PREVALENCE OF INTESTINAL PARASITES IN SCHOOL CHILDREN FROM TWO MEXICAN STATES AFTER 7 YEARS OF ALBENDAZOLE ADMINISTRATION.

Thesis Submitted for the Degree of Philosophy
at the University of Glasgow

by

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DECLARATION

I declare that the work described in this thesis has been carried out by myself. It is entirely of my own composition and has not in whole or part been submitted for any other degree. Brief quotes of the material contained in this thesis are allowed without authorisation but giving the correspondent credit. When complete information for academic purposes is required, a special authorisation must be requested to the Head of the Division of Infection and Immunity of the University of Glasgow, Joseph Black Building, G12 8QQ, Glasgow U.K.

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SUMMARY

A total of 1476 stool samples was collected from 492 school children aged 6-10 years old (240 females and 252 males) representing 41.5% of 1185 enrolled school children from 1 sub-urban and 11 rural communities of the States of Sinaloa (n=341) and Oaxaca (n=151). The school children from Sinaloa were found to be more susceptible to protozoan infections than helminth infections and children from Oaxaca are at the same risk to protozoan and helminth infections. The school children from Oaxaca are currently more affected by helminth infections than those from Sinaloa. Protozoan infections were found to be equally present in both States. The current prevalences of *G. lamblia, E. coli, E. nana, I. butschlii, T. trichiura, A. lumbricoides, E. vermicularis*, and *H. nana* in school children continue to be similar with those found by Diaz *et al.* from 1987 to 1994 in the general population from different rural and sub-urban communities. The current prevalences of *G. lamblia, E. coli, E. nana,* and *I. butschlii* are currently higher in Oaxaca than those reported in the general population by Navarrete *et al.*, (1993), Soriano, (1998), and LESPO (1999), although the prevalence of *A. lumbricoides* has shown an important decrease with time. Results showed no difference in the levels of intensities of *H. nana, A. lumbricoides,* and *T. trichiura* in the school children between Sinaloa and Oaxaca. Younger school children harboured higher eggs counts than older children from both States The gender of the school children had no influence on the prevalence of the intestinal parasitic infections in this study. The prevalence of protozoan infections showed a decrease with age but helminth infections remained low but stable in Sinaloa. The prevalence of protozoan and helminth infections showed different trends with age in Oaxaca. The number of family members was found not to be associated with the levels of intensity of *A. lumbricoides, H. nana,* and *T. trichiura* in Sinaloa and Oaxaca. There was no association between malnutrition according to the nutritional indicators H/A (15.%), W/A (11.6%), and W/H (6.6%), and intestinal parasitic infections in school children from Sinaloa. However, school children with malnutrition according to the ratios W/A (57.8%) and W/H (32.6%) showed a higher prevalence of intestinal parasitic infections than uninfected children. The prevalence of malnutrition according to the ratios H/A and W/A was higher in school children from Oaxaca than in Sinaloa. The school children from both States are subjected to sub-optimal daily nutrient intakes. Although this shortage of nutrient intakes showed no association with the prevalence of intestinal parasitic

infections but was found to be associated with malnutrition. The financial status of the parents was found not to be an influencing factor in the prevalence of these infections in Sinaloa. In Oaxaca, the mothers contributed significantly with the family monthly income which was found to be negatively associated to the prevalence of intestinal parasitic infections. The level of education of the parents in both States was found not to be associated with the prevalence of intestinal parasitic infections and family monthly income. The uninfected school children from both States were living in better housing conditions than infected school children. An association was found between intestinal parasitic infections and frequency of respiratory infections, abdominal pain, and fever in the school children from Sinaloa, and abdominal pain, fever, diarrhoea, and allergies in Oaxaca. An investigation of the association between *T. trichiura* and iron status in the school children from the community of El Higueral Sinaloa revealed the following results: 28 school children with *T. trichiura* (experimental group) and 24 uninfected school children (control group) were studied. The level of education of the parents was a determining factor for the presence of *T. trichiura* in these children. No association was found between *T. trichiura* and malnutrition. A high proportion of uninfected and *Trichuris*-infected school children were receiving sub-optimal daily intakes of energy, calcium, niacin, zinc, vitamin B12, riboflavin and pantothenic acid. Low daily intakes of energy and protein were found to be negatively associated with malnutrition. *T. trichiura* was found to be associated with respiratory infections, abdominal pain, and diarrhoea. *T. trichiura* was found not to be a risk factor for the presence of low but normal concentrations of serum ferritin in the experimental group, but higher levels of intensity of *T. trichiura* tended to be associated with low concentrations of haemoglobin (Hb), mean cell heamoglobin (MCH), mean cell haemoglobin concentration (MCHC) and high concentration of total iron binding capacity (TIBC). Low daily intakes of vitamin B12 and riboflavin were found to be risk factors for the presence of low normal values of serum ferritin. No significant difference was found in the biochemical indicators of iron between the experimental and control groups before and after the albendazole and placebo treatments ($p>0.05$). The control group showed no significant increase in Hb and serum ferritin concentrations. These changes were accompanied by significant association between Hb and haematocrit (Ht), Hb and red blood cells (RBC). No association was found between ferritin and other iron indicators. An increase in the frequency of allergies and a decrease of vomiting and diarrhoea were observed after treatment. These changes were not significant. Ferritin was found not to be associated with morbidity in this group. No significant increases in Hb, MCH, TIBC and significant increases in MCHC and ferritin were observed. These changes were accompanied by a significant and positive association between Hb with Ht, Hb with MCH, and Hb with MCHC, and significant and negative associations between Hb, MCH, MCHC with daily intakes of vitamin B12 and riboflavin, and TIBC with transferrin saturation. No association was found between ferritin and other iron indicators in this group. An increase in the frequency of respiratory infections and a decrease in vomiting, abdominal pain, and diarrhoea were observed in this group. These changes were not significant. No association was found between ferritin and morbidity ($p>0.05$). Albendazole showed a low efficacy according to the estimated cure rate (28%) and egg count reduction (69%). Ferritin acts as a reactant of the acute phase but it showed no association with morbidity in these children. Vitamin B12 and riboflavin are probably influencial factors in erythropoiesis or are a proxi for low intakes of other micronutrients associated with erythropoiesis. No evidence of suboptimal daily intakes of iron, folate, and vitamin was found. The levels of intensity of *T. trichiura* and sub-optimal nutrient intakes may be disturbing the erythropoietic process in these children. The association between trichuriasis and respiratory infections may be evidence of an impaired immune response to face these infections. The current balance between protozoan and helminth infections in school children demand additional actions by the Mexican Ministry of Public Health to take decisions about the treatment really required for particular regions of the country (antiprotozoan and anthelmintic treatments). It will be necessary to re-design a schedule of albendazole treatment when it is particularly administered to school children in communities where *T. trichiura* is persistent. This will help to optimise human and economic resources.

CHAPTER 1

PUBLIC HEALTH SIGNIFICANCE OF INTESTINAL PARASITIC INFECTIONS IN HUMANS.

1.1 Significance of Public Health and its Importance in Human Development.

The definition of public health has been very controversial, due to the complexity and number of different factors involved. In 1920, Winslow defined public health as the process to prevent diseases, prolong life, and increase health. Hanlon, in 1974, proposed that public health should be devoted to the common achievement of the highest physical, mental, and social status of well-being, accompanied with knowledge, and available resources in a defined time and place. However, the World Health Organisation (WHO) has proposed that a status of entire physical, social, and mental well-being can be regarded a current definition of public health, and this has been widely accepted world-wide. Although the physical, social, and mental aspects have been regarded as part of that definition, social development has not yet been regarded as part of health status in most developing countries (Alvarez, 1988), because the high levels of poverty and un-justice.

Developing countries will be at disadvantage as compared with developed countries when the health status index is studied. Emphasising the great difference in the public health significance between a developed and developing country, public health has become a profitable business in some developed countries. In the USA the percentage of the national gross product designated to health increased by 1.6% between 1970 and 1977 (Alleyne, 1983). However, this focus on medical attention is not appropriate in developing countries because the economic status of their population can not afford it. Between 1960-1970 in Guatemala, expenditure on health care was twenty times lower than those estimated in the UK. The economic situation of a country is intimately associated with public health standards. The higher economic standards of a country the higher is the public expenses on health. The availability of human and economic resources in developing countries is strictly limited, and their communities have to create improvised structures to occupy empty spaces. As an example, patients, elderly people, orphan children, mental disabled people, and other social problems have to be the concern of families without the support from the appropriate health and social institutions (Alleyne, 1983).

It has been confirmed that parasitic disease results from the association of a complex of multiple factors including economic status, unemployment, poor education, poor sanitary conditions, lack of basic services (eg drinking water supply), limited access to medical services, lack of organised participation of the society, family stability and culture, integrating a social status known as social deprivation (Beghin *et al.*, 1980). Therefore, improved public health is needed for each individual, because healthy people are more creative, impacting in physical well-being and productivity in developed countries. However, the impact on developing countries will be dependent on factors such as social classes (high levels of poverty), interdependence in the system of employment (the more trained is an individual the more difficult to replace him/her in a job) , health attention (limited access), and assigning of economic resources to health services. It is well known that public health problems limit economic development in developing countries, and it has been proposed that expenditures on health quality be regarded as an investment, because human well-being will contribute positively to national productivity. If health is improved, mortality and morbidity will be reduced, and an increase in demographic growth can then be seen as an increased human capital with huge potential for the economic and social development (Conly, 1975). Trained people in developing countries understand that improvement in social conditions will improve public health.

Finally, the gap between the public health status of developing and developed countries has increased, and it has become a serious threat for health world-wide. Therefore, personal and community actions must be urgently linked and applied, based on high quality information about socio-economic-models, and reliable national epidemiological trends, to establish decisions and priorities destined to improve the development of public health in developing countries (Alleyne, 1983).

1.2 Major Intestinal Parasitic Infections as a Threat to Human Public Health.

Mata *et al.* (1982) listed 22 protozoan parasites of high prevalence in humans on a global scale and two of them, *Entamoeba histolytica*, and *Giardia lamblia*, responsible for amoebiasis and giardiasis respectively, infect the human intestinal tract (Solomons, 1993). They also listed 75 helminths with 11 of them having a high prevalence in the world. Of these, 5 species live in the human intestinal tract (*Ascaris lumbricoides*, *Trichuris trichiura, Ancylostoma duodenale*, *Necator americanus,* and *Strongyloides stercoralis*, responsible for ascariasis, trichuriasis, hookworm disease, and strongyloidiasis respectively. All of them are regarded as major human pathogenic parasites due to their association with mal-digestion and mal-absorption processes, and mortality in humans.

1.3 Global Distribution of Human Intestinal Parasites

1.3.1 Human Pathogenic Protozoa

Entamoeba histolytica (*E. histolytica*) has been found in different populations of the world, but its prevalence is usually higher in the tropics and subtropics than in temperate climates. In the Western hemisphere, amoebiasis has been found from Anchorage, Alaska to the Magallanes strait, and in the Eastern hemisphere from Finland to South Africa (Markell and Voge, 1992). In Mexico a review of epidemiological surveys carried out in the States of Distrito Federal, Coahuila, Chiapas, San Luis Potosi, Puebla, Sinaloa, and Morelos from 1981 to 1991 revealed a national mean prevalence of 30.8% (Tay *et al.*, 1994) (appendix 1).

Giardia lamblia (*G. lamblia)* is world-wide in its distribution and it is affected by the same factors that influence the distribution of *E. histolytica*. Giardiasis should be considered in the differential diagnosis of any traveller's diarrhoea (Markell and Voge, 1992). Numerous outbreaks were reported from North America, Europe, and Latin America two decades ago (Craig and Faust, 1961; Markell and Voge, 1992). Its prevalence is higher in warm than in cold climates. In Mexico a review of epidemiological surveys carried out in the States of Distrito Federal, Coahuila, Chiapas, San Luis Potosi, Puebla, Sinaloa, and Morelos from 1981 to 1991 revealed a national mean prevalence of 32.3% (Tay *et al.*, 1994)

Balantidium coli (*B. coli*) is also world-wide in its distribution, but is rare in warm climates. Although in the USA, balantidiasis is sometimes found in epidemic form in patients in mental hospitals, it is almost unknown in the general population (Markell and Voge, 1992).

Infections with *Isospora belli* (*I. belli*) were considered to be of exceedingly rare occurrence prior to the Second World War. However, during and since that conflict, large numbers of infections have been described from all parts of the world, mainly in warm climates. *I. belli* is not easily recognised and is usually confused with *Isospora hominis* (*I. hominis*) (Markell and Voge, 1976; Neva and Brown, 1994). Most of the cases have been diagnosed in the islands of the Pacific South West, Brazil, South Africa, Chile, Venezuela, USA, Egypt, Macedonia, Iraq, East of the Mediterrean, South and Central America, Caribbean Area, South of Asia (Craig and Faust, 1961; Neva and Brown, 1994).

Since 1980, another protozoan, *Cryptosporidium spp.* has become a common pathogen in outbreaks of gastroenteritis in individuals with Acquired Immunodeficiency Syndrome (AIDS). *I. belli* and *Cryptosporidium spp* have been implicated in AIDS-related diarrhoea in the USA (Solomons, 1993). *E. polecki,*, and *I. hominis,* have an unknown distribution as a result of the difficulties in their identification. Few reports and vague symptoms have been described in humans (Markell and Voge, 1999).

1.3.2 Human-Non Pathogenic Protozoa

Other species of amoebae (*Entamoeba coli*, *Iodamoeba butschlii*, *Endolimax nana*, *Dientamoeba fragilis* and *Entamoeba gingivalis*) have a similar geographical distribution to *E. histolytica.* All live in the large intestine, except *E. gingivalis* which is found in the mouth. *Endolimax nana* (*E. nana*) is usually encountered with about the same prevalence as *Entamoeba coli*, and both are commoner than *Iodamoeba butschli*i (*I. butschlii*) and *E. histolytica* (Neva and Brown, 1994)

Species of flagellates such as *Chilomastix mesnili*, *Trichomonas hominis*, *Enteromonas hominis*, and *Retortamonas intestinalis* are cosmopolitan in distribution, but are less common than *G. lamblia* (Markell and Voge, 1981). Tay *et al.*, (1994), reported in Mexico a national mean prevalence of 30% and 10.2% for *E. coli* and *E. nana* respectively.

