundy, D.A.P., Hall, A. & Savioli, L. (1998). *Guidelines for the Evaluation of Soil-Transmitted Helminthiasis and Schistosomiasis at Community Level*. WHO Document WHO/CTD/SIP/98.1. Geneva: World Health Organization.

Murphy, J.F., O'Riordan, J., Newcombe, R.G., Coles, E.C. & Pearson, J.F. (1986). Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. *Lancet* **1**, 992-995.

Musher, D.M., Hamill, R., J. & Olson, O.E. (1990). Effects of human immunodeficiency virus (HIV) infection on the course of syphilis and on the response to treatment. *Annals of Internal Medicine* **113**, 872-881.

Nawalinski, T., Schad, G.A. & Chowdhury, A.B. (1978). Population biology of hookworms in children in rural West Bengal. *American Journal of Tropical Medicine and Hygiene* **27**, 1152-1161.

Ndumbe, P.M., Andela, A., Nkemnkeng-Asong, J., Watonsi, E. & Nyambi, P. (1992). Prevalence of infections affecting the child among pregnant women in Yaounde, Cameroon. *Medical Microbiology and Immunology* **181**, 127-130.

Needham, C.S. & Lillywhite, J.E. (1994). Immunoepidemiology of intestinal helminthic infections. 2. Immunological correlates with patterns of *Trichuris* infection. *Transactions of the Royal Society for Tropical Medicine and Hygiene* **88**, 262-264.

Needham, C.S., Lillywhite, J.E., Beasley, N.M.R., Didier, J.M., Kihamia, C.M. & Bundy, D.A.P. (1996). Potential for diagnosis of intestinal nematode infections through antibody detection in saliva. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 526-530.

Nelson, C.T. & Demmler, G.J. (1997). Cytomegalovirus infection in the pregnant mother, fetus and newborn infant. *Clinics in Perinatology* **24**, 151-160.

Nelson, G.S. (1990). Hookworms in perspective. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 417-430.

Nelson, M., Black, A.E., Morris, J.A. & Cole, T.J. (1989). Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. *American Journal of Clinical Nutrition* **50**, 155-167.

Nesheim, M.C. (1989). Ascariasis and human nutrition. In: *Ascariasis and its Prevention and Control*, Crompton, D.W.T., Nesheim, M.C. & Pawlowski, Z.S. (editors). London: Taylor & Francis, pp. 87-100.

Nesheim, M.C. (1993). Human nutrition needs and parasitic infection. *Parasitology* **103** (supplement), S7-S18.

Ng, P.C. & Fok, T.F. (1996). Infections in the neonate. *Current Opinion in Infectious Diseases* **9**, 181-186.

Nilsson, L. & Solvell, L. (1967). Clinical studies on oral contraceptives - a randomized double blind, crossover study of 4 different preparations. *Acta Obstetrica Gynecologia Scandanivica* **46** (supplement **8**), 1-36.

Nordeide, M.B. (1997). *Table de Composition D'aliments du Mali. Projet de Recherche, SSE Environnement et Developpement au Mali.* Institute de Nutrition, Universite d'Oslo.

Norton, R. (1994). Maternal nutrition during pregnancy as it affects infant growth, development and health. *Subcommittee on Nutrition News* **11**, 10-14.

Nwosu, A.B.C. (1981). Human neonatal infections with hookworms in an endemic area of Southern Nigeria - a possible transmammary route. *Tropical and Geographical Medicine* **33**, 105-111.

Nyamwaya, D. (1987). A case study of the interaction between indigenous and Western medicine among the Pokot of Kenya. *Social Science and Medicine* **25**, 1277-1287.

Obisesan, K.A., Olaleye, O.D. & Adeyemo, A.A. (1997). The increasing prevalence of HIV-1 and HIV-2 infections in a low risk antenatal population in south west Nigeria. *International Journal of Gynecology and Obstetrics* **56**, 271-273.

Odebiyi, A.I. (1989). Food taboos in maternal and child health: the views of traditional healers in Ife-Ife, Nigeria. *Social Science and Medicine* **28**, 985-996.

Ogbeide, O. (1974). Nutritional hazards of food taboos and preferences in Mid-West Nigeria. *American Journal of Clinical Nutrition* **27**, 213-216.

Ogunbode, O., Akinyele, I.O. & Hussain, M.A. (1979). Dietary iron intake of pregnant Nigerian women with anaemia. *International Journal of Gynaecology and Obstetrics* **17**, 290-293.

Ogunniyi, S.O. (1990). Aetiological factors in low birth weight in a Nigerian population. *Tropical Doctor* **20**, 87-88.

Ogunniyi, T.A.B. & Amole, B.O. (1990). Socio-cultural problems in the execution of a guinea worm control programme. *Tropical and Geographical Medicine* **42**, 377-379.

Ohto, H., Lin, H.H., Kawana, T., Etoh, T. & Tohyama, H. (1987). Intrauterine transmission of hepatitis B virus is closely related to placental leakage. *Journal of Medical Virology* **21**, 1-6.

Okada, K., Kamiyami, I., Inomata, M., Mitsunobu, I., Miyakawa, Y. & Mayumi, M. (1976). e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. *New England Journal of Medicine* **294**, 746-749.

Olusi, T., Gross, U. & Ajayi, J. (1996). High incidence of toxoplasmosis during pregnancy in Nigeria. *Scandinavian Journal of Infectious Diseases* **28**, 645-646.

Onadeko, M.O., Joynson, D.H.M. & Payne, R.A. (1992). The prevalence of *Toxoplasma* infection among pregnant women in Ibadan, Nigeria. *Journal of Tropical Medicine and Hygiene* **95**, 143-145.

Opala, J. & Boillot, F. (1996). Leprosy among the Limba: illness and healing in the context of world view. *Social Science and Medicine* **42**, 3-19.

Oski, F.A. (1979). The non-hematologic manifestations of iron deficiency. *American Journal of Diseases in Childhood* **133**, 315-322.

Ottesen, E.A. (1990). Immune responses in human hookworm infection. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 404-413.