1.3.3 The Major Human Intestinal Pathogenic Helminths.

1.3.3.1 The Major Human Pathogenic Nematodes.

Ascaris lumbricoides (*A. lumbricoides*) is the most prevalent helminth of humans, and its distribution extends throughout the countries of the tropics and subtropics where poor and socio-economically people are afflicted (Crompton *et al.*, 1989). The information about the occurrence of *A. lumbricoides* since 1975, indicated that the infection was present in 153 countries and territories (with an approximate population of 4,550 million) out of a list of 218 which form part the world with a total population estimated in 4,653 million (Crompton *et al.*, 1989). Recent estimations have considered that 1,472 million people are infected by ascariasis and 335 million people show morbidity (Crompton, 1999). Although the distribution of ascariasis is undoubtedly world-wide and cosmopolitan it is clear that when quantitative information about prevalence is considered, most of the infection is located in developing countries (Crompton *et al.*, 1989).

Trichuris trichiura (*T. trichiura*) is cosmopolitan in distribution but is more common in the warm, moist regions of the world, where both the incidence and the intensity of the infection may be high. Recent estimates have indicated that 1,049 million people is infected by *T. trichiura*, and 220 million people develop morbidity (Crompton, 1999). *T. trichiura* has been found to be more or less coextensive with *A. lumbricoides*, in areas of high rainfall, high humidity, and dense shade (Neva and Brown, 1994).

Ancylostoma duodenale (*A. duodenale*) was considered to be essentially a parasite of Southern Europe (Markell and Voge, 1992). Recently, the migration of infected persons from endemic areas has introduced this parasite into many new foci where *Necator americanus* (*N. americanus*) had been the predominant human hookworm. *A duodenale* has been the dominant species in Mediterranean region, Northern Asia, and West cost of South America (Neva and Brown, 1994). *N. americanus* is the prevailing species in the Western Hemisphere, in Central and South Africa, Southern Asia, Indonesia, Australia, and the Islands of the Pacific (Neva and Brown, 1994).

Enterobius vermicularis is also cosmopolitan in distribution, and is more common in temperate and cold climates than in warm climates (Beaver *et al.*, 1984). Stoll estimated that 208.8 million people were infected in the world, and 18 million of these in Canada, and the USA (Neva and Brown, 1994). Although this parasite is more prevalent in the lower socio-economic groups, mental institutions, and orphanages, is also common in wealthy and educated people. Children are more commonly infected than adults (Neva and Brown, 1994).

Strongyloides stercoralis is primarily adapted to warm climates, but it has been reported sporadically in temperate and cold regions. Its distribution parallels that of the human hookworms, but its prevalence is lower in temperate zones (Neva and Brown, 1994). It is especially prevalent in tropical and subtropical areas, where warmth, moisture, and lack of sanitation favour its free-living cycle. In the USA it occurs in the rural South and immigrants from tropical countries. During its free living existence, the rhabditiform larvae develop into sexually mature stage (free living males and females) in the soil. They become infective and may enter to new host or repeat the free living generation. In autoinfection the larvae develops rapidly into the filariform stage and penetrates the intestinal mucosa or the perianal skin to establish a development cycle within the host. Autoinfection explains the presence of strongyloidiasis in patients living in non-endemic areas. Also, there is evidence that some animals, such as dogs or monkeys, may serve as non-human reservoirs of infection, with strains of parasite capable of infecting humans (Neva and Brown, 1994).

1.3.3.2 The Main Intestinal Cestoda of Human Beings.

Hymenolepis nana is practically cosmopolitan in its distribution, but is more common in warm regions and low rainfall than in cold regions and high rainfall. It has been particularly common in Southern Europe, Russian, and India and Southern USA and Latin America (Beaver, *et al.* 1984).

Diphyllobothrium latum is also world-wide in distribution occurring in Northern temperate areas of the world where raw or improperly cooked fish is eaten. It is present in Europe in the Baltic countries, Switzerland, Romania, Russian, Israel, Northern Manchuria and Japan, South America in Chile and Argentina, and in North America (Michigan, Minnesota, California, Florida, Alaska, and Canada (Neva and Brown, 1994).

Taenia saginata is cosmopolitan in beef-eating countries. Humans acquire the infection from eating improperly cooked beef containing the cysticerci. Cattle are infected from grazing land contaminated by fertilisation with night-soil or sewage-laden water. On the other hand, the prevalence of *Taenia solium* in humans is transmitted by pork meat containing the cysticerci, varies throughout the world. This infection is rare in the USA, but is common in Central and South America countries, Africa, India, and China (Neva and Brown, 1994)

1.3.3.3 The Major Intestinal Trematoda of Human Beings.

Schistosoma mansoni is spread less widely in Africa than is *Schistosoma haematobium* but occurs intensely in the Nile Delta, North of Cairo and in one belt across Africa, South of the Sahara from Mali to Ethiopia, and in another belt South to Mozambique. It is also present in Madagascar and Angola. Scattered foci have been reported in Arabia and Yemen. The parasite probably was introduced by the slave trade into the Western Hemisphere and is established in large areas of Brazil, Venezuela, Surinam, Puerto Rico, Viequez, Antigua, Dominican Republic, Martinique, Montserrat, Nevis, and St. Lucia (Neva and Brown, 1994). *S. japonicum* is confined to the Far East. It is highly endemic in the Yangtze River valley of Central China, and it has also been reported in Philippines, Indonesia. *S. intercalatum* has been reported in certain regions of Central and Western Africa (Zaire, Gabon, Cameroon, Central African Republic, Equatorial Africa, and Mali) (Neva and Brown, 1994) and *S. mekongi*, discovered in the Mekong River Valley between Laos, Thailand, and Cambodia (Neva and Brown, 1994)

1.3.3.4 Other Minor Human Intestinal Pathogenic Helminths

Other normally minor pathogenic intestinal helminths are sporadically found in humans such as *Trichostrongyloidea* spp., *Ternidens deminutus, Cesophagostomum apiostomum, Toxocara canis, Toxocara cati, Fasciolopsis buski, Echinostomes* spp., *Heteropfyids* spp., *Hymenolepis diminuta, Capillaria philippinensis, Dipylidium caninum, Watsonius watson, Gastrodiscoides hominis,* and *Moniliformis moniliformis*, causing some gastrointestinal disorders. Because these infections in humans are acquired from reservoirs hosts (apes, dogs, cats, mammals, raw fish, brackish water fish, snails, infected insects), and are confined to limited geographical areas they are commonly misdiagnosed (Markell and Voge, 1992). The real impact on the world's public health situation of these kinds of infections is still unknown.

1.4 Prevalence, Morbidity, and Mortality Rates of the Major Intestinal Parasitic Infections in Humans.

1.4.1 Human Intestinal Parasitic Protozoan Infections.

1.4.1.1 Amoebiasis

Infections by *E. histolytica* have also been an important concern of public health in different parts of the world since 1920 (Markell and Vogue, 1999). Walsh and Warren (1979) reckoned that amoebiasis produced about 400 million cases of infection per year, 30,000 fatal cases per year, and 1.5 million cases of disease per year. The actual current prevalence of amoebiasis throughout the world remains unknown. More recent surveys have revealed the amoebiasis varies from 0.2% to 50% in different places such as Alaska, Canada, Soviet Union, Mexico, India, Indonesia, some African countries, Caribbean and South American countries (Neva and Brown, 1994).

1.4.1.2 Giardiasis

According to a large number of parasitological studies carried out in important regions of the world in 1948, giardiasis was found in 10.4% of 134,966 persons. Belding and Piekarski (Craig and Faust, 1961), reported a world-wide prevalence of 16.6% in children, and 5.5% in adults. Estimations published by Walsh and Warren (1979) indicated that giardiasis was infecting around 200 million people per year and caused disease in 500,000 people per year. WHO reported that the prevalence of giardiasis could be four times higher for developing countries than that of 7.4% reported for industrialised countries (WHO, 1987).

1.4.2 Human Intestinal Parasitic Helminths Infections.

1.4.2.1 Ascariasis.

Stoll in 1947 had calculated that 644.4 million of people were infected with ascariasis distributed as follows: 3 million in North America, 42 million in Tropical America, 59 million in Africa, 32 million in Europe, 19.9 million in the ex U.R.S.S, 488 million in Asia, and 0.5 million in Oceanic (Craig and Faust, 1961). Some years later, the annual prevalence (1977-1978) of ascariasis in Africa, Asia and Latin America, was estimated to be 800 million to 1 billion of cases, and causing disease in 1 million cases, although with a low death rate of 20,000 cases (Walsh and Warren, 1979). In addition, the prevalence of ascariasis in Kenya has apparently not decreased in over 60 years (Chunge *et al.* 1985). Botero in 1981 published that prevalence of ascariasis in Latin America appeared not to have changed substantially from the 1930's. Ascariasis was reported uncommon but still present in urban populations in Western Europe, and some South-eastern areas in the USA mainly in pre-school children.(Beaver *et al.*, 1984). In

México, ascariasis was found to be one of the main causes associated with morbidity in some regions, mainly in school children (De La Loza Saldívar *et al.,* 1983). The problem of ascariasis has been highlighted by the fact that there are more countries with prevalence of 50% or more than those reporting a decline in prevalence (Stephenson and Holland, 1987; Crompton, *et al.*, 1989). More recent estimates have revealed that ascariasis is causing infection in 1,472 million people, morbidity in 335 million people, and an annual mortality of 60, 000 (WHO, 1998; Crompton, 1999).

1.4.2.2 Trichuriasis

Stoll in 1947 had estimated that 355.1 million people were infected by trichuriasis, distributed in 227 million in Asia, 27.2 million in the former U.R.S.S. 34 million in Europe, 28 million in Africa, 38 million in Tropical America, 0.4 in North America, and 0.5 million in the Pacific Islands (Markell and Voge, 1976). Several years later, the annual prevalence (1977-1978) of trichuriasis was estimated to be 500 million of cases, and causing disease in 100,000 cases in Africa, Asia and Latin America, although it caused a low death rate (Walsh and Warren, 1979). However, others reported that up to 1000 million may harboured the infection (Bundy, 1986). Pawlowski in 1984 pointed up about 63% of the infected persons are found in the tropical and subtropical regions of Asia, 11% in Africa, and about 14% in the Americas (Stephenson and Holland, 1987). In México trichuriasis was found to be listed in the ten main transmissible diseases causing morbidity in some regions (De La Loza-Saldívar, *et al.*, 1983). Recent estimations have revealed that trichuriasis is infecting around 1,049 million people, causing morbidity in 220 million people, and an annual mortality of 10,000 (WHO, 1998; Crompton, 1999).

1.4.2.3 Hookworms Infections.

Estimations based on the Stoll's figures revealed that 72.5 million people were infected by *A. duodenale* distributed in 9 million people in Africa, 1.4 million in Europe, 2.8 million in the former U.R.S.S., 59 million in Asia, and 0.3 in Oceania. In addition, the same estimations revealed that 384.3 million people were infected by

Necator americanus distributed in 1.8 million in North America, 42 million in Tropical America, 40 million in Africa, 300 million in Asia, and 0.5 million in Oceania (Craig and Faust, 1961). Published annual estimations (1977-1978), indicated that hookworms were responsible of 700 million-900 million of infections, 50,000-60,000 cases of deaths, and 1.5 million cases of disease (Walsh and Warren, 1979). On the other hand, Schad and Banwell (1984) reported that hookworms were infecting 900 million people representing over one-fifth of the world's population.

Different findings in México estimated that hookworm varies between 6% and 71% in the tropical regions, and it was caused mainly by *Necator americanus* (Aguilera, 1984).

Recent estimates have revealed that hookworms are causing infection in 1,298 million people, morbidity in 159 million, and mortality in 65,000 people per year (Montresor *et al*, 1998; Crompton, 1999).

1.4.2.4 Strongyloidiasis

In 1947 Stoll estimated that 34.9 million people were infected by *S. stercoralis* distributed in 2 million in Asia, 0.9 million in the former U.R.S.S, 8.6 million in Africa, 0.4 million in Tropical America, 0.4 million in North America, and 100,000 in Pacific Islands (Craig and Faust, 1961). Other surveys reported prevalences as high as 60% in tropical areas of Brazil, Colombia, and Ecuador (Beaver. *et al.* 1984; Walls, *et al.* 1986) and variations between 0% and 26% in some tropical areas of Mexico (Aguilera, 1984). In the USA a strongyloidiasis occurs in the rural South (Neva and Brown, 1994). Grove in 1984 estimated that around 50-100 million people were affected by *S. stercoralis*. More recent estimates have revealed that *S. stercoralis* is infecting about 70 million people (Crompton, 1999).

1.4.2.5 Schistosomiasis.

It was estimated that schistosomiasis was endemic in 74 tropical developing countries (Stephenson and Holland, 1987). The intestinal schistosomiasis is caused by *S.*

mansoni, *S. japonicum*, *S. intercalatum*, and *S. mekongi*. The *S. mansoni* was estimated to infect over 57 million people and *S. japonicum* about 69 million people (Peters and Gilles, 1977). Walsh and Warren (1979) stated that schistosomiasis was infecting about 200 million people in Africa, Asia, and Latin-America with an annual morbidity of 20 million cases and mortality between 500-1000 million cases. More recent estimates published by Crompton (1999) have revealed that *S. mansoni* is infecting about 83.3 million people, *S. japonicum* about 1.5 million people, *S. intercalatum* about 1.7 million people, *S. haematobium* about 113 million, and *S. mekongi* about 0.9 million people.

1.4.2.6 Other Neglected Important Human Intestinal Parasitic Helminths Infections.

Hymenolepiases caused by *Hymenolepis nana* has not been considered an important infection in the world public health because a real impact of this infection on human health has not been observed. However, the prevalence of *H. nana* is commonly high, particularly in children, in areas where the major intestinal parasitic infections in humans are also highly prevalent and they co-exist in combinations (polyparasitism) (Neva and Brown, 1994). We do not know the extent to which *H. nana* is associated to morbidity in those areas, or impacting on the health status of the affected population. This infection is maintaining the same or increasing levels of prevalence at least in different communities of Latin America, where it is used as indicator of the levels of parasitic contamination (Neva and Brown, 1994). It was estimated that more than 20 million people are infected by this parasite and some surveys revealed a prevalence by countries of 0.2% to 3.7%, and 10% in children in some areas (Neva and Brown, 1994). Current estimates point out that hymenolepiasis is affecting around 75 million people (Crompton, 1999).

1.5 World Health Public Policy.

Human intestinal parasitic infections are among the most important, most common, and most persistent health problems in developing countries today. Because policy makers, and government officials are now beginning to take action to improve health in vulnerable countries, the study and control of parasitic infections must continue. However, priorities, and decisions are having to be taken on insufficient scientific evidence. Better information can change those priorities, and provide greater benefits to those who are now relatively neglected. There have been some attempts to estimate the physical, social, and economic costs of diseases, the price to control them, including estimates of prevalence, morbidity, mortality, and feasibility of control of common infectious diseases (Walsh and Warren, 1979; Stephenson and Holland, 1987). However, in most of the cases, much of the data needed to determine costs of disease and benefits of controlling them, are not available, are inadequate, or are of questionable accuracy. Hospitals in developing countries are too busy with patient care to keep adequate and accurate statistics; patients having more than one disease are common, some diseases are misdiagnosed, other are not appropriately registered, analysed, or interpreted, and hospital patients are not representative of the entire community. The extent of the morbidity due to human intestinal parasitic infections has received a very little systematic study. For example, it is unknown how much diarrhoeal disease, which is a major cause of death in children under five years old, is due to intestinal parasites capable of causing diarrhoea (*E. histolytica, G. lamblia, T. trichiura*) (Beaver *et al.*, 1984). Nor is it known as to the extent to which intestinal parasites are contributing to the malnutrition status of a population. Similarly, there is little idea of how much the respiratory symptoms which are very common in children in the tropics are the result of the migration of *A. lumbricoides* larvae through the lungs, producing extremely potent allergens, and causing severe asthma, and other allergic phenomena in sensitised persons (Coles, 1985).

Therefore, all factors (human and economic) involved in collection and management of data, must be taken into account in policy decisions by governments as well as by national and international funding agencies. Appropriate data interpretation will provide a very important information for the planning, implementation, monitoring, and evaluation of future control programmes. In many regions, priorities to institute control measures must be assigned, and measures that use the limited human and financial resources more effectively and efficiently must be chosen.

As a country progresses in controlling some diseases, the shortage of evidence will stimulate the necessary research to improve data and epidemiological knowledge, so that it will become possible to implement further actions to reduce the most important health problems in that country (Stephenson and Holland, 1987).

1.6 Progress and Problems in Control Strategies.

1.6.1 Benefits.

Intestinal helminth infections are starting to be recognised as an important public health problem, particularly in developing countries where they have been consistently underestimated (Albonico *et al.* 1999). Progress has been made in the understanding of ecology, epidemiology, and related morbidity and in developing of new tools for control strategies. This knowledge has provided rational basis for the solution of this problem of public health. In recent years the WHO, (1997), has recognised albendazole, levamisole, mebendazole, and pyrantel as essentials in the control of helminthiasis in different countries (WHO, 1996; Crompton, 1999). Recent results from the control of soil-transmitted nematodes through chemotherapy have shown the significant advances in different regions of the world. A first epidemiological evaluation carried out in The Seychelles archipelago after 3 mebendazole campaigns in a sample of about 1000 children from all schools showed that the prevalence of intestinal parasites had dropped from 60.5% to 33.8%, accompanied by a reduction in the intensity of *A. lumbricoides*, *T. trichiura*, and hookworm (WHO, 1996). In Sri Lanka the results from an evaluation carried out 2 years after a biannual deworming with a single 500 mg dose of mebendazole launched in 1994 to target more than 200,000 children 3-14 years has have shown reduced prevalence and intensity of intestinal helminth infections (Albonico *et al.*, 1999). In Zanzibar the local government developed the National Plan for the Control of Helminth Infections by school based activities in 1994 administering 500 mg of mebendazole every 4 months. After one year of treatment the programme reduced significantly the intensities of *A. lumbricoides*, hookworm and *T. trichiura* in a target population of 70,000 schoolchildren (Albonico *et al.*, 1999). The National Health System in Mexico has organised periodic health campaigns which is promoting a mass anthelminthic chemotherapy using a twice yearly administration of a single dose of albendazole in school age children. The results from baseline data obtained from 9,337 from 32.3% (1st. Week, October 1993) to 14.8% (5th Week, February 1995) and the prevalence and intensity of *A. lumbricoides* have decreased from 23% (1st. Week, October 1993) to 9.1% (5th Week, February 1995) and 3010 eggs per gram of faeces (1st. Week, October 1993) to 1032 epg (5th Week, February 1995) (WHO, 1996). Other efforts using praziquantel have led to a significant reduction of *S. japonicum* in China, Indonesia, and the Philippines. The number of cases of blindness has fallen from 0.1% in 1971 to less than 0.05% in 1995, regarding the growth of the population of 7 countries of Sub-Saharan Africa taking part from the start of the onchocerciasis control programme (33 million in 1970 and 61 million in 1995) (Crompton, 1999).

1.6.2. Problems.

All above has shown that efforts and progress are already producing benefits. Unfortunately the control programmes are susceptible to serious problems in technical and financial resources. Selecting the target for treatment may be difficult and costly in practice. The selective treatment of heavily infected children could be expensive, because it is difficult to identify them and time consuming, and probably results in low efficacy, because light and moderate infection may be also associated to morbidity (Albonico *et al.*, 1999). In addition, the treatment of children can be more cost-effective than population treatment in reducing the number of disease cases because is usually the population group with the highest levels of intensity of infection. In a high transmission area has been pointed out that enhancing coverage is a more cost-effective strategy that increasing frequency of treatment, but the presence of insidious helminth infections could change this point of view.

In the case of the used anthelminthic drugs it is important to mention that many counterfeit products exist and may be used inadvertently in control programmes. With generic products there may be variations in pharmaceutical formulations, bio-availability and therapeutic equivalence. A careful balance between quality of product and cost-effectively must be made. Programme managers must take steps to avoid fake or counterfeit drugs and should take action that quality has been assured by independent analysis. On the other hand a wide variability of drug efficacy is found even in trials in which the same drug is given at the same dosages. The lack of use of a standardised parasitological technique, the different length of follow-up after treatment, presence of single and multiple infections before treatment, the different prevalence and intensity rates, the age-group enrolled, the sample size, and lack a placebo group account for the difference in efficacy of the same drug in epidemiological studies. In addition, the searching process for the appropriate drug must include cost of the total treatment, storage, delivery and treatment on site and monitoring (Albonico *et al.*, 1999). The unit cost of the drug itself does not guaranties the best cost-minimisation, cost-effectiveness, cost-utility and cost-benefit analysis (Albonico *et al.*, 1999).

Previous studies on effectiveness have shown that measures of coverage or cure rates do not reflect any long-term reduction in morbidity (Albonico *et al.*, 1999). More recently, the number of heavy infections prevented or cured in a population have been used as outcome indicators, given the strong relationship between intensity of infection and morbidity (Medley *et al.*, 1993). However, diverse statistical measurements of intensity (arithmetic or geometric means) have been used, making the results of efficacy trials more difficult to compare and interpret.

There is evidence that school-based health programmes provide significant benefits by periodic anthelminthic treatment for school-age children, however in many developing countries school enrolment and attendance rates could very low and this implies that a school-programme will not reach those children. Such children will be disadvantaged because they are not receiving education and health benefits.

Health education is mentioned as an important tool to make people aware and responsible for their own health, to create the demand for, and to involve the community in, latrine building, use and maintenance, but precise strategies are not well elaborated. Helminth control is usually viewed as a health intervention in support of health education rather than health intervention be supported by health education. However health education may be hampered by receiving low attention, or planned in basis on wrong needs, poor training of school-teachers and teaching material (Albonico *et al.*, 1999). In addition, some chemotherapeutic interventions are often hampered by re-infections which may lead to higher worm burdens than those before treatment. Therefore culturally non-acceptable and defective latrines and septic tanks, overflowing soak-away pits and the discharge of untreated sewage into rivers, streams and ponds can minimise the progress made by a control programme and ruins prospects for sustainability. Despite the well-recognised role of effective sanitation in preventing transmission of intestinal helminths the impact of improved water supply and sanitation has not been well studied (Huttly, 1990). Some studies in different regions in the world have associated a reduction between the prevalence and intensity of infection with an improvement in sanitation and education (Tanner *et al.*, 1987; Sorensen *et al.*, 1994). However, other studies have failed to find such associations (Muller *et al.*, 1989). The effect of improved sanitation on helminth infections is slow to develop and may take decades to achieve measurable impact (Huttly, 1990). However is not an argument against improvements in sanitation because the provision of sanitation facilities may result in an immediate impact on the control of short-lived helminths (Henry, 1988).

Strategies to change behaviour such as shoes-wearing to protect feet from contact with contaminate soil has been recognised as an important preventive measure against hookworm infections since 1920's (Smillie, 1924). Recently Chongsuvivatwong *et al.* in 1996 found that the only protective measure for hookworm infection was the wearing of shoes. However, there continues to be debates to whether promotion of footwear use is a feasible and effective hookworm control measure given the difficulties inherent in any strategy attempting to change behaviour (Albonico *et al.*, 1999).

Chemotherapy may faces the problem of susceptibility of subjects to strongyloidiasis which can reduce the effectively of the programme. Morbidity is known to be substantial in individuals immuno-suppressed by chemotherapy, radiotherapy or genetic disorders (Genta, 1989). The possibility of auto-infection occurring with *S. stercoralis* plays an important role in chronic disease, leading to increased morbidity among people living in endemic areas. Until recently, treatment for *S. stercoralis* has resulted in low cure rates or been hampered by a high frequency of side-effects (Albonico, 1999).

On the other hand a control programme for intestinal nematode infections con not be planned, implemented or sustained without recent and reliable information about the infections of interest and risk factors involved in transmission and re-infection rates (epidemiology), the people at risk (target), chemotherapy to be used, health education, sanitation, monitoring and evaluation, and identification of useful morbidity indicators (signs and symptomatology, anthropometry and micronutrients status) (Albonico *et al.*, 1994; Albonico *et al.*, 1995). It will be necessary to carry out specific surveys to obtain the information needed. The more recent findings about these components of a control programme will have significant implications for health planners both in adjusting the appropriate drug treatment schedules already existing to control morbidity, or replacement of more cost-effective drugs. The lack of both resources and multi-sectoral collaboration make difficult the complete knowledge about these components of a control programme (Albonico *et al.*, 1999).

The drug resistance would be another problem in a control programme (Albonico, 1999; WHO, 1996). Although there have been recent reports of the development of anthelminthic drug resistance in human hookworms (De Clerk *et al.*, 1997) and schistosomes (Stelma, 1995), convincing evidence of drug resistance in humans helminths is not well documented. In addition, there are not confirmed reports of drug resistance in S-THs to the currently used anthelminthic drugs by control programmes. However, the existence of drug resistance is commonly reported in the veterinary literature (Albonico *et al.*, 1999). Widespread treatment of farm animals with anthelminthics has been widely practised to increase productivity and to prevent disease and death. Nowadays veterinary medicine is facing an important and increasing problem of drug resistance of the major anthelminthics. Resistance to benzimidazoles is most commonly reported in Australia, South America and South Africa, the prevalence of resistance to benzimidazoles varies between 40 and 90%. In addition, resistence to levamisole (24-84%) and ivermectin (73%) has been recently reported in South American countries (Geerts *et al.*, 1997). In addition, pyrantel resistance by *Haemonchus contortus* in small ruminants particularly sheep has been reported by WHO (1996). There is evidence that structural changes in the ß-tubulin molecule of the nematode reduce the binding affinity of benzimidazoles. The mutant resistant gene is already present in the nematode population and its distribution is enhancing by selection pressure (Roos, 1990). Drug resistance will be favoured by no genes flow (isolated population), homogeneous treatment, frequent treatment shorter than generation time of the nematode, and underdosing or treatment with partially effective drugs. By extrapolation from some studies on the development of pesticide resistance in insects, it seems that 5-100 generations would be required under appropriate conditions before drug resistance appear in a nematode population (Comins, 1984). However drug resistance should be carefully distinguished from drug tolerance that is an innate insusceptibility of a parasite resistance should be carefully distinguished from drug tolerance that is an innate insusceptibility of a parasite to a drug even before previous exposition to the drug. Several measures such as the treatment of a proportion of the people in an infected population (pre-school and school children, mother of child-bearing age) which will ensure that some nematodes remain in the untreated population and serve as reservoir of drug-susceptible genes diluting the selection pressure, low frequent treatments and given at intervals greater than the generation time of the nematodes, changes of drug or using combinations of anthelminthic drugs will help to reduce and delay drug resistance (WHO, 1996). Measures to recognise and monitor drug resistance will reduce the threat to the health benefits of any chemotherapy-based control programme.

Much progress has been achieved in the control of human helminth infections but guidelines should be developed to help health planners set targets, to determine frequency of treatment for sustaining control programmes, and to facilitate the assessment of morbidity and mortality in relation to the intensity of soil-transmitted nematode infections (Albonico *et al.*, 1999). The challenge for the next century will include the developing of sustainable approaches for controlling human helminth infections and associated diseases being aware of the growing demographic trends and increasing urbanisation that will be occurring, particularly in developing countries.

CHAPTER 2

Public Health Significance of Human Intestinal Soil-Transmitted Nematode Infections in Mexico.

2.1 Important Human Soil-Transmitted Nematode Infections in Mexico.

The global estimates presented in chapter one, reveal strong evidence that the human intestinal soil-transmitted helminthiases (S-THs) are likely to be highly prevalent in Mexico.

Helminthiases have been for many decades an important cause of concern in public health in Mexico. The prevalence of helminthiases varies in relation to social and economic development, climate and soil conditions, educational and cultural factors. However, many Mexicans are at risk of acquiring infections by S-THs (Lara and Alvarez, 1974; Salazar, *et al.*, 1976; Guerrero, 1983; Duarte *et al.*, 1984). A review of published data in a twenty-year period (1955-1995) about the distribution of helminthiases in Mexico, revealed that the most common S-THs (Quihui, 1995, unpublished data), reported in different regions of Mexico were *Ascaris lumbricoides, Trichuris trichiura*, *Necator americanus*, *Ancylostoma duodenale*, and *Strongyloides stercoralis*. In developed countries, these parasites are present in low prevalence, but in Mexico a number of factors seem to be associated with an increase in the levels of transmission and morbidity rates of these infections, and other parasitic infections. It is important to point out that the presence of parasitic carriers in the immigrant human populations from Central, and South America, or people flowing from the countryside to the large cities, could be an important factor associated with transmission of these infections in Mexico (Flores *et al.*, 1983; Martinez *et al.*, 1987). Anaemia, growth failure, protein-losing enteropathies, mal-digestion and malabsorption, chronic or recurrent abdominal pain, abdominal distension, diarrhoea, intestinal obstruction, allergic outbreaks, and other numerous secondary complications have been associated with these infections (Lara, 1984; Garcia 1987). In spite of the great impact that S-THs have on Mexican public health, implementation of control actions by the appropriate organisations have been difficult in a large country like Mexico (appendix 1). Currently, a national deworming campaign has been established (Ministry of Public Health, 1993) but it is well known that factors such as lack of reliable epidemiological information, limited human and economic resources, lack of co-operation of the people, the inappropriate use of economic resources from the Mexican government, lack of an organised surveillance will reduce the efficacy of the present campaign.