Pan, W.H. & Habicht, J.P. (1991). The non-iron-deficiency-related difference in hemoglobin concentration distribution between blacks and whites and between men and women. *American Journal of Epidemiology* **134**, 1410-1416.

Panth, M., Shatrunga, V., Yasodhara, P. & Sivakumar, B. (1990). Effect of vitamin A supplementation on haemoglobin and vitamin A levels during pregnancy. *British Journal of Nutrition*, **64**, 351-358.

Pappagallo, S. & Bull, D.L. (1996). Operational problems of an iron supplementation programme for pregnant women: an assessment of UNRWA experience. *Bulletin of the World Health Organization* **74**, 25-33.

Parks, Y.A. & Wharton, B.A. (1989). Iron deficiency and the brain. *Acta Pediatrica Scandinavica Supplement* **361**, 71-77.

Pass, R.F., Little, E.A., Stagno, S., Britt, W.J. & Alford, C.A. (1987). Young children as a probable source of maternal and congenital cytomegalovirus infection. *New England Journal of Medicine* **316**, 1366-1370.

Pastorek, J.G., Miller, J.M. & Summers, P.R. (1988). The effect of hepatitis B antigenemia on pregnancy outcome. *American Journal of Obstetrics and Gynecology* **158**, 486-489.

Paul, A.A., Muller, E.M. & Whitehead, R.G. (1979). The quantitative effects of maternal dietary intake on pregnancy and lactation in Gambian women. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 686-692.

Pawlowski, Z.S. (1995). Anthelminthic therapy and iron supplementation of pregnant women. *American Journal of Clinical Nutrition* **62**, 1023-1024.

Pawlowski, Z.S., Schad, G.A. & Stott, G.J. (1991). *Hookworm Infection and Anaemia. Approaches to Prevention and Control.* Geneva: World Health Organization.

Pelto, G.H. (1987). Cultural issues in maternal and child health and nutrition. *Social Science and Medicine* **25**, 553-559.

Pepin, J., Dunn, D., Gaye, I., Alonso, P., Egboga, A., Tedder, R., Piot, P., Berry, N., Schellenberg, D., Whittle, H. & Wilkins, A. (1991). HIV-2 infection among prostitutes working in the Gambia: association with serological evidence of genital ulcer diseases and with generalized lymphadenopathy. *AIDS* **5**, 69-75.

Perine, P.L. (1991). Syphilis. In: *Hunter's Tropical Medicine*, 7th Edition, Strickland, G.T. (editor). Philadelphia: W.B. Saunders Company, pp. 302-306.

Perlas, L.A., Kuizon, M.D., Tajaon, R.T. & Desnacido, J.A.T.I. (1992). Iron status of pregnant Filipino women as measured by serum ferritin. *Southeast Asian Journal of Tropical Medicine and Public Health* **23**, 691-697.

Perry, G.S., Byers, T., Yip, R. & Margen, S. (1992). Iron nutrition does not account for the hemoglobin differences between black and whites. *Journal of Nutrition* **122**, 1417-1424.

Pinus, J. (1985). Surgical complications of ascariasis in Brazil. In: *Ascariasis and its Public Health Significance*, Crompton, D.W.T., Nesheim, M.C. & Pawlowski, Z.S. (editors). London: Taylor & Francis, pp. 161-165.

Pitts, M., McMaster, J., Hartmann, T. & Mausezahl, D. (1996). Lay beliefs about diarrhoeal diseases: their role in health education in a developing country. *Social Science and Medicine* **43**, 1223-1228.

Poppitt, S.D., Prentice, A.M., Jequier, E., Schutz, Y. & Whitehead, R.G. (1993). Evidence of energy sparing in Gambian women during pregnancy: a longitudinal study using whole-body calorimetry. *American Journal of Clinical Nutrition* **57**, 353-364.

Powers, H.J. & Bates, C.J. (1987). Micronutrient deficiencies in the aetiology of anaemia in a rural area of the Gambia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 421-425.

Powers, H.J., Bates, C.J. & Lamb, W.H. (1985). Haematological response to supplements of iron and riboflavin to pregnant and lactating women in rural Gambia. *Human Nutrition: Clinical Nutrition* **39C**, 117-129.

Prada, J.A. & Tsang, R.C. (1998). Biological mechanisms of environmentally induced causes of IUGR. *European Journal of Clinical Nutrition* **52** (supplement 1), S21-S28.

Prasad, A.N. & Prasad, C. (1991). Iron deficiency: non-haematological manifestations. *Progress in Food and Nutrition Science* **15**, 255-283.

Pratt, E. (1983). Food habits and nutrition in developing countries. *Journal of the Sierra Leone Medical and Dental Association* **4**, 34-38.

Prema, K, Ramalakshmi, B.A., Madhavapeddi, R. & Babu, S. (1982). Immune status in anaemia pregnant women. *British Journal of Obstetrics and Gynaecology* **89**, 222-225.

Prentice, A. (1991). Functional significance of marginal calcium deficiency. In: *Modern Lifestyles, Lower Energy Intake and Micronutrient Status*, Peitrzik, K. (editor). London: Springer-Verlag, pp. 139-154.

Prentice, A. (1994). Maternal calcium requirements during pregnancy and lactation. *American Journal of Clinical Nutrition* **59** (supplement), S477-S483.

Prentice, A.M. (1980). Variations in maternal dietary intake, birthweight and breast-milk output in the Gambia. In: *Maternal Nutrition During Pregnancy and Lactation*, Aebi, H. & Whitehead, R.G. (editors). Bern, Switzerland: Hans Huber Publication, pp. 167-183.

Prentice, A.M., Cole, T.J., Foord, R.A., Lamb, W.H. & Whitehead, R.G. (1987). Increased birth-weight after prenatal dietary supplementation of rural African women. *American Journal of Clinical Nutrition* **46**, 912-925.

Prentice, A.M., Whitehead, R.G., Roberts, S.B. & Paul, A.A. (1981). Long-term energy balance in child-bearing Gambian women. *American Journal of Clinical Nutrition* **34**, 2790-2799.