2.2 Distribution, Abundance, and Impact of the Soil-Transmitted Helminths Infections (S-THs) in The Mexican Population.

2.2.1 Distribution, Prevalence, and Intensity of the Soil-Transmitted Helminths Infections in Mexico from 1955 to 1995.

The review of the published data on the prevalence of helminths infections in Mexico from 1955 to 1995 (Quihui, 1995, unpublished data), revealed that *A. lumbricoides* was the S-TH species with the highest national average prevalence (16.7%). Hookworm infections (*N. americanus* and or *A. duodenale*), *Trichuris trichiura*, and *S. stercoralis* presented average national prevalences of 14.3%, 12.6%, and 4.6% respectively. Another recent review by Tay *et al.* (1995) about helminth infections in Mexico, revealed that *A. lumbricoides*, *T. trichiura*, hookworm infections, and *S. stercoralis*, were the human helminths with the highest prevalence in Mexico, (average prevalence of 11.2%, 1.7%, 0.15%, and 0.06% respectively.

The review of the data published between 1955-1995 revealed that the prevalence trends of these helminths species, decreased and increased with time although it could result from the absence or unpublished national epidemiological information in a defined time, non-standardised parasitological techniques, non-well designed studies, non well-trained technical personnel, no representative sample size, and no homogeneity in the demographic features of the sampled population, however there is no evidence of control programme (Quihui, 1995, unpublished results). Analysis showed that the prevalence of *A. lumbricoides*, hookworms, and *T. trichiura* appeared to decrease from 1955-1965 to 1976-1985 (between 35%-20% and 10%-5% respectively), and after this, they increased markedly during the period 1986-1995 (between 10%-

20%) (Fig. 2.1). *S. stercoralis* showed a slight decrease from 1955-1965 to 1966-1975 (between 10%-5% and 5%-0% respectively), but increased again from 1976-1985 to 1986-1995 (between 5%-10% in both periods).

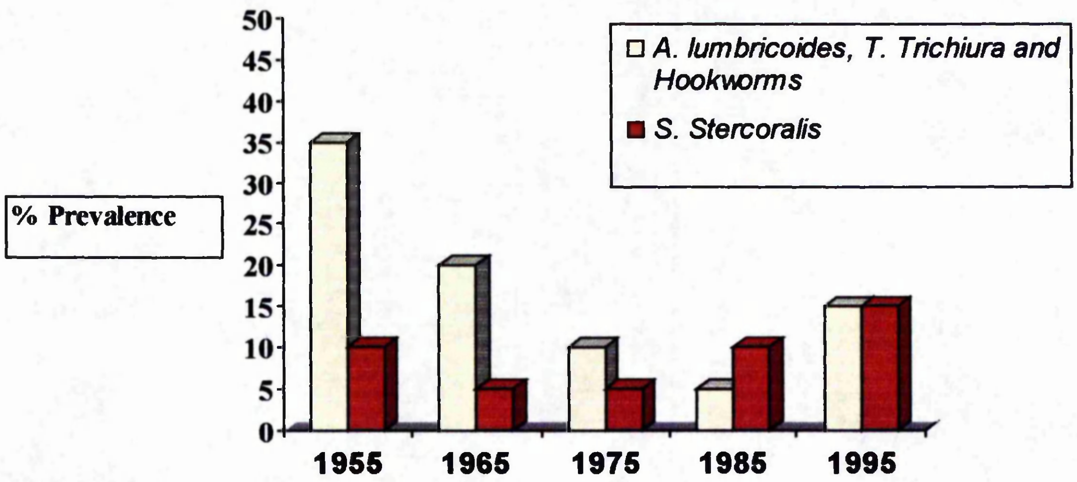

Fig. 2.1 Trends of the Mexican Mean Prevalence of the Different Human Helminth Infections in the General Population from 1955 to 1995.

The analysis of distribution among the different helminths species in the same state showed that *A. lumbricoides* presented the highest prevalence in Chiapas (36.3%), *T. trichuria* in Veracruz (57.2%), and hookworms in Guerrero (63.1%); and the analysis of the same helminths species in the different Mexican states showed that *A. lumbricoides* presented the highest prevalence in Veracruz (50.7%), *T. trichiura* in Veracruz (57.2%), hookworm infections in Guerrero (63.1%), and *S. stercoralis* in Oaxaca (10.7%) and Tabasco (10%) (Table 2.1) (appendix 1).

Table 2.1 Mean Prevalence of Each Soil-Transmitted Helminth Species for each State in Mexico for the Period 1955-1995.

Region divided by State	*Ascaris lumbricoides*	Hookworm Infections	*Trichuris trichiuria*	*Strongyloides stercoralis*
Southeast Region				
Chiapas	36.3	30	32	9.8
Veracruz	50.7	47.3	57.2	5.6
Oaxaca	18.0	9.6	8.7	10.7
Yucatan	10.5	0.1	11.3	0.7
Quintana Roo	1.5	*	0.2	*
Campeche	2.1	*	0.5	*
Tabasco	35.1	17.6	25.7	10.0
Southwest Region				
Guerrero	47.3	63.1	27.6	8.3
Jalisco	12.8	*	7.3	5
Michoacan	9.8	*	1.4	*
Colima	2.6	*	0.2	*
Nayarit	5.7	*	0.3	*
Central Region				
Distrito Federal	19.0	1.8	10.6	1.5
Queretaro	11.8	1.8	2.3	1.8
Puebla	15.8	16.2	19.5	1.3
Estado de Mexico	*	0.7	1.5	7.3
Morelos	26.9	7.1	31.1	0.9
Hidalgo	5.9	*	0.05	*
Guanajuato	0.7	*	0.008	*
Tlaxcala	1.6	*	0.02	*
Aguascalientes	0.6	*	0.007	*
Northwest Region				
Baja California Norte	0.05	*	0.01	*
Baja California Sur	0.3	*	0.01	*
Coahuila	0.8	*	0.001	*
Chihuahua	0.3	*	0.3	*
Durango	0.8	*	0.001	*
Sinaloa	2.6	2	6.4	3.5
Sonora	3.1	*	0.16	*
Zacatecas	2.7	*	0.02	*
Northeast Region				
Nuevo Leon	12.5	0.02	3	0.2
San Luis Potosi	4.1	*	3.3	*
Tamaulipas	0.9	*	0.1	*

***No reported or published cases**

Partially taken from Quihui, 1995, (unpublished data)

When Mexico was divided into five regions on the basis of the geographical situation of Mexican States (appendix 1), and the analysis of the distribution of helmith species by region was carried out, *A. lumbricoides*, *T. trichiura*, hookworms infections (mean prevalence between 25%-35%) and *S. stercoralis* (between 5%-10%) were the predominant helminth species in the Southeast region (Chiapas, Veracruz, Oaxaca, Yucatan, Quintana Roo, Campeche, and Tabasco). However, these helmith infections showed lower mean prevalences in the Central region (between 5%-15% for *A. lumbricoides*, *T. trichiura*, hookworms infections, and between 0%-5% for *S. stercoralis*), Southwest region (between 5%-15% for *A. lumbricoides*, and *T. trichiura*, and between 5%-10% for *S. stercoralis*), Northeast region (between 0%-10% for *A. lumbricoides* and *T. trichuria*, and between 0%-5% for *S. stercoralis*), and Northwest region (between 0%-5% for *A. lumbricoides* and *T. trichiura*) (appendix 1).

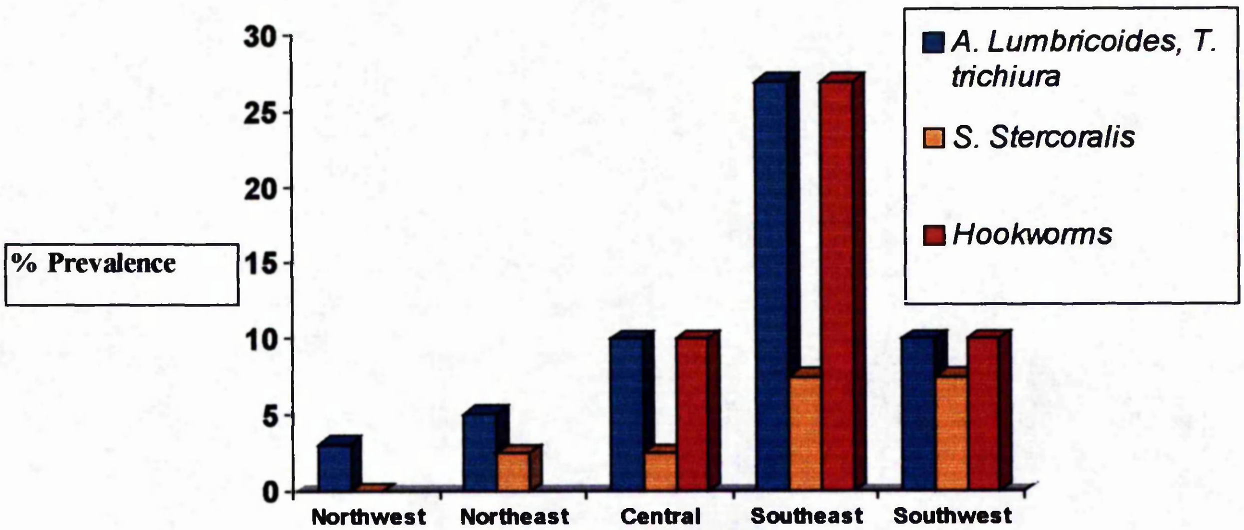

Fig. 2.2 Trends of the Mexican Mean Prevalence of the Different Human Helminth Infections by Mexican Regions from 1955 to 1995.

Considering demographic factors, analysis showed that these infections are present from early years in all age-groups (from 0 to >19 years old). The prevalences of *A. lumbricoides*, hookworms infections, *T. trichiura*, increased with age, reaching peaks in the 7-14 and 15-19 age-groups (between 20%-30% and 25%-35% respectively), and after they decreased in the > 19 age-group (between 15%-25%). However, the prevalence of *S. stercoralis* increased with age in a similar way, reaching a peak in the 7-14 age-group (between 5%-10%), but it maintained its prevalence in the older 19 age-group (between 5%-10%)

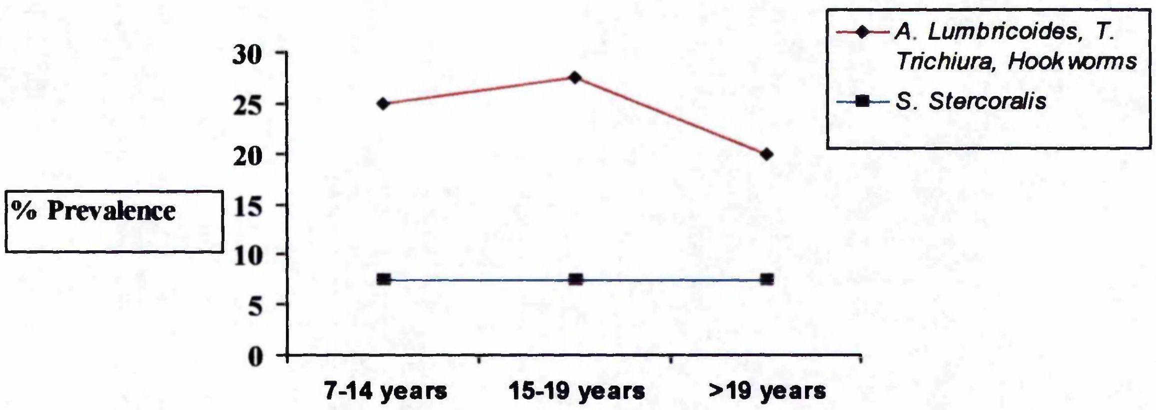

Fig. 2.3 Trends of the Mexican Mean Prevalence of the Different Human Helminth Infections with age from 1955 to 1995.

Information about intensity data obtained from few Mexican published scientific records from 1955 to 1995 is shown in the Table 2.2 (Quihui, 1995, unpublished data).

Table 2.2 Distribution of the Level of Intensity for *Ascaris lumbricoides*, *Trichuris trichiura*, and *Necator Americanus* in the General Population from the States of Hidalgo, Chiapas, and Distrito Federal.

% of the Sampled Population (n=150)	Level of the Intensity of the Infection (epg)	
10.5	*A. lumbricoides*	> 50,000
23.4	*A. lumbricoides*	20,000-50,000
66.1	*A. lumbricoides*	< 20,1000
9.4	*T. trichiura*	> 5000
14.6	*T. trichiura*	2,100-5000
76.1	*T. trichiura*	< 2,100
1	*N. americanus*	> 5,000
14.7	*N. americanus*	2,100-5,000
84.2	*N. americanus*	< 2,100

2.2.2 Mortality and Morbidity Rates

2.2.2.1 Rates of Morbidity.

The analysis of data from published Mexican scientific records on morbidity revealed that *A. lumbricoides*, and *T. trichiura* were associated with morbidity in 2.6% and 0.13% cases respectively in the general population attending the Mexican Institute of Social Security between 1972-1981. A higher rates of morbidity per 100,000 cases associated with *A. lumbricoides* and *T. trichiura* were reported in the Southeast region (5.5% and 1.5% respectively) than that reported in the rest of the Mexican regions (Northwest, 0.07% and 1% respectively; Northeast, 1.7% and 1% respectively; Southwest 3% and 1% respectively; and Central region 2% and 0.5% respectively) (appendix 1) (Fig. 2.4) (Saldivar *et al.*, 1983).

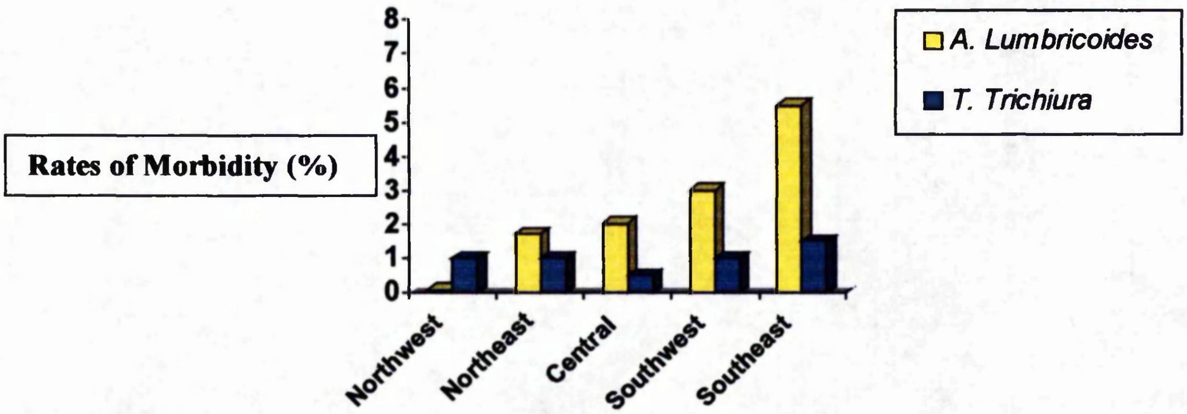

Fig. 2.4 Rates of Morbidity Associated with Ascariasis and Trichuriasis in the General Population Reported by the Mexican Institute of Social Security between 1972-1981.

A review of the public health significance of hookworm infections (Carrada, 1986), showed that *N. americanus* was the predominant hookworm species in Mexico. However in 1960 health researchers from the Health and Tropical Diseases Institute of the Ministry of Public Health, described a discrete presence of *A. duodenale* in some Mexican geographical regions, although prevalence data were not revealed (Ministry of Public Health, 1993). In adddition, medical units of the Mexican Institution of Social Security reported about 49,905 cases of necatoriasis (*N. americanus*), and the rate by 100,000 patients showed a decrease from 79.6 (12,269 cases) to 6.4 (1,807 cases) of necatoriasis from 1975 to 1984. In 1982, 1,231 cases of necatoriasis were detected in males (rate 9.0 per 100,000) and 1,177 cases in females (8.5 per 100,000). The distribution by age showed that 1,439 cases (61.4%) of necatoriasis were present in children from 0 to 14 years old (608 cases in children aged 1 to 4 years and 831 cases in school age children), 725 cases (30.1%) in young people from 15 to 44 years, and 180 cases in adults older than 45 years (7.5%). The children were the most vulnerable group to hookworm infection (Fig. 2.5).

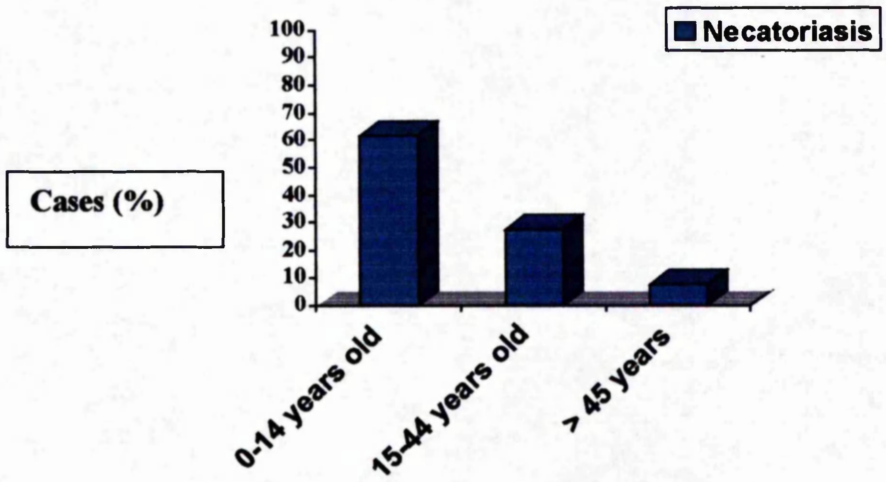

Fig. 2.5 Distribution of 2344 cases of Necatoriasis in the Mexican General Population by Age Reported by the Mexican Institute of Social Security According to the Ministry of Public Health (1993).

The Mexican States with the highest rates of necatoriasis on the basis of clinical cases included Veracruz, Chiapas, Campeche, Tabasco, Guerrero, Nayarit, and Oaxaca (Carrada, 1986) (Table 2.3).

Table 2.3 Rates of Necatoriasis per 100, 000 Cases Reported in the Different Mexican States by Carrada (1986).

MEXICAN STATE	RATES OF NECATORIASIS PER 100,000 CASES
VERACRUZ	55.5 (1002 cases)
Chiapas	83.8 (223 cases)
Campeche	51.7 (91 cases)
Tabasco	23 (71 cases)
Guerrero	9.3 (40 cases)
Nayarit	8.9 (31 cases)
Oaxaca	5.3 (16 cases)

2.2.2.2 Rates of Mortality

A. lumbricoides has been reported as the only helminth associated in cases of death in Mexico. Mortality rates of 24.6% and 3.9% associated with ascariasis were found in 1953 and 1975 respectively from a total of 1379 autopsy cases carried out in Distrito Federal (Quihui, 1995, unpublished data).

2.3 Progress and Problems Associated with Mexican Information about Intestinal Helminth Infections in the Population and the Current Deworming Programme.

2.3.1 Availability and Reliability of Data about Soil-Transmitted-Helminth Infections in the Mexican Population.

Although, we know that the S-TH infections are probably a main factor associated with morbidity and mortality in Mexico, published information and research surveys to evaluate the impact of these helminthiases in the Mexican communities are poor, and not trustful, and we are not even close of knowing the real figures of their prevalence and intensity.

It is important to emphasise that Mexico is a country with an extensive variety of climates, socio-economic, and culture of its inhabitants, which favour the diversity, spreading, and persistence of human intestinal parasites. In 1990, the national percentage of illiterates older than 10 years was about 12.4%. In Distrito Federal the percentage of illiteracy was found to be 4% and seven times higher in Chiapas, Oaxaca, and Guerrero. 19.5% of households in Mexico had floor of bare-earth, and the households from the States of Chiapas, Oaxaca, and Guerrero contributed almost half of this percentage. In addition, 20.6%, and 26.4% of households lacked piped water and proper sanitation respectively (XI Censo General de Poblaci6n y Vivienda, 1990, INEGI) .

Unfortunately, most of the research about helminth infections in Mexico, has been undertaken without an appropriate strategy (number of stool samples, not defined age and gender groups of population, inappropriate statistical analysis, lack of information of risk factors), or effective technology to determine the real prevalence, morbidity, and intensity of helminth infections, the use of well-trained technicians, or statistical representative sample (Quihui, 1995, unpublished information, Tay *et al.*, 1995) (appendix 2)

It is also highly probable that a large number of inhabitants in Mexican tropical areas are suffering multiparasitism. Such people constitute the main geographical targets, where urgent action of the appropriate health authorities is required. In addition, most of the data about human intestinal helminth infections obtained from the population who received attention in the different Mexican health institutions are not published in a proper national information system, but if publication does exist, the information is found partially dispersed in different Mexican papers which usually makes reviewing difficult. The problem is more complicated when knowledge about rates of morbidity and mortality are required because they are in general uncertain and not trustworthy. Available current information about morbidity rates has been estimated on the basis of informed cases. However, some identified factors such as declining of the infected people to medical attention and self-medication, make the estimations of actual figures of morbidity more difficult (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

2.3.2 Development of Control Programmes for Human Soil-Transmitted Helminths in Mexico.

2.3.2.1 Significance of a Control Programme for Soil-Transmitted Helminthiases.

Control programmes for helminth infections depend on a simple strategy to prevent and to control these infections by using broad-spectrum anthelminthic drugs. According to Warren (1990), an effective control programme depends on the following stages: helminths do not multiply in their definitive hosts, clinical symptomatology is only experienced by heavily infected individuals mainly school-age children, and broad-spectrum anthelmintic drugs are administered in one simple dose, to reduce significantly intensity and morbidity. These stages have highlighted the difference between the meanings of the words "control" and "eradication". A control programme is the implementation of specific measures by a disease control authority to limit the incidence of a disease (Gemmell *et al.*, 1986). Eradication implies the reduction of the incidence of a specific disease to the point of continued absence of transmission within a specified area by means of a time-limited campaign (Yekutiel, P. 1980). There are four major benefits of chemotherapy at the community level that deserve special attention. First, because chemotherapy is generally popular, it can serve as an entry point into a community to encourage the villagers to reduce the risk factors associated with transmission of infection (environmental sanitation, personal hygiene, etc.) (Stephenson *et al.*, 1980). Secondly, community treatment is a form of prevention, because it helps to reduce the probability of transmission of infection from those faeces containing potentially infective eggs and larvae. Thirdly, although re-infection is inevitable in endemic areas, periodic treatment will help to decrease morbidity in those groups at particularly vulnerable periods of re-infections in their lives. Finally, since polyparasitism is common, chemotherapy with broad-spectrum anthelmintic drugs will allow reduction of more than one parasite. (Stephenson, 1984).

2.3.2.2 Planning a Control Programme for Soil-Transmitted Helminthiases.

When a chemotherapy programme is formulated, it must identify the target group, the anthelmintic drug to be used, the frequency and duration, and strategy to reach the target group. Children and women in their child-bearing years are usually the groups most at risk from severe helminthiases (ascariasis, trichuriasis, and hookworm infection) (Stephenson and Holland, 1989). An additional target group might be migrant groups (Brown, 1982). The frequency and time at which drugs should be given will depend on the initial prevalence and intensity, probability of re-infection, realistic goals of the programme, costs, and ease of reaching the target population. Treatment for every 3 months has sometimes been recommended to keep infections to a minimum, but in many areas this would be too expensive or time consuming to accomplish with available resources. However, providing mass treatment in children once or twice per year in areas where ascariasis, trichuriasis, and hookworm infections are common would probably produce substantial decreases in morbidity even though some re-infection would occur.

The system used to reach the target population is often the most difficult and expensive in a control programme for soil-transmitted helminths. The local primary schools have become a very effective mean of reaching the school-age children (Stephenson, *et al.*, 1983; WHO, 1996b). This approach is useful when most children attend the primary school, and teachers are available to co-operate and conduct health education lessons on improved sanitation and personal hygiene, and health messages may then reach the children's parents.

2.3.2.2.1 Schools as a Significant Setting Giver of Public Health.

The school has been found to be an effective setting to improve the health of pupils, staff, families and members of the community, because offers opportunities to achieve significant health and education benefits, and commitment to raise the social status of women and girls with the limited resources (WHO, 1996b). The number of young people who do not attend school could be reducing the role of schools as a significant setting to promote health. However, primary schools enrolments are increasing in nearly every part of the world. This is true in absolute number and enrolments rates. Schools worldwide reach about 1000 million young people and through them their families and communities. The only major exception is in Sub-Saharan Africa where the enrolments are declining. At present, more schools than ever before are considering whether their policies and practices contribute positively or negatively to the health of students and WHO has call them health-promoting schools. Teaching children and adolescents how to become not only employees but also responsible citizens is a fundamental mission for today's schools. It means to prepare them to become women and men with equal rights, mutually supportive, have a well-developed intelligence to make critical decisions.

Administrators and programme managers think of school health in terms of infrastructure, sytems, capacities and save of resources. Scientifics, researchers and planners think of school health in terms of interventions, theories and technical factors. The WHO programme has supported health and education promotion in schools but it has been recognised there is more to be done. A health promoting school engages health and education officials, teachers, students, parents, and community leaders to make the school a healthy place; implements policies, and practices to provide a healthy environment, health education and health services; and to improve the health of school personnel, families and community members and students.

The first network was initiated by WHO's Regional Office for Europe, the Council of Europe, and the Commission of the European communities. This network has grown in five years to include 34 countries, 500 core schools, and 1600 affiliated schools together comprising some 400,000 students. In 1995, the regional networks of health promoting schools were started by the Regional Office for the Western Pacific. In 1996, the Global School Health initiative assisted WHO Regional Offices to develop networks in the Eastern Mediterrean, Africa and Latin America. In the Eastern Mediterrean Region (Bahrain, Egypt, Jordan, Morocco and Sudan) the health educators have arranged for pupils to visit health institutions, shopping centres to learn about available foods, food safety and hygiene, and factories. Students have also gained knowledge and skills regarding health and how to practise what they had learnt about health promoting behaviour. WHO in the Western Pacific Region (China, Lao People's Democratic Republic, Malaysia, Papua New Guinea, Republic of Korea, Singapore, Solomon Islands and Viet Nam) has outlined in the document New Horizons in Health three themes for future work named preparation for life, protection of life, and quality of life. These concepts were taken up by the Ministers of Health of this region (WHO, 1996b). In 1991, the primary schools in Shangai established a health action project in cooperation with the local community authorities. The health standards extended to social and personal behaviour and since the start of this programme there has been a marked decrease in dental caries and short-sightedness among pupils. In Papua New Guinea, improved standards of toilets in the school and the community was the top priority for the health-promoting school programme, and adequate nutrition as a second priority. As part of health education effort in Fiji, sanitation, waste disposal, toilet hygiene and personal hygiene have been continually highlighted in the school children, and a School Health Guardian Programme implemented in Manila, involved health instruction, a safe and healthy school environment, useful community-school projects, and regular health promotion activities.

The health promoting school initiative called the development of health education (updating the school curriculum, improving the training and skills of the teachers, and preparing appropriate educational material), environment health (adequate sanitation and safe water supply, improving physical infrastructure and pupil-teacher-parent relationships) and health and nutrition services (better coordination between the health services, the parent-teacher associations, non-governmental organisations, and community institutions) at school in the Region of the Americas in a meeting held in Costa Rica in 1993. In Latin America ministries of education are carrying out programmes of school health education at the primary-school level. Most countries in the region can rely on the health services to detect problems in areas such as hearing, sight, posture, malnutrtion, and caries. Some programmes are built into the curriculum on subjects such as hygiene, diet, accidents, and waste disposal. However, ther is no system of surveillance for risky practices at school, and this makes difficult to design strategies, activities and teaching materials to promote health. In Bolivia, educational reform calls for close coordination between the health and education sectors using the technique rapid diagnosis and analysis to evaluate the country's capacity to undertake health promotion and education at primary-school level. In Costa Rica the Commission for Education and Health prepared questionnaires to evaluate habits and practices that might endanger the health health of schoolchildren and adolescents and is coordinating the formation of a national network of such schools. In Mexico, the national school health programme is promoting a change in attitude, habits, and behaviour in order to inculcate a culture of health in the schoolchildren, their families and the community; to contribute to the early detection and treatment of health problems; and to increase the effectiveness of health activities through health and educational institutions (WHO, 1996b). The Caribbean island states has a school health project which was teaching life skills in the English-speaking Caribbean, to improve health education and sexuality in the schools with particular reference to Dominica, Saint Kitts and Nevis, and the British Virgin Islands. In Belize the schools have encouraged children to take simple steps to prevent diseases, adequate nourishment, and awareness about good hygiene. In Argentinan schools in communities such as San Cristobal, Villa Crespo, Ezpeleta and Berazategui were promoting healthy habits and health care; to manage the school as a health-giving physical and social environment by making changes in the physical structure and teaching learning methods; to optimise the links between school and health centre; and to convert the motivation (sensitisation) of the pupils and paremts to production (change) (WHO, 1996b).