Prentice, A.M., Whitehead, R.G., Watkinson, M. & Lamb, W.H. (1983). Prenatal dietary supplementation of African women and birth-weight. *Lancet* **1**, 489-492.

Preziosi, P., Prual, A., Galan, P., Daouda, H., Boureima, H. & Hercberg, S. (1997). Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. *American Journal of Clinical Nutrition* **66**, 1178-1182.

Prince, A.M., Szmuness, W., Millian, S.J. & David, D.S. (1971). A serologic study of cytomegalovirus infections associated with blood transfusions. *New England Journal of Medicine* **284**, 1125-1131.

Pritchard, D.I., Ali, N.M.H. & Benke, J.M. (1984). Analysis of the mechanism of immunodepression following heterologous antigens stimulation during concurrent infection with *Nematospiroides dubius*. *Immunology* **51**, 633-642.

Pritchard, D.I., McKean, P.G. & Schad, G.A. (1990a). An immunological and biochemical comparison of hookworm species. *Parasitology Today* **6**, 154-156.

Pritchard, D.I., Quinnell, R.J., Moustafa, M., McKean, P.G. & Slater, A.F.G. (1991). Hookworm (*Necator americanus*) infection and storage iron depletion. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 235-238.

Pritchard, D.I., Quinnell, R.J., Slater, A.F.G., McKean, P.G., Dale, D.D.S., Raiko, A. & Keymer, A.E. (1990b). Epidemiology and immunology of *Necator americanus* infection in a community in Papua New Guinea: humoral responses to excretory-secretory and cuticular collagen antigens. *Parasitology* **100**, 317-326.

Pritchard, D.I., Quinnell, R.J. & Walsh, E.A. (1995). Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunology* **17**, 71-75.

Pugh, R.N.H. (1979). Periodicity of output of *Schistosoma haematobium* eggs in the urine. *Annals of Tropical Medicine and Parasitology* **73**, 89-90.

Puolakka, J., Janne, O., Pakarinen, A., Jarvinen, P.A. & Vihko, R. (1980). Serum ferritin as a measure of iron stores during and after normal pregnancy with and without iron supplements. *Obstetrica Gynecologia Scandinavica* **95** (supplement), 43-51.

Quinnell, R.J., Slater, A.F.G., Tighe, P., Walsh, E.A., Keymer, A.E. & Pritchard, D.I. (1993). Reinfection with hookworm after chemotherapy in Papua New Guinea. *Parasitology* **106**, 379-385.

Quinnell, R.J., Woolhouse, M.E.J., Walsh, E.A. & Pritchard, D.I. (1995). Immunoepidemiology of human necatoriasis: correlations between antibody responses and parasite burdens. *Parasite Immunology* **17**, 313-318.

Rauyajin, O., Kamthornwachara, B. & Yablo, P. (1995). Socio-cultural and behavioural aspects of mosquito-borne lymphatic filariasis in Thailand: a qualitative analysis. *Social Science and Medicine* **41**, 1705-1713.

Reddy, V.A.P., Bates, C.J., Goh, S.G.J., Rowland, M.G.M., Greenwood, A.M., Greenwood, B. & Paul, A.A. (1987). Riboflavin, folate and vitamin C status of Gambia women during pregnancy: a comparison between urban and rural communities. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 1033-1037.

Reinhardt, M.C. (1980). The African newborn in Abidjan - maternal and environmental factors influencing the outcome of pregnancy. In: *Maternal nutrition during pregnancy and lactation*, Aebi, H. & Whitehead, R.G. (editors). Bern, Switzerland: Hans Huber Publication, pp. 132-149.

Ridwan, E., Schultink, W., Dillon, D. & Gross, R. (1996). Effects of weekly iron supplementation of Indonesian pregnant women are similar to those of daily supplementation. *American Journal of Clinical Nutrition* **63**, 884-890.

Roberts, N.S., Copel, J.A., Bhutani, V., Otis, C. & Gluckman, S. (1985). Intestinal parasites and other infections during pregnancy in southeast Asian refugees. *Journal of Reproductive Medicine* **30**, 720-725.

Roberts, S.B., Paul, A.A., Cole, T.J. & Whitehead, R.G. (1982). Seasonal changes in activity, birthweight and lactation performance in rural Gambian women. *Transactions of the Royal Society of Medicine and Hygiene* **76**, 668-678.

Robertson, L.J., Crompton, D.W.T., Sanjur, D. & Nesheim, M.C. (1992). *Trichuris trichiura* and the growth of primary schoolchildren in Panama. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 656-657.

Roche, M. & Layrisse, M. (1966). The nature and causes of 'hookworm anaemia'. *American Journal of Tropical Medicine and Hygiene* **15**, 1030-1100.

Rodier, M.H., Berthonneau, J., Bourgoin, A., Giraudeau, G., Agius, G., Burucoa, C., Hekpazo, A. & Jacquemin, J.L. (1995). Sero-prevalence of toxoplasma, malaria, rubella, cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou, Republic of Benin. *Acta Tropica* **59**, 271-277.

Rogers, I.T. (1992). *The Prevalence of Intestinal Parasites Among Children in Kroo Bay, Sierra Leone*. Dissertation for B.Sc. Basic Medical Science. University of Sierra Leone.

Roingeard, P., Diouf, A., Sankale, J-L., Boye, C., Mboup, S., Diadhiou, F. & Essex, M. (1993). Perinatal transmission of hepatitis B virus in Senegal, West Africa. *Viral Immunology* **6**, 65-73.

Rolschau, J., Date, J. & Kristoffersen, K. (1979). Folic acid supplement and intrauterine growth. *Acta Obstetrica et Gynecologica Scandinavica* **58**, 343-346.

Ronday, M.J., Stilma, J.S., Barbe, R.F., McElroy, W.J., Luyendijk, L., Kolk, A.H., Bakker, M., Kijlstra, A. & Rothova, A. (1996). Aetiology of uveitis in Sierra Leone, West Africa. *British Journal of Ophthalmology* **80**, 956-961.