South-East Asia Region (India, Nepal, and Sri Lanka) was encouraging the "little doctor programmes" to ensure the children learn hygiene, good sanitation and healthy life-styles and they become a good teachers to their elders, and the African Region (Benin, Cameroon, Ghana, and Namibia) was re-orientating the health services to look for strategies to attract investment in school health to ensure its future.

All above experiences have shown that schools provide a cost-effective of reducing and preventive health problems in children, and this concept is in an excelent position to introduce worm control. Studies made by WHO and the World Bank have concluded that the yield by health programmes developed in the school setting can be measured in long-term economic and social development and the well-being of the whole population (WHO, 1996b)

2.3.2.3 Basis for Establishing the Current National Deworming Campaign in Mexico.

2.3.2.3.1 Outlined Objectives.

Currently an active deworming campaign is being undertaken in Mexico since October 1993 (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993). The information used as a basis for establishing this campaign included previous epidemiological information about distribution of intestinal parasites in Mexico, childhood mortality associated with diarrhoea and parasitism, and defined objectives according to World Health Organisation determinations Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993). This information was useful for selecting the municipalities from a total of 1,862 distributed in the whole country. Necessary dispositions were carried out to link the mass chemotherapy campaign to the programme of the Health National Week (Semana Nacional de Salud), already established in Mexico in 1986 (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

The appropriate health authorities of the Ministry of Public Health determined that the control programme in Mexico should reach the following objectives (Albonico *et al.*, 1999):

a) To provide anthelminthic chemotherapy for 95% of children aged 2-14 years in municipalities with high risk of intestinal infections.

b) To reduce the frequency of serious health complications (morbidity and mortality) associated with ascariasis, trichuriasis, hookworm infections, and strongyloidiasis.

c) To reduce the negative impact of these helminthiases on infantile growth and development, including learning skills.

d) To decrease the egg output from soil-transmitted nematodes among groups with highest infection prevalence.

e) To decrease the prevalence of infection among school-age children living in specific areas (municipalities with high risk of intestinal nematode infections)

f) To decrease the re-infection rates among school-age children living in areas receiving mass anthelminthic chemotherapy.

The above objectives are expected to be reached in the basis of social mobilization headed by the national, state and county consensus, the participation of private enterprise and organisations, participation of social and community leaders, television and radio (interviews and spots), graphic educational material (posters) and a "health telegram". The actions taken included informing and training decision-makers, training for state trainers (300 epidemiological and health administrators), the design and production of 500 educational packages (videos, slides. manual), the training of operational personnel at local level and the training of teachers and volunteers (n=50,000) (Albonico *et al*, 1999).

2.3.2.3.2 Results Obtained from a Review of Previous Epidemiological Information as Part of the Planning of the National Deworming Campaign in Mexico.

The evaluation of the review about the prevalence and intensity of S-TH infections carried out by the Ministry of Public Health (1993), revealed that epidemiological surveys with representative samples of the general population were limited; and they had been carried out in a few Mexican States (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993). In spite of its relevance to morbidity and mortality rates, information about levels of intensity of these infections was not found.

Table 2.3 shows the epidemiological information estimated by the Ministry of Public Health. *A. lumbricoides*, *T. trichiura*, and hookworm infections in Mexico were present with prevalences between 20% and 80%, and *S. stercoralis* between 0% and 26%, particularly in rural areas on both Mexican Western and Eastern coasts.

A. lumbricoides and *T. trichiura* were found to be the S-TH with a higher prevalence than those showed by hookworms infections, and *S. stercoralis* with prevalences between 3% and 20% in urban communities (Table 2.3).

Table 2.4 Estimations of the Prevalences for *A. lumbricoides*, hookworms infections, *T. trichiura*, and *S. stercoralis* in Different Mexican States carried out by the Ministry of Public Health (1993).

Region divided by State	*A. lumbricoides*	Hookworm Infections	*T. trichiuria*	*S. stercoralis*
Southeast Region				
Chiapas	42.4-56.4	20	37.5	15
Veracruz	70	12-70.1	*	5.1
Oaxaca	24	9.6	17.3	21
Yucatan	23	0.1	29.4	1.3
Quintana Roo	*	*	*	*
Campeche	*	*	*	*
Tabasco	70	92	32.7	2.2-16
Southwest Region				
Guerrero	17.8	55.8	78.4	5.8
Jalisco	10.6	*	2	5.1
Michoacan	16.9	*	4.6	*
Colima	2.6	*	*	*
Nayarit	5.7	*	*	*
Central Region				
Distrito Federal	13-68.4	0.4-3.4	6-9	1.9
Queretaro	22.8	1.8	6	1.5
Puebla	2.5-58.2	0.31-62.4	5.8-39.7	2.1
Estado de Mexico	12.6-17	1.2	1.4-21.7	3.7
Morelos	70	*	*	*
Hidalgo	*	*	*	*
Guanajuato	*	*	*	*
Tlaxcala	*	*	*	*
Aguascalientes	*	*	*	*
Northwest Region				
Baja California Norte	*	*	*	*
Baja California Sur	*	*	*	*
Coahuila	1.2	*	0	*
Chihuahua	*	*	*	*
Durango	*	*	*	*
Sinaloa	*	*	*	*
Sonora	*	*	*	*
Zacatecas	*	*	*	*
Northeast Region				
Nuevo Leon	2.1-30	0.02	0.3-6	0.16-0.2
San Luis Potosi	4.5	*	6.5	*
Tamaulipas	*	*	*	*

***No reported cases**

The prevalence of hookworm infection was estimated between 6% and 72% in rural populations, and between 0% and 2% in urban communities from tropical areas. No evidence was found of hookworm infections in the North of the country. The prevalence of *S. stercoralis* was estimated between 2% and 22% in the humid regions and between 0%-and 4% in urban populations (Table 2.3). Some similarities and differences can be observed between the estimations of the prevalences of these infections from the Ministry of Public Health (1993) (Table 2.3) and those from Quihui

(1995, unpublished data) (Table 2.2)

2.3.2.3.3 Target Population and Justification.

The population in Mexico was around 81.2 million in 1990, of which 10 million were younger than 5 years old (XI Censo General de Poblacion y Vivienda, 1990, INEGI) and 9% of these children suffered chronic diarrhoea. However, the actual extent to which soil-transmitted helminths could be associated with chronic diarrhoea in Mexico is not known. Because there is evidence that children are the most vulnerable population to acquire S-TH infections, and they represent the new generation and the future of a country, the Ministry of Public Health decided to provide anthelminthic treatment to all individuals aged from 5 to 14 years living in the municipalities of highest risk (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

2.3.2.3.4 Drug Selection.

Currently several drugs are effective against intestinal helminths, and all of them have advantages and disadvantages in effectivity, side effects, and cost. The cyclic amines (pyrantel and oxantel) are well tolerated, and cause side effects in low percentage of patients (WHO, 1996a). Piperazine and tetrachloroethilene cause strong side effects, but they are still prescribed because of their low cost (Fernandez, 1958; Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993; Albonico *et al.* 1999). Levamisole is well tolerated in low doses against ascariasis and hookworm infections and cause from mild to transient side effects (WHO, 1996). The benzimidazoles such as albendazole and mebendazole are the most widely used, are well tolerated, and cause transitory effects in patients (WHO, 1996a). The macro-cyclic lactone ivermectin is used against *A. lumbricoides* and *S. stercoralis* but mild adverse reactions have been reported (WHO, 1996a)

The Ministry of Public Health in Mexico decided to choose albendazole for the national campaign encouraged by the historical background of this anthelminthic in different countries such as in Zimbabwe (1989) where administrations of a single-dose of albendazole on the 3rd, 11th, and 20th month in the general population, reduced hookworm infection from 20% to 11%; in Kenya (1989) a single dose of albendazole in school children resulted in a significantly reduction in the levels of intensity for *A. lumbricoides*, *T. trichiura*, and hookworm infection (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993), in Japan where the prevalence of *A. lumbricoides* decreased from 33% to 0.08% and hookworm infections from 9.6% to 0.02% between 1955 and 1980 after periodical massive albendazole treatments were re-started in the 1950's; in Montserrat (West India), where single doses of albendazole administered to children (2 and 15 years), reduced significantly the prevalences and intensities of *A. lumbricoides* and *T. trichiura* (Chan *et al.*, 1992 in Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

In addition, other factors such as activity spectrum, dose regime, administration facility, low rates of side effects, availability of infrastructure, cost, and international recognition by the World Health Organisation were taken into account to select albendazole as the appropriate anthelmintic, to ensure the success of the national deworming campaign in Mexico (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

2.3.2.3.5 Administration of Albendazole and Strategy to Reach the Target.

Authorities of the Mexican Health System (Mexican Institute of Social Security, Secretary of Health, Institute of Social Security for the State Employees), decided to incorporate the control programme into the National Programme titled "Semana Nacional de Salud" (Health National Week), which has been in operation since 1986 (Consejo Nacional de Vacunación, 1992) in October, 1993. This strategy was planned to reach children in the whole country (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

Currently, a single-dose of albendazole (400 mg.) (tablet or suspension) is administered to school children twice yearly before a dry season and after the rain season. The duration of the Mexican control programme has not been defined yet (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

2.3.2.3.6 Selection of Communities and Goals to be Reached.

The target population was estimated at around six million individuals at higher risk, aged from 5 to 14 years according to the Census of Population in Mexico (Censo General de Poblacion, 1990). Factors such as mortality levels in children younger than 5 years old in association with diarrhoeal diseases and prevalence of intestinal infections were taken into account to estimate the target population (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

A total of 887 municipalities with a total population of 5,957,602 individuals aged 5-14 years were selected to be covered by the Mexican Deworming Campaign (Table 2.4) (Ministry of Public Health, 1990). From the selected population (5,957,602) the campaign will be covering 24.7% inhabiting the Southeast region, 30% from the Central region, 12.4% from the Southwest region, 22.3% from the Northwest region, and 10.6% from the Northeast region (Table 2.4) (appendix 1). The Ministry of Public Health (1993) considered that the most of the selected population at higher risk of acquiring intestinal helminth infections were living in the Southeast, Central, and Northwest regions (Table 2.4) (appendix 1).

Table 2.5 Selected Municipalities and Population from 5 to 14 Years Old to be Covered by The Mexican Deworming Campaign (Ministry of Public Health, 1993).

Region divided by State	Total of Municipalities	Selected Municipalities	Population to be Reached
Mexico (Country)	1,892	887	5,957,602
Southeast Region	486	275	1,471,423
Chiapas	111	77	364,058
Veracruz	207	115	449,246
Oaxaca	30	22	336,139
Yucatan	106	49	191,081
Quintana Roo	7	3	31,552
Campeche	8	4	45,081
Tabasco	17	5	54,266
Southwest Region	342	151	736,431
Guerrero	75	39	195,240
Jalisco	124	55	233,259
Michoacan	113	45	221,406
Colima	10	4	39,829
Nayarit	20	8	46,697
Central Region	588	286	1,790,380
Distrito Federal	16	6	606,939
Queretaro	18	7	54,657
Puebla	217	130	309,751
Estado de Mexico	121	46	340,915
Morelos	33	12	62,146
Hidalgo	84	48	199,852
Guanajuato	46	17	152,388
Tlaxcala	44	16	41,095
Aguascalientes	9	4	22,637
Northwest Region	296	116	1,326,145
Baja California Norte	4	1	42,118
Baja California Sur	4	1	6,590
Coahuila	38	16	272,780
Chihuahua	67	25	231,570
Durango	39	16	135,445
Sinaloa	18	8	294,958
Sonora	70	27	271,824
Zacatecas	56	23	112.978
Northeast Region	150	58	633,223
Nuevo Leon	51	17	341,085
San Luis Potosi	56	25	99,445
Tamaulipas	43	16	192,693

2.3.2.3.7 Planning a Surveillance Programme for the Mexican Deworming Campaign.

According to regulations from the Ministry of Public Health (1993), the effectiveness of the Mexican Campaign would be evaluated by longitudinal surveys with demographic and parasitological information.

The objectives of the surveillance programme included: a) to estimate the prevalence and intensity of helminth infections before albendazole treatment; b) to estimate the re-infection rate of helminth infections after albendazole treatment; c) to monitor the helminth infections trends with time; d) to identify groups at higher risk for additional treatment; e) to establish priorities for the control programme; f) to identify the risk factors involved in the transmission of helminth infections (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

The prevalence and intensity would be evaluated by analysing one simple faecal sample every six months using the quantitative technique of Kato Katz. The indicators of cure rates (interruption of eggs and/or larvae excretion) and eggs reduction rate (comparison of eggs excretion per gram of faeces before and after treatment) will be used to determine the effectiveness of the deworming campaign (Epidemiologia de la Helminthiasis Intestinales in Mexico. Bases para su Control, 1993).

CHAPTER 3

A Brief Description of the Main Pathological Intestinal Parasitic Infections

3.1 Introduction

Mexico has made a significant progress in public health as a result of the economic development over the last forty years. However, parasitic infections are still a persistent national health problem, especially in rural communities, and predominantly affecting the pre-school and school populations.

A review of published epidemiological information from 1955 to 1995 has revealed that ascariasis, trichuriasis, hookworm infections, hymenolepiasis, amoebiasis, and giardiasis are still the most important intestinal parasitic infections in Mexico, and they are followed by other less frequently occurring infections such as strongyloidiasis and taeniasis (Quihui, 1995, unpublished data). In addition, these infections are accompanied by a high prevalence of other non-pathogenic intestinal parasites equally important because they provide information about the possible risk factors involved in the transmission of these type of infections (Lara *et al.*, 1974; Salazar *et al.*, 1976 Tay *et al.*, 1976; Lara *et al.*, 1990; Diaz *et al.*, 1994)

Most of the intestinal parasitic infections are seldom observed nowadays in the developed countries, but in Mexico the environmental conditions, lack of basic services such as sanitation, clean water supply, health and educational services, and poverty are still predominant factors involved in the transmission and prevalence of protozoan and helminth infections, particularly in populations living in the rural areas.

Anaemia, growth failure, protein losing enteropathies, malabsorption, chronic or recurrent abdominal pain, intestinal obstruction, headache, vomiting, fever, allergies, nausea, and numerous secundary complications have been associated with these infections (Neva and Brown, 1994).

Anaemia has also been a serious problem of public health in developing countries. The helminthiases such as hookworm infections, strongyloidiasis, and trichuriasis have been associated with anaemia and they represent a strong risk factor for the prevalence of iron deficiency or anaemia mainly in children and woman of childbearing age (WHO, 1994; Neva and Brown, 1994)

Because of the availability of simple, cost-effective, wide-spectrum, and well tolerated drugs, control programmes using anthelminthic drugs have been carried out in different developing countries such as Republic of South Africa, Seychelles, Sri Lanka, Zanzibar, including Mexico (Mexican Ministry of Health, 1993; Crompton, 1999). Certainly, the establishing of control programmes will help to reduce worm burdens, prevalence, and morbidity in the general population and it will result in a higher quality of life, health, and productivity of populations at highest risk to these kind of infections.

3.2 A Brief Description of the Main Pathological Intestinal Helminth Infections.

3.2.1 *A. lumbricoides.*

3.2.1.1 Morphology and Transmission.

Ascariasis is caused by the large roundworm *A. lumbricoides* (Nematoda). The fertilised eggs are ovoidal, with a thick, transparent shell and an albuminoid outer layer. These eggs measure from 45 to 75 μm in length by 35 to 50 μm. Unfertilised eggs measure from 88 to 93.5 by 38.5 to 44 μm. Females often reaching a length of more than 300 mm and a diameter of 6 or 7 mm. The mature males are smaller than the females (150 to 310 mm in length by 2 to 4 mm in diameter) and may also be identified by their curved posterior ends, which house the copulatory spicules.

Human infection with *A. lumbricoides* is acquired by swallowing fully embryonated eggs accidentally picked up from contaminated soil, or from contaminated food or drink. Young children are more commonly infected than adults. Ascariasis is primarily propagated by the soil around the house with eggs present in the faeces of the small children. Children spread the infection to their parents, siblings, and neighbouring children (Beaver *et al.*, 1984).

3.2.1.2 Factors Associated with Ascariasis Transmission.

3.2.1.2.1. Demographic Factors.

Evidence has suggested that prevalence of ascariasis is low in infants younger one year old (Bilo and Bilo-Groen, 1983), The prevalence usually rises in pre-school and schoolchildren and reaches a plateau which may well remain stable throughout life (Elkins *et al.*, 1986), although in other studies the prevalence has decreased and then remained stable in adult populations (McCullough, 1974; Richard-Lenoble *et al.*, 1982; Thein-Hlaing *et al.*, 1984).

In addition, some observations indicate that prevalence is higher in females than males, usually independent of age. This statement is supported by observations in Tanzania, Indonesia, Central Iran, Papua New Guinea, Philippines and Thailand (Mc Cullough, 1974; Shield *et al.*, 1980; Harisunata and Charoenlarp, 1980). However, evidence for this association between prevalence and host sex is not always evident (Elkins *et al.*, 1986). Probably, these findings are related with other factors rather than age.

Other surveys have detected an apparent relationship between the level of prevalence of ascariasis and the ethnicity of the subjects. Pampiglione and Ricciardi (1974) showed that nomadic Babinga group Pygmies in Central African Republic presented lower prevalence than the sedentary Bantu population. Kan (1985) showed that ascariasis is lower in Chinese as compared with Malay or Indian people. This difference may be due to cultural factors or other unidentified factors rather than ethnicity (Crompton, *et al.*, 1989).

Several studies have demonstrated that ascariasis is usually higher in children from large than small families (Prakash, *et al.*, 1980). It seems to indicate that the chance of acquiring ascariasis is higher for those children from relatively large families than that for children belonging to small families (Adekunle *et al.*, 1986).

3.2.1.2.2 Environmental Factors

Results from different studies have shown that the prevalence of ascariasis is usually higher in rural rather than urban communities. In Africa and India the average rural prevalence was 21% while the average urban prevalence was 12% (Crompton and Tulley, 1987). Seo (1980) observed higher prevalence values of ascariasis in rural communities than in urban communities in South Korea. However, different patterns have been observed in Morocco, and Peru (Lumbreras and Naquira, 1985). Rapid migration of populations from rural areas to urban areas apparently are involved in these changes.

The climate has been assumed to be influential in the distribution and prevalence of ascariasis. In Africa, the prevalence is generally low in dry countries (Crompton and Tulley, 1987). Factors such as warmth and moisture enhance the embryonation process leading to the development of the infective larval stage. However, the availability of water can also affect the host population density, and perhaps the opportunities for the successful transmission of *A. lumbricoides* as well as the survival of the infective eggs (Crompton, *et al.*, 1989). Also, it is well documented that seasonal fluctuations influence the prevalence of ascariasis. In Saudi Arabia, Gelphi and Mustafa (1967) observed that raised temperatures and reduced humidity are accompanied by an increase in seasonal pneumonitis and eosinophilia.

3.2.1.2.3 Socio-economic Factors (Housing and Sanitation)

An investigation carried out amongst Panamanian children revealed that the prevalence of ascariasis were significantly higher in children living in housing made of wood and bamboo rather than concrete blocks (Holland *et al.*, 1988). This same pattern is applied to children living in crowded conditions and to those bound to the poorest water supplies and worst sanitation (Crompton *et al.*, 1989)

3.2.1.3 Human Distress Associated with Ascariasis.

3.2.1.3.1 Symptoms Associated with Larval and Adult Migration.

The important organs involved during this period are the liver, lungs, through which *A. lumbricoides* larvae must pass in order to develop into adult worms. Larval migration clearly can cause pneumonitis, including asthma, cough, substernal pain, fever (39.5°C or 40°C), skin rash, and eosinophilia (Coles, 1985). Pulmonary ascariasis is said to be relatively mild and short lived, lasting about five days, and to be more common where ascariasis is only seasonally transmitted (Gelpi and Mustafa, 1967), and rare where transmission is more or less continuous throughout the year (Spillman, 1975). The most serious complications are due to intestinal obstruction by a bolus of worms or the migration of the adult worms into the hepatic duct, the appendix, a surgical wound or pancreatic duct (WHO, 1981). Although these complications are observed in a small percentage of all infected cases, they have a high fatality rate, and are likely to cause malnutrition in children who are not undernourished (Pinus, 1985)

3.2.1.3.2 Malabsorption of Protein, Carbohydrate, and Fat in Children

Ascariasis has been found to be associated with a significant reduction in mean faecal nitrogen excretion after deworming. (Venkatachalam and Patwardhan, 1953). Tripathy *et al.* (1972) demonstrated intestinal loss of dietary fat in infected subjects. However, other studies have not found a relationship between ascariasis and nitrogen or fat malabsorption in children. Also, Tripathy *et al.*, (1971) found impaired D-xylose absorption in 3 of 5 cases prior treatment, and improved slightly in 2 cases immediately after deworming. Decreased carbohydrate absorption is extremely critical in term of energy intake and growth in children from countries where typical diets contains up to 75% of their calories from carbohydrates sources. Bray (1953) showed no differences in nitrogen absorption in children before or after deworming. Freij, *et al* (1979) found no difference in nitrogen and fat absorption in a small number of lightly infected children.

3.2.1.3.3 Malabsorption of Vitamin A in Children.

Vitamin A has been reported to be affected by ascariasis in several clinical studies with children and contribute to xerophthalmia. Sivakumar and Reddy (1975) found a group of infected children to have lower mean serum vitamin A value than uninfected controls. This study did not include a statistical analysis. They suggested that in areas where xerophthalmia is common, vitamin A deficiency might be exarcebated by the presence of *A. lumbricoides*. Mahalanabis *et al.* (1976) found that patients with ascariasis showed poorer absorption of radioactively labelled vitamin A than did uninfected controls. Bhattacharyya *et al.* (1982) reported reductions in serum vitamin A in infected children compared with those uninfected. Taren (1986) and Taren *et al.* (1987) showed that ascariasis was associated with lower plasma vitamin A and ß-carotene values (Crompton, *et al.*, 1989).

However, contradictory results have been obtained. Reddy *et al* (1986) showed no an effect of deworming on the utilisation of a large single dose of vitamin A by children. The mechanism of the possible effect of ascariasis on vitamin A absorption is not known, but the influence of ascariasis on the absorption of fat may be related to the reduced utilisation of vitamin A (Crompton, *et al.*, 1989)

3.2.1.3.4 Impact on Growth.

There is evidence that the presence of *A. lumbricoides* in the intestinal tract influences the ability of the host to digest and absorb certain nutrients. Obviously, the presence of ascariasis associated with the limited or lack of nutrients could have some detrimental effect upon the host (Nesheim 1985; Stephenson, 1987). Some studies have showed improvements in growth after treatment in children (Gupta *et al.*, 1977; Willett *et al.*, 1979, Stephenson *et al.*, 1980; Foo, 1986), but others could not confirm these findings (Freij *et al.*, 1979; Greenberg, *et al.*, 1981; Kloetzel *et al.*, 1982; Gupta and Urrutia, 1982).

3.2.1.3.5 Ascariasis and other Nutrition-Related Conditions.

Little importance has been attached to the relationship between ascariasis and mal-absorption of nutrients other than protein, fat and vitamin A, due to the fact that few clinical studies have demonstrated such relationship. Blumenthal and Schultz (1976) found that ascariasis had adverse effect on levels of albumin in serum and vitamin C in plasma in children. However, Morgan *et al.*, (1972) found opposite results.

3.2.1.3.5.1 Proposed Mechanisms Responsible for the Possible Nutritional Outcomes.

A few studies have shown the probable mechanisms by which *A. lumbricoides* can cause malnutrition, stunting, xerophthalmia, vitamin deficiencies, and other nutrition-related conditions. The following mechanisms have been hypothesised: 1) consumption by roundworms of nutrients needed by the host; 2) interference with intestinal absorption due to mucosal damage by the parasites; 3) loss of nutrients, fluid, and electrolytes through diarrhoea and vomiting; and 4) production of proteolytic substances by the parasites 5) reduced appetite and food intake. Jejunal abnormalities in infected persons were found by Tripathy *et al.* (1972), Lagundoye (1972), and Maxwell *et al.* (1968). Layrisse and Varges (1975) described changes in intestinal microflora, which aid the biosynthesis and digestion of nutrients. Chatterjee (1972) hypothesised that trypsin and chymotrypsin (pancreatic proteases) are neutralised by "ascarase", an anti-enzyme polypeptide product of *A. lumbricoides*. Finally, nutrient utilisation, reductions in nutrient digestion and absorption can be observed in infected children compared to those uninfected. It is not surprising that appetite for food declines when abdominal and epigastric pain, diarrhoea, fever, malaise, nausea, steatorrhoea are accompanying ascariasis. However, the difficulties of obtaining precise food intake measurements in free-living human populations make this hypothesis difficult to test (Crompton *et al.*, 1989).

3.2.2 *Trichuris trichiura.*

3.2.2.1 Morphology of the Aetiological Agent.

The whip-worm *Trichuris trichiura* is the cause of trichuriasis, and lives attached to the wall of the human caecum, but in heavy infections can be found in the appendix, colon or posterior level of the ileum. The male, approximately, measures 30 to 45 mm in length and has its caudal extremity coiled through 360° or more. The female measures 35 to 50 mm in length and is bluntly rounded at the posterior end. The eggs are characteristically barrel-shaped, un-segmented when oviposited in the large intestine. They have a bipolar, intralaminar prominence. These eggs measure 50 to 54 μm by 22 to 23 microns in width which are discharged in the stool in the non-segmented stage. The eggs will not develop in direct sunlight and perish below -9°C and above 52°C. Ideal conditions include shade, warm, moist, and soil. After ingestion by man the larvae hatch and penetrate the intestinal villi and remain there temporarily. After a period of growth (one week), the larvae re-enter the intestinal lumen and migrate to the caecum. Adult develop in 30 to 90 days and after copulation the females start to lay eggs (Stephenson and Holland, 1987).

3.2.2.2 Prevalence and Geographic Distribution.

This human whip-worm is cosmopolitan in distribution but is more common in the warm, moist regions of the world, where both the incidence and the intensity of the infection may be high. In certain hyperendemic areas, 90% of the population may be infected (Beaver *et al.* 1984). Past estimations indicated that between 500 and 700 million of people were infected world-wide (Peters, 1978; Walsh and Warren, 1979), but others reported that up to 1000 million could harbour the infection (Bundy, 1986). In addition, it was estimated that 63% of the infected persons inhabited in the tropical and sub-tropical regions of Asia, about 11% in Africa, and about 14% in the Americas (Pawlowski, 1984). More recent estimates have indicated that about 1,049 million people are infected with *T. trichuria* (Crompton, 1999).

3.2.2.3 Modes of Transmission and Risk Factors

Trichuriasis results from swallowing eggs in infective stage obtained directly or indirectly from the contaminated soil. Areas of high prevalence and heavy worm burden are usually polluted by small children, who are often more commonly infected than are adults. In areas of high endemicity, small children develop heavy infections, and the greatest prevalence typically occurs in children of primary school age who contaminate soil with their faeces. Later they pick up the infective eggs, in various ways transfer them to their mouths, and spread the infection to their parents, siblings, and neighbouring children (Neva and Brown, 1994)

3.2.2.3.1 Demographic and Environmental Risk Factors.

Evidence has suggested that the number of clinically significant cases is often higher in the 5 to 15 year age-group (Wolfe, 1978; Pawlowski, 1984; Bundy *et al.*, 1985a). In St. Lucia, the prevalence increased rapidly with age in early childhood and remained high and relatively constant throughout adulthood. In contrast the average worm burden declines significantly in adults (Bundy *et al.*, 1987), possible associated with factors such as acquired immunity, changes in behaviour, or a combination of both processes are involved in this (Anderson and May, 1985; Bundy *et al.*, 1987).

Some surveys have detected an apparent relationship between the level of prevalence of trichuriasis and the ethnicity of the subjects. Studies from Malaysia reported a higher incidence of heavy trichuriasis amongst Indian children than in Malays or Chinese (Kamath, 1973; Kan, 1982; Gilman *et al.*, 1983). This difference may be due to cultural factors or other unidentified factors rather than ethnicity.

In addition, evidence has suggested that children harbour the heaviest worm burdens, which supports the conclusion that trichuriasis is mainly a disease of childhood (Bundy, 1986) but it is has been reported that chance of trichuriasis during childhood is higher for those children from relatively large families than that for children belonging to small families (Adekunle *et al.*, 1986). Therefore, children in the families become the main carriers, and spreaders of this infection to their parents, siblings, and other children and persons in the community.

The climate is also an influential factor in the distribution and prevalence of trichuriasis. Warmth and moisture will enhance the development of the infective larval stage. Trichuriasis is more or less coextensive with ascariasis, but the former is more prevalent in areas of high rainfall, high humidity, and dense shade. This is probably associated with *Trichuris trichiura* eggs being much less resistant to desiccation and heat than are those of *A. lumbricoides*. The infective stage will not develop on hard clay, ashes, or cinders, and direct sun's rays, intense cold, putrefying medium, or action of many chemical agents (Beaver, *et al.*, 1984).