Rosenberg, E.M. (1980). Demographic effects of sex-differential nutrition. *Nutritional Anthropology. Contemporary approaches to diet and culture*, Jerome, N.W., Kandel, R.F. & Pelto, G.H. (editors). New York: Redgrave Publishing Company, pp. 181-203.

Ross, J.S. (1991). Perinatal Care: the Kuranko context of choice. *Health Care for Women International* **12**, 249-260.

Rossignol, J.F. & Maisonneuve, H. (1983). Albendazole: placebo-controlled study in 870 patients with intestinal helminths. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **77**, 707-711.

Rougereau, A., Gore, J., N'Diaye, M. & Person, O. (1982). Ferritin and iron status in Senegalese women. *American Journal of Clinical Nutrition* **36**, 314-318.

Rush, D. & Kristal, A. (1982). Methodologic studies during pregnancy: the reliability of the 24-hour dietary recall. *American Journal of Clinical Nutrition* **35**, 1259-1268.

Sabin, A.B. (1941). Toxoplasmic encephalitis in children. *Journal of the American Medical Association* **116**, 801-807.

Sachs, L. & Krantz, I. (1997). Interdisciplinary health research - a symposium. *Social Science and Medicine* **44**, 1209-1210.

Sahwi, S.Y., Zaki, M.S., Haiba, N.Y., Elsaid, O.K., Anwar, M.Y. & AbdRabbo, S.A. (1995). Toxoplasmosis as a cause of repeated abortion. *Journal of Obstetrics and Gynecology* **21**, 145-148.

Sanchez, P.J. & Wendel, G.D. (1997). Syphilis in pregnancy. *Clinics in Perinatology* **24**, 71-90.

Sangare, L., Meda, N., Lankoande, S., Van Dyck, E., Cartoux, M., Compaore, I.P., Catrayc, J., Sanou, P.T. & Soudre, R. (1997). HIV infection among pregnant women in Burkino Faso: a nationwide sero-survey. *International Journal of STD and AIDS* **8**, 646-651.

Sanjur, D. (1982). *Social and Cultural Perspectives in Nutrition*. Englewood Cliffs, New Jersey: Prentice-Hall.

Sanjur, D. (1989). Socio-economic and cultural factors in ascariasis. In: *Ascariasis and its Prevention and Control*, Crompton, D.W.T., Nesheim, M.C. & Pawlowski, Z.S. (editors). London: Taylor & Francis, pp. 301-320.

Santiso, R. (1997). Effects of chronic parasitosis on women's health. *International Journal of Gynecology and Obstetrics* **58**, 129-136.

Saowakontha, S., Pongpaew, P., Schelp, F.P., Rojsathaporn, K., Intarakha, C., Pipitgool, V., Mahaweerawat, U., Lumbiganon, P., Sriboonlue, P., Sanchaisuriya, P., Vudhivai, N., Supawan, V., Hinz, E., Hempfling, A., Bohning, D.M. & Migasena, P. (1992). Nutritional health and parasitic infection of rural Thai women of child bearing age. *Nutrition Research* **12**, 929-942.

Sawitz, W. (1942). The buoyancy of certain nematode eggs. *Journal of Parasitology* **28**, 95-102.

Schad, G.A. (1990). Hypobiosis and related phenomena in hookworm infection. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 71-88.

Schad, G.A. (1991). Hooked on hookworm: 25 years of attachment. *Journal of Parasitology* **77**, 179-186.

Schad, G.A. (1994). *Biology of Hookworm Infection Specific to Women*. WHO Informal Consultation on Hookworm Infection in Women, 5-7 December 1994. Working Paper No. 2. Geneva: World Health Organization.

Schad, G.A. & Anderson, R.M. (1985). Predisposition to hookworm infection in humans. *Science* **228**, 1537-1540.

Schad, G.A., Nawalinski, T.A. & Kochar, V. (1983). Human ecology and the distribution and abundance of hookworm population. In: *Human Ecology and Infectious Diseases,* Croll, D.A. & Cross, J.H. (editors). New York: Academic Press, pp. 187-223.

Schad, G.A. & Warren, K.S. (1990). *Hookworm disease: current status and future directions*. London: Taylor & Francis.

Scheirer, C.J., Roy, W.S. & Hare, N. (1976). The analysis of ranked data derived from completely randomised factorial designs. *Biometrics* **32**, 429-434.

Schoenbaum, E.E., Davenny, K. & Holbrook, K. (1992). The management of HIV disease in pregnancy. *Bailliere's Clinical Obstetrics and Gynecology* **6**, 101-124.

Scholl, T. & Hediger, M. (1994). Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. *American Journal of Clinical Nutrition* **59** (supplement), S492-S501.

Scholl, T.O., Hediger, M.L., Fischer, R.L. & Schaerer, J.W. (1992). Anaemia vs iron deficiency: increased risk of preterm delivery in a prospective study. *American Journal of Clinical Nutrition* **55**, 985-992.

Schopfer, K., Lauber, E. & Krech, U. (1978). Congenital cytomeglovirus infection in newborn infants of mothers infected before pregnancy. *Archives of Disease in Childhood* **53**, 536-539.

Schweiztzer, I.L. (1973). Viral hepatitis B in neonates and infants. *American Journal of Medicine* **55**, 762-771.

Scrimshaw, C.N. & Hurtado, E. (1988). Anthropological involvement in the Central American Diarrhoeal Disease Control Project. *Social Science and Medicine* **27**, 97-105.

Sempos, C.T., Johnson, N.E., Smith, E.L. & Gilligan, C. (1985). Effect of intra-individual and inter-individual variation in repeated dietary records. *American Journal of Epidemiology* **121**, 120-130.

Sen-Hai, Y. (1994). *Infantile Hookworm Disease*. WHO Informal Consultation on Hookworm Infection in Women, 5-7 December 1994. Working Paper No. 10. Geneva: World Health Organization.

Sever, J.L. (1989). HIV: Biology and Immunology. *Clinical Obstetrics and Gynecology* **32**, 423-428.

Shah, K. (1991). The evolution of the use of arm circumference for assessing maternal nutritional status. In: *Maternal Nutrition and Pregnancy Outcomes. Anthropometric Assessment*, Krasovec, K. & Anderson, M.A. (editors). Washington: Pan American Health Organization, pp. 132-137.

Shaw, R.H. (1982). *Temne Divination: the Management of Secrecy and Revelation*. Ph.D. Thesis. School of Oriental and African Studies, University of London.

Shetty, P.S., James, W.P.T. & Ferro-Luzzi, A. (1994). Malnutrition and the immune response. 1. Malnutrition in the community: recent concepts. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 612-614.

Shulman, C.E., Graham, W.J., Jilo, H., Lowe, B.S., New, L., Obiero, J., Snow, R.W. & Marsh, K. (1996). Malaria is an important cause of anaemia in primigravidae: evidence from a district hospital in coastal Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 535-539.

Siegenberg, D., Baynes, R.D. & Bothwell, T.H. (1991). Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytate on nonheme iron absorption. *American Journal of Clinical Nutrition* **53**, 537-541.

Sierra Leone Government. (1992). *1985 Population and Housing Census of Sierra Leone. Summary report.* Freetown: Central Statistics Office.

Singla, P.H., Chang, S., Khanna, S. & Agarwal, K.N. (1978). Effect of maternal anaemia on the placental and the newborn infant. *Acta Paediatrica Scandinavica* **67**, 645-648.

SLRC/MOH (1997). *The Sierra Leone Red Cross Blood Bank Annual Report 1996.* Freetown: Sierra Leone Red Cross/Ministry of Health.

Smith, G. (1990). The ecology of the free-living stages: a reappraisal. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 147-164.

Smithie, B.M. (1992). *The Aetiology of Diarrhoea in Sierra Leonean Children.* Dissertation for M.Sc. University of Liverpool.

Sofowora, A. (1982). *Medicinal Plants and Traditional Medicine in Africa.* Chichester: John Wiley and Sons Ltd.

Spence, M.R. (1988). The treatment of gonorrhea, syphilis, chancroid, lymhogranuloma venereum and granuloma inguinale. *Clinical Obstetrics and Gynecology* **31**, 453-465.

St. George, J.S. (1976). Intestinal parasitic infestation among parturients in Trinidad and Tobago. *International Surgery* **61**, 222-225.

Stagno, S., Pass, R.F., Cloud, G., Britt, W.J., Henderson, R.E., Walton, P.D., Veren, D.A., Page, F. & Alford, C.A. (1986). Primary cytomegalovirus infection in pregnancy. *Journal of the American Medical Association* **256**, 1904-1908.

Stagno, S., Pass, R.F., Dworsky, M.E., Henderson, R.E., Moore, E.G., Walton, P.D. & Alford, C.A. (1982). Congenital cytomegalovirus infection. The relative importance of primary and recurrent maternal infection. *New England Journal of Medicine* **306**, 945-949.

Stagno, S., Reynolds, D.W., Huang, E-S., Thames, S.D., Smith, R.J. & Alford, C.A. (1977). Congenital cytomegalovirus infection: occurrence in an immune population. *New England Journal of Medicine* **296**, 1254-1258.

Stapleton, M. (1989). Diarrhoeal disease: perceptions and practices in Nepal. *Social Science and Medicine* **28**, 593-604.

Stephenson, L.S. (1987a). Human malnutrition and helminths. In: *Impact of Helminth Infections on Human Nutrition. Schistosomes and Soil-Transmitted Helminths*, Stephenson, L.S. & Holland, C. London: Taylor & Francis, pp. 1-20.

Stephenson, L.S. (1987b). The design of nutrition-parasite studies. In: *Impact of Helminth Infections on Human Nutrition. Schistosomes and soil-transmitted helminths*, Stephenson, L.S. & Holland, C. London: Taylor & Francis, pp. 21-48.

Stephenson, L.S. (1987c). Ascariasis. In: *Impact of Helminth Infections on Human Nutrition. Schistosomes and soil-transmitted helminths*, Stephenson, L.S. & Holland, C. London: Taylor & Francis, pp. 89-127.

Stephenson, L.S., Crompton, D.W.T., Latham, M.C., Schulpen, T.W.J., Nesheim, M.C. & Jansen, A.A.J. (1980). Relationships between *Ascaris* infection and growth of malnourished preschool children in Kenya. *American Journal of Clinical Nutrition* **33**, 1165-1172.

Stephenson, L.S. & Holland, C. (1987). *Impact of Helminth Infections on Human Nutrition. Schistosomes and Soil-Transmitted Helminths.* London: Taylor & Francis.

Stephenson, L.S., Kinoti, S.N., Latham, M.C., Kurz, K.M. & Kyobe, J. (1989a). Single dose metrifonate or praziquantel treatment in Kenyan children. I. Effects on *Schistosoma haematobium*, hookworm, hemoglobin levels, splenomegaly and hepatomegaly. *American Journal of Tropical Medicine and Hygiene* **41**, 436-444.

Stephenson, L.S., Latham, M.C., Adams, E.J., Kinoti, S.N. & Pertet, A. (1993a). Weight gain of Kenyan children infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* is improved following once or twice yearly treatment with albendazole *Journal of Nutrition* **123**, 656-665.

Stephenson, L.S., Latham, M.C., Adams, E.J., Kinoti, S.N. & Pertet, A. (1993b). Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *Journal of Nutrition* **123**, 1036-1046.

Stephenson, L.S., Latham, M.C., Kurz, K.M., Kinoti, S.N. & Brigham, H. (1989b). Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections. *American Journal of Tropical Medicine and Hygiene* **41**, 78-87.

Stephenson, L.S., Latham, M.C., Kurz, K.M., Kinoti, S.N., Oduori, M.L. & Crompton, D.W.T. (1985). Relationship of *Schistosoma haematobium*, hookworm, and malarial infections and metrifonate treatment to growth of Kenyan school children. *American Journal of Tropical Medicine and Hygiene* **34**, 1109-1118.

Stirrat, G.M. (1982). *Obstetrics*. London: Grant McIntyre Ltd.

Stockman, J.A. & Oski, F.A. (1978). Physiological anaemia of infancy and the anaemia of prematurity. *Clinics in Haematology* **7**, 3-18.

Stoltzfus, R.J., Dreyfuss, M.L., Chwaya, H.M. & Albonico, M. (1997). Hookworm control as a strategy to prevent iron deficiency. *Nutrition Reviews* **55**, 223-232.

Stray-Pederson, B. (1993). Treatment of toxoplasmosis in the pregnant mother and newborn child. *Scandinavian Journal of Infectious Diseases* **84** (supplement), 23-31.

Suharno, D., West, C.E., Muhilal, Karyadi, D. & Hautvast, G.A.J. (1993). Supplementation with vitamin A and iron for nutritional anaemia in pregnant women in West Java, Indonesia. *Lancet* **342**, 1325-1328.

Suitor, C.W. (1994). Nutritional assessment of pregnant women. *Clinical Obstetrics and Gynecology* **37**, 501-514.

Susser, M. (1991). Maternal weight gain, infant birth weight, and diet: causal sequences. *American Journal of Clinical Nutrition* **53**, 1384-1396.

Tabor, E. (1985). Hepatitis B virus and primary hepatocellular carcinoma. In: *Hepatitis B*, Gerety, R.J. (editor). Orlando: Academic Press Inc., pp. 247-267.

Taggart, N.R., Holliday, R.M., Billewicz, W.Z., Hytten, F.E. & Thomson, A.M. (1967). Changes in skinfolds during pregnancy. *British Journal of Nutrition* **21**, 439-451.

Taylor, D.J. & Lind, T. (1979). Red cell mass during and after normal pregnancy. *British Journal of Obstetrics and Gynecology* **86**, 364-370.

Taylor, D.J., Mallen, C., McDougall, N. & Lind, T. (1982a). Effect of iron supplementation on serum ferritin levels during and after pregnancy. *British Journal of Obstetrics and Gynaecology* **89**, 1011-1017.

Taylor, D.J., Mallen, C., McDougall, N. & Lind, T. (1982b). Serum ferritin in women of reproductive age. *British Journal of Obstetrics and Gynaecology* **89**, 1000-1005.

Temmerman, M., Lopita, M.I., Sanghvi, H.C.G., Sinei, S.K.F., Plummer, F.A. & Piot, P. (1992). The role of maternal syphilis, gonorrhoea and HIV-1 infections in spontaneous abortion. *International Journal of STD and AIDS* **3**, 418-422.

Thein-Hlaing, Than-Saw & Myint-Lwin (1987). Reinfection of people with *Ascaris lumbricoides* following single 6-month and 12-month interval mass chemotherapy in Okpo village, rural Burma. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 140-146.

Thein-Hlaing, Thane-Toe, Than-Saw, Myat-Lay-Kyin & Myint-Lwin (1991). A controlled chemotherapeutic intervention trial of the relationship between *Ascaris lumbricoides* infection and malnutrition in children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 523-528.

Thompson-Clewry, P. (1972). Food nutrition and Health in Sierra Leone. *Home Economics (Nutrition)* **11**.

Thomson, J. (1997). Anaemia in pregnant women in eastern Caprivi, Namibia. *South African Medical Journal* **87**, 1544-1547.

Tomkins, A. (1993). Environment, season and infection. In: *Seasonality and Human Ecology: 35th Symposium Volume of the Society of the Study of Human Biology*, Ulijaszek, S.J. & Strickland, S.S. (editors). Cambridge: Cambridge University press, pp. 123-134.

Torrance, C. (1992). Absorption and function of iron. *Nursing Standard* **6**, 25-28.

Tramont, E.D. (1995). *Treponema pallidum* (syphilis). In: *Principles and Practice of Infectious Diseases*, 4th Edition, Mandell, G.L., Bennett, J.E. & Dolin, R. (editors). New York: Churchill Livingston, pp. 2117-2133.

Ulijaszek, S.J. (1992). Human energetics methods in biological anthropology. *Yearbook of Physical Anthropology* **35**, 215-242.

Ulijaszek, S.J. & Strickland, S.S. (1993). Food consumption. In: *Nutritional Anthropology*. London: Smith-Gordon, pp. 107-118.

Ulmer, H.U. & Goepel, E. (1988). Anemia, ferritin and preterm labor. *Journal of Perinatal Medicine* **16**, 459-465.

UNDP (1993). *Development Co-operation, Sierra Leone. 1992 Report*. Freetown, Sierra Leone.

UNDP (1995). *Human Development Report 1995*. New York, Oxford: Oxford University Press.

UNICEF (1996). *The State of the World's Children 1996*. Oxford: Oxford University Press.

USAID Office of Nutrition (1978). *Sierra Leone National Nutrition Survey, 1978*. Washington D.C.: Bureau of Science and Technology.

USDA (1998). *USDA Nutrient Database for Standard Reference*. World Wide Web: http://www.nal.usda.gov/fnic/foodcomp/data/.

van den Broek, N. (1996). The aetiology of anaemia in pregnancy in West Africa. *Tropical Doctor* **26**, 5-7.

van den Broek, N. (1998). Anaemia in pregnancy in developing countries. *British Journal of Obstetrics and Gynaecology* **105**, 385-390.

Variyam, E.P. & Banwell, J.G. (1982). Hookworm disease: nutritional implications. *Review of Infectious Diseases* **4**, 830-835.

Villar, J. & Belizan, J.M. (1982). The relative contribution of prematurity and fetal growth retardation to low birth weight in developing and developing countries. *American Journal of Obstetrics and Gynecology* **143**, 793-798.

Villar, J., Klebanoff, M. & Kestler, E. (1989a). The effect of fetal growth of protozoan and helminthic infection during pregnancy. *Obstetrics and Gynecology* **74**, 915-920.

Villar, J., Krestler, E. & Pareja, G. (1989b). Measurement error in clinical perinatal data. *American Journal of Obstetrics and Gynecology* **160**, 380-382.

Viteri, F.E. (1994). The consequences of iron deficiency and anaemia in pregnancy on maternal health, the foetus and the infant. *Subcommittee on Nutrition News* **11**, 14-18.

Viteri, F.E. (1997). Iron supplementation for the control of iron-deficiency in populations at risk. *Nutrition reviews* **55**, 195-209.

Viteri, F.E., Liu, X-N., Tolomei, K. & Martin, A. (1995). True absorption and retention of supplemental iron is more efficient when iron is administered every three days rather than daily to iron-normal and iron-deficient rats. *Journal of Nutrition* **125**, 82-91.

Wagner, C. (1995). Biochemical role of folate in cellular metabolism. In: *Folate in Health and Disease*, Bailey, L.B. (editor). New York: Marcel Dekker, pp. 23-42.

Wakelin, D. (1985). Genetic control of immunity to helminth infections. *Parasitology Today* **1**, 17-23.

Wakelin, D. (1986). Genetic and other constraints on resistance to infection with gastrointestinal nematodes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 742-747.

Wakelin, D. (1996). Gastrointestinal nematodes. In: *Immunity to Parasites*. London: Edward Arnold, pp. 122-145.

Walker, A.R.P., Walker, B.F., Jones, J., Verardi, M. & Walker, C. (1985). Nausea and vomiting and dietary cravings and aversions during pregnancy in South African woman. *British Journal of Obstetrics and Gynaecology* **92**, 484-489.

Walker, A.R.P., Walker, B.F. & Labadarios, D. (1994). The benefits of iron supplementation and prophylaxis in Africa. *Nutrition Research* **14**, 513-521.

Waller, D.P. (1993). Methods in ethnopharmacology. *Journal of Ethnopharmacology* **38**, 189-195.

Waller, P.J. (1990). Resistance in nematode parasites of livestock to the benzimidazole anthelminthics. *Parasitology Today* **6**, 127-129.

Weatherall, D.J. & Wasi, P. (1984). Anaemia. In: *Tropical and Geographical Medicine* Warren, K.S. & Mahmoud, A.A.F. (editors). New York: McGraw-Hill Book Company, pp. 61-69.

Webster, J., Hodges, M., Crompton, D.W.T. & Walters, D.E. (1990). Intestinal parasitic infections in children from Freetown, Sierra Leone. *Journal of the Sierra Leone Medical and Dental Association* **5**, 144-155.

Weinberg, E.D. (1984). Pregnancy-associated depression of cell-mediated immunity. *Review of Infectious Diseases* **6**, 814-831.

Weinberg, E.D. (1987). Pregnancy-associated immune suppression: risks and mechanism. *Microbial Pathogenesis* **3**, 393-397.

Weiss, M.G. (1988). Cultural models of diarrheal illness: conceptual framework and review. *Social Science and Medicine* **27**, 5-16.

Welffens-Ekra, C.H., Kibora, M., Comoe, Koume, P., N'Diaye, A., Koffi, M. & Diarra, S. (1990). Efficacite et tolerance de l'Helmintox chez la femme enceinte en Cote D'Ivoire. *Medecine d'Afrique Noire* **37**, 800-803.

West Suitor, C. (1994). Nutritional assessment of pregnant women. *Clinical Obstetrics and Gynecology* **37**, 501-514.

Westwood, M., Kramer, M.S., Munz, D., Lovett, J.M. & Watters, G.V. (1983). Growth and development of full-term non-asphyxiated small-for-gestational-age newborns: follow-up through adolescence. *Pediatrics* **71**, 376-382.

White, P.T. (1977). *Farmer Health Survey.* IADP Eastern Region, Kenema, pp. 12.

Whitehead, R.G. & Paul, A.A. (1982). Diet and the pregnant and lactating women. In *Nutrition and health - a perspective*, Turner, M.R. (editor). Lancaster: MTP Press Ltd, pp. 159-168.

Whitley, R.J. & Kimberlin, D.W. (1997). Treatment of viral infections during pregnancy and the neonatal period. *Clinics in Perinatology* **24**, 267-283.

Whittaker, P.G. & Lind, T. (1993). The intravascular mass of albumin during human pregnancy: a serial study in normal and diabetic women. *British Journal of Obstetrics and Gynaecology* **100**, 587-592.

Whittaker, P.G., Lind, T. & Williams, J.G. (1991). Iron absorption during normal human pregnancy: a study using stable isotopes. *British Journal of Nutrition* **65**, 457-463.

Whitworth, J.A.G., Morgan, D., Maude, G.H., McNicholas, A.M. & Taylor, D.W. (1991). A field study of the effect of ivermectin on intestinal helminths in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 232-234.

WHO (1968). *Nutritional Anaemias: A Report of a WHO Scientific* Group. WHO Technical Report Series No. 405. Geneva: World Health Organization.

WHO (1977). *Food and Nutrition Summaries for Countries of the African Region.* Document AFR/NUT/77. Brazzaville: World Health Organization.

WHO (1978). *The Promotion and Development of Traditional Medicine.* WHO Technical Report Series No. 622. Geneva: World Health Organization.

WHO (1981). *Ethical Review of Procedures for Research Involving Human Subjects.* Geneva: World Health Organization.

WHO (1988). Progress in the control of viral hepatitis: memorandum from a WHO meeting. *Bulletin of the World Health Organization* **66**, 443-455.

WHO (1989). *Control of Anaemia in Pregnancy. Report of the African Regional Consultation on Control of Anaemia in Pregnancy.* Brazzaville: World Health Organization.

WHO (1991a). *Basic Laboratory Methods in Medical Parasitology.* Geneva: World Health Organization.

WHO (1991b). *Hepatitis B as a Sexually Transmitted Disease. Report of a WHO Consultation.* Geneva: World Health Organization.

WHO (1991c). Maternal anthropometry for prediction of pregnancy outcomes: memorandum from a USAID/WHO/PAHO/MotherCare meeting. *Bulletin of the World Health Organization* **69**, 523-532.

WHO (1992a). *The Prevalence of Anaemia in Women. A Tabulation of Available Information.* Document WHO/MCH/MSH/92.2. Geneva: World Health Organization.

WHO (1992b). *Low Birth Weight - a Tabulation of Available Information. Updated version, September, 1996.* Maternal Health and Safe Motherhood Programme. Document MCH/92.2. Geneva: World Health Organization.

WHO (1993). *Prevention and Management of Severe Anaemia in Pregnancy. Report of a Technical Working Group.* Document WHO/FHE/MSM/93.5. Geneva: World Health Organization.

WHO (1994). Expanded Programme on Immunization. Achieving the major disease control goals. *Weekly Epidemiological Record* **69**, 34-35.

WHO (1995a). Pregnant and lactating women. In: *Physical Status: the Use and Interpretation of Anthropometry. Report of a WHO Expert Committee.* WHO Technical Report Series 854. Geneva: World Health Organization, pp., 37-120.

WHO (1995b). The newborn infant. In: *Physical Status: the Use and Interpretation of Anthropometry. Report of a WHO Expert Committee.* WHO Technical Report Series 854. Geneva: World Health Organization, pp., 121-160.

WHO (1996a). *Report of the WHO Informal Consultation on the Use of Chemotherapy for the Control of Morbidity due to Soil-Transmitted Nematodes in Humans.* Document WHO/CTD/SIP/96.2. Geneva: World Health Organization.

WHO (1996b). *Report of the WHO Informal Consultation on Hookworm Infection and Anaemia in Girls and Women.* Document WHO/CTD/SIP/96.1. Geneva: World Health Organization.

WHO/ UNICEF (1978). *Primary Health Care.* Geneva: World Health Organization.

WHO/UNICEF/UNU (1995). *Report of the 1993 Consultation on Indicators and Strategies for Iron Deficiency and Anaemia Programme.* Geneva: World Health Organization.

Willett, W.C., Kilama, W.L. & Kihamia, C.M. (1979). *Ascaris* and growth rates: a randomized trial of treatment. *American Journal of Public Health* **69**, 987-991.

Williams, M.O. (1974). Intestinal animal parasites of man in Sierra Leone. *Bulletin of the Sierra Leone Medical and Dental Association* **2**, 62-64.

Williams, M.S. (1988). *Prevalence of Malnutrition in Children 0-5 Years Attending the Under-Five's Clinics of the Army Medical Services, Freetown.* Dissertation for the Diploma in Community Medicine and Health. University of Sierra Leone.

Williams, R.A.M. (1996). The prevalence of some soil-transmitted helminth (S-TH) infections in Sierra Leone. *Journal of Pure and Applied Science* **5**, 38-44.

Williams, R.A.M. & Hodges, M. (1995). Effects of levamisole chemotherapy on growth of primary school children and the prevalence and intensity of common soil-transmitted helminth infections in Sierra Leone. *Journal of the Sierra Leone Medical and Dental Association* **8**, 68-76.

Wilson, C.B., Remington, J.S., Stagno, S. & Reynolds D.W. (1980). Development of adverse sequelae in children born with subclinical *Toxoplasma* infection. *Pediatrics* **66**, 767-774.

Wilson, C.S. (1973). Food taboos of child birth and nutrition. *Ecology of Food and Nutrition* **2**, 267-274.

Wilson, F., Lindsay, G., Crompton, D.W.T. & Hodges, M. (1991). Intestinal helminth infections in mothers and their infants in Sierra Leone. *Journal of the Sierra Leone Medical and Dental Association* **6**, 22-30.

Wolf-Gould, C.S., Taylor, N., McCue Horwitz, S. & Barry, M. (1991). Misinformation about medications in rural Ghana. *Social Science and Medicine* **33**, 83-89.

Wong, M.S., Bundy, D.A.P. & Golden, M.H.N. (1988). Quantitative assessment of geophagus behaviour as a potential source of exposure to geohelminth infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 621-625.

Working Group (1991b). Height. Summary and recommendations In: *Maternal Nutrition and Pregnancy Outcomes. Anthropometric Assessment*, Krasovec, K. & Anderson, M.A. (editors). Washington: Pan American Health Organization, pp. 112-115.

Working Group on Fortification of Salt with Iron (1982). Use of common salt fortified with iron in the control and prevention of anemia - a collaborative study. *American Journal of Clinical Nutrition* **35**, 1442-1451.

Wright, A.J.A. & Southon, S. (1990). The effectiveness of various iron-supplementation regimens in improving the Fe status of anaemic rats. *British Journal of Nutrition* **63**, 579-585.

Wu Leung, W.T. (1968). *Food Composition Table for use in Africa.* Rome: Food and Agriculture Organization.

Wurie, A.T., Wurie, M. & Hodges, M. (1996). Hepatitis B in Freetown. *Journal of the Sierra Leone Medical and Dental Association* **9**, 1-5.

Yarjah, M. (1996). *Intestinal Helminth Infections Among Pregnant Women at the Kissy and Bo Displaced Camps, Sierra Leone.* Unpublished report.

Yip, R. (1994). *Assessing Population Iron Status: Issues Relevant to Developing Countries.* WHO Informal Consultation on Hookworm Infection in Women, 5-7 December 1994. Working Paper No. 14. Geneva: World Health Organization.

Yip, R. (1996). Iron supplementation during pregnancy: is it effective? *American Journal of Clinical Nutrition* **63**, 853-855.

Yoder, P.S. (1981). Knowledge of illness and medicine among Cokwe of Zaire. *Social Science and Medicine* **15B**, 237-245.

Yoder, P.S. (1982). Biomedical and enthomedical practice in rural Zaire. *Social Science and Medicine* **16**, 1851-1857.

Yu, S.H. & Shen, W. X. (1990). Hookworm infection and disease in China. In: *Hookworm Disease: Current Status and New Directions*, Schad, G.A. & Warren, K.S. (editors). London: Taylor & Francis, pp. 44-54.