3.2.2.3.2 Socio-economic Factors.

A study with Panamanian children revealed that the prevalence of trichuriasis was significantly higher in children living in the poorest housing conditions (Holland *et al.*, 1988). In addition, family overcrowding and poor socio-economic conditions influenced in the high prevalence of this infection (Holland *et al.*, 1988)

3.2.2.4 The Clinical Spectrum of Trichuriasis in Humans.

As these parasites do not migrate beyond the intestinal mucosa, their influence on humans is related mainly to the activity of the adult worms in the mucosa of the large intestine. The severity of this infection is dependent not only on the intensity of the infection itself and its location in the gastrointestinal tract, but also on the state of the host including age, general health, iron reserves and experience of past infections (Pawlowski, 1984). Trichuriasis usually shows a very strong, positive correlation between intensity, and pattern of symptoms (Jung and Beaver, 1951; Gilman *et al.*, 1983), although, some light infections may result in massive infantile trichuriasis, can develop (Kouri and Valdez, 1952), and is characterised by severe symptoms including anaemia (Bundy, 1986). In moderate infections, diarrhoea, abdominal pain, nausea, and vomiting can result in both decreased food intake and increased nutrient losses, making a child already compromised by protein-energy malnutrition more vulnerable to illness. On the other hand, rectal prolapse is thought to be a consequence of either straining at defaecation in the presence of a massive number of worms and/or irritation of nerve endings with increased peristalsis (Ramirez-Weiser, 1971). Clubbing of the fingers may occur as a secondary effect of increased vagal stimulation caused by chronic diarrhoea (Kitis *et al.*, 1979).

3.2.2.4.1 Trichuriasis and Nutritional Status of the Host.

Some studies have found trichuriasis to be significantly associated with reductions in growth, mostly in the hosts considered to be heavily infected. Studies by Gilman *et al.*, (1983), Cooper and Bundy, (1988), Cooper *et al.*, (1990), produced results that suggested trichuriasis has some impact on child growth and nutrition. Particularly, Cooper *et al.*, (1990) demonstrated accelerated growth following chemotherapeutic expulsion of worms. Robertson *et al.*, (1992), suggested that light infections of trichuriasis may exert a detrimental effect on the growth of even well-nourished children, and Simeon *et al.*, (1994) concluded that benefits in growth after treatment of trichuriasis depended on the nutritional status as well as the infection intensity. In addition, trichuriasis associated with a dysentery syndrome was reported to cause poor growth development in children (Callender *et al.*, 1993). In some cases concurrent infections of ascariasis and trichuriasis were found to be exacerbating childhood malnutrition (Cooper and Bundy, 1988, Cooper *et al.*, 1990; Robertson *et al.*, 1992; and Hadju *et al.*, 1996). In contrast, Bowie *et al.*, (1978) considered diet as the most important factor for improvements in weight gain following treatment of children with moderate trichuriasis.

3.2.2.4.2 Blood Loss and Anaemia in Trichuriasis

Trichuriasis and iron deficiency anaemia have been associated widely in the tropics. Since 1896, it was suspected that *T. trichiura* sucked blood from the mucosa of the large intestine (Otto, 1935). Crystals of human haematin (Fernan-Nuñez, 1927), or blood in the oesophagi of *T. trichiura* have been found in some studies (Burrows and Lillis, 1964). Early studies by Otto (1935) showed that cases of heavy trichuriasis (10,000 eggs per gram of faeces) were associated with anaemia. Massive trichuriasis was found in four Panamanian children aged 4 to 13 years who died of severe anaemia according to a report by Getz in 1945. The number of worms recovered at autopsy (1,100, 1,700, 4,100, and 4000) was inversely related to haemoglobin levels. Studies by Jung and Jelliffe (1952), and Belding (1961), suggested low haemoglobin levels and anaemia as symptoms of the infection. Brown (1954), reported that 8 patients (children) with relatively heavy infections (17,000 to 224,000 epg) had haemoglobin levels in the anaemic range of 50 to 100 g/L. Wong and Tan, (1961) demonstrated that 19 of the 20 children with trichuriasis had bleeding ulcers in the rectal mucosa, and after treatment ulcers began to heal and haemoglobin levels rose. Layrisse *et al.* (1967) found that heavy trichuriasis (more than 5 million eggs per day and 32,000 per gram of faeces) caused a blood loss of 4 ml. per day (0.005 ml of blood per worm per day) which represented more than 1.5 mg of haemoglobin iron loss when the haemoglobin concentration in the peripheral blood was more than 120g/L. However, the amount of daily haemoglobin iron loss due to trichuriasis that could induce iron-deficiency varies from one person to another according to diet, physiological blood loss such as menstruation, requirements for growth, and haemoglobin formation. If diet only permitted a maximum of iron absorption of 1.5 mg per day used for new tissue and haemoglobin formation, the higher daily losses of iron haemoglobin due to trichuriasis and normal blood loss without parasitic infection (0.2 to 5 ml per day) (Roche *et al.*, 1955; Blahd *et al.*, 1958) would unbalance the iron metabolism, with detriment of iron storage and haemoglobin values. However, epidemiological investigation on human groups other than children has proven anaemia to be scarcely a feature of uncomplicated trichuriasis. Clinical studies by Mahmood (1966), Martinez and Beaver (1968), and Lotero *et al* (1974) showed no evidence of enough blood loss to cause anaemia even when the infection was severe. Further recent studies have also produced more controversial results. Data by Greenberg and Cline (1979) did not support the hypothesis that trichuriasis caused anaemia through blood loss in lightly infected children. One explanation for this paradox could be a failure in some of the community studies to quantify adequately the worm burden, or to take into account its overdispersed distribution. Robertson *et al* (1992) and Ramdath *et al* (1994) found that trichuriasis over 5000 eggs per gram of faeces were associated with a low-grade anaemia, whereas infections of less intensity are associated with neither anaemia nor low iron status. However, it is possible that anaemia may be associated with less intense trichuriasis in developing countries where children are malnourished and are exposed to multiple infections.

3.2.2.4.3 Proposed Mechanisms Involved in the Nutritional Outcomes in Trichuriasis.

Different mechanisms have been proposed by some authors to explain both the clinical effects and malnutrition observed in humans during trichuriasis. Worm crowding may block the lumen of the appendix or cause inflammation and irritation of the epithelium of the caecum, appendix, and colon leading to appendicitis, colitis and proctitis, with further diarrhoea (Stephenson and Holland, 1987). In another study involving associations between *T. trichiura* and *E. histolytica* infections, Gilman, *et al.*, (1983) proposed that *T. trichiura* may disrupt the intestinal epithelial continuity altering the ability of the large intestine to resist infections with enteropathogenic bacteria resulting in the complex of severe diarrhoea, anaemia, malnutrition, clubbing of the fingers, rectal prolapse. In addition, these lesions may be accompanied by mechanisms of reduced appetite as consequence of increases in cytokines, especially in tumour necrosis factor (TNFα) and interleukin-1 (IL-1) (Matsui, *et al.*, 1993) already observed in children with *T. trichiura* dysentery syndrome by Mc Donald *et al.*, (1994). It has been found that, interleukin-1 and tumor necrosis factor α (TNF-α) suppress the food intake by altering firing rates of glucosa-sensitive neurones in the lateral hypothalamus (Mc Donald *et al.*, 1994).

3.2.2.4.4 Proposed Mechanisms Involved in the Possible Development of Anaemia in Trichuriasis.

It is not clear how blood loss by sucking process or bleeding from the lesions in the colon can cause anaemia mainly in vulnerable children. Burrows and Lillis (1964) have stated that the whip-worms attach to the caecum epithelium to suck blood of the host, and as the number of worms increases, anaemia will be one of the results of the infection. Passage of blood from the anus as a highly sign of dysentery has been observed. Bundy *et al.*, (1985c) reported clinical history of blood in stools from 14 cases of intense trichuriasis for periods exceeding six months (Jamaica). Beer *et al.* (1974) considered that this mechanism of the red cell loss was the leakage of erythrocytes into the damaged gut lumen from dilated lamina propria blood capillaries and from impaired blood forming petechial haemorrhages. Hale *et al.* (1979) observed a microscopic bloody flux from surface of colonic mucosa in pig trichuriasis. However, colonoscopic studies by Bundy and Cooper (1989) did not found any relation between formed petechiae and the degree of anaemia.

3.2.3 Hookworm Infection.

3.2.3.1 Morphology and Epidemiology.

The species in man include *Necator americanus* (*N. americanus*) and *Ancylostoma duodenale* (*A. duodenale*), and rarely, *A. braziliense*, *A. caninum* and *A. ceylonicum*. The latter three species are hookworm parasites of the dog and cat. *A. braziliense* and *A. caninum* can infect but do not develop to maturity in humans. But *A. ceylonicum* found mainly in Asia and the Pacific can develop to adults in humans (Neva and Brown, 1994)

Adult hookworms are small, cylindrical, fusiform, greyish white nematodes.

The females (9 to 13 mm by 0.35 to 0.6 mm) are larger than males (5 to 11 mm by 0.3 to 0.45 mm). *A. duodenale* is larger than *N. americanus*. The worm has a relatively thick cuticle. There are single male and paired female reproductive organs. The posterior end of the male has a broad, translucent, membranous caudal bursa used for attachment to the female during copulation. The chief morphologic differences in the species are in the shape, buccal capsule, and male bursa. In the buccal capsule *N. americanus* has a conspicuous dorsal pair of semilunar cutting plates and *A. duodenale* two ventral pairs of teeth. The egg has bluntly rounded eggs and a single thin transparent hyaline shell. It is non-segmented at ovoposition and in two to eight cell stages of division in fresh faeces (Neva and Brown, 1994).

N. americanus, and *A. duodenale* penetrate human skin by third-stage larvae which develop from eggs passed in human stools into the environment. After completing a period of migration through the tissues, larval hookworms return by means of the respiratory system to the alimentary tract. However, *A. duodenale* has the particular feature to behave as an opportunistic species whose larvae can establish an infection by the swallowing, transplacental, transmammary mechanisms (WHO, 1996). It has been suggested that some larvae *A. caninum* may be stored as arrested larvae in the parenteral tissues of their hosts and presumably after reactivation by hormones associated with lactation, the arrested larvae are shed in milk and become infective to suckling puppies. This observation has suggested that larvae of *A. duodenale* can become arrested in people in parts of China and India (WHO, 1994). It has been observed that the most of eggs and adults worms expelled (one single worm was identified as *N. americanus*) in stools from some Chinese or Indian children following chemotherapy or examined at autopsy were *A. duodenale*. Apparently, they were infected before birth while *in utero*. The highly occurrence of *A. duodenale* in neonatal children in regions where *N. americanus* is the dominant species, suggests that transmammary transmission rather than cutaneous infections, is the usual basis for neonatal infections by *A. duodenale* (WHO, 1994).

It has been estimated that 1.2 billion people are infected by hookworms and cause a daily blood loss of more than 1 million litres (Neva and Brown, 1994; Crompton, 1999).

Factors that facilitate the maintenance and dispersal of hookworm infection include defaecation on the soil by infected individuals, presence of shaded sandy soil or loam instead of tightly packed clay soil, a warm climate, appropriate moisture to prevent desiccation of eggs and larvae, and a population with barefoot or wearing simple sandals. These conditions are found in the tropics, subtropics, and even temperate climates (Neva and Brown, 1994)

3.2.3.2 Pathology and Symptomatology in Human Hookworm Infections.

3.2.3.2.1 General Clinical Spectrum.

When larvae penetrate the skin and migrate through the lungs can result in bronchitis or pneumonitis in sensitive individuals. The shortest pre-patent period reported in humans infected percutaneously with *A. duodenale* is about 56 days. (WHO, 1994). Severe gastroenteritis develops about 6 weeks after infection. This is associated with nausea, vomiting, epigastric discomfort, and diarrhoea. The hookworms attach to the mucosa of the small intestine by their buccal capsules. The favourite site is the upper small intestine (jejunal mucosa), but in heavy infections the worms can be present as far as the lower ileum. Adult worms bite into the tissues and suck blood and mucosal substances by the tractile pull of the contracting oesophagus. An anti-coagulating secretion facilitates blood sucking. *A. duodenale* infections can persist for 6 to 8 years and longer, and *N. americanus* disappear within 2 years and other live 4 or 5 years. The maximum daily egg output by a mature female has been estimated at 20,000 for *A. duodenale* and 10,000 for *N. americanus*. The most prominent characteristic in moderate or heavy chronic hookworm infection is a progressive, secondary, microcytic, hypochromic anaemia of the iron deficiency type. Depending on the degree of anaemia the patient experiences dyspnea, weakness, and dizziness. The appetite may be enormous or poor and associated with pica. The heart shows hypertrophy and the pulse is rapid. Serum proteins levels are reduced, and there may be oedema of varying degrees secondary to hypoproteinemia. Heavily infected children may be physically, mentally, and sexually retarded.

Eosinophilia and leukocytosis are marked in early infection and start decreasing as anaemia persists (Neva and Brown, 1994). The clinical picture does not permit differentiation from anaemia by nutritional deficiencies or chronic diseases. Final diagnosis depends upon finding the eggs in the faeces. (Neva and Brown, 1994)

3.2.3.2.2 Iron Deficiency Anaemia and Hookworm Infection.

The most important outcome of hookworm infection is iron deficiency anaemia as a result of intestinal blood loss (Roche and Layrisse, 1966; Banwell and Schad, 1978; Shad and Banwell, 1984). A single *N. americanus* causes a loss of approximately 0.03 ml of blood per day, and *A. duodenale* causes about 0.15 ml. per worm per day. A person who passes 2000 hookworm eggs per gram of faeces, blood faecal losses have been estimated in 1.3 mg of iron per day for *N. americanus infection*, and about 2.7 mg. of iron per day in an infection with *A. duodenale*, assuming a haemoglobin level of 140 g/L. (Farid *et al.*, 1965; Roche and Layrisse, 1966; Mahmood, 1966; Martinez-Torres, *et al* 1967; Miller, 1979). Since only 10% or less of iron consumed in food is typically absorbed into the body in tropical areas, a person with 2000 worms in hookworm infection must consume at least 13 mg of extra-iron required per day in an infection with *N. americanus*, and 27 mg of extra-iron required per day for *A. duodenale*, to compensate for iron losses (Stephenson and Holland, 1987).

Since the 1920's the correlation between haemoglobin level and worm load has been apparent (Darling, 1922; Smillie, 1922). However this relationship has always not been observed in every study carried out (Layrisse and Roche, 1964; Roche and Layrisse, 1966) and a few workers have doubted about the importance of hookworm disease in the aetiology of iron deficiency anaemia (Foy and Kondy, 1957). Many factors as nutritional status, sample size (individual variation), intensity (light and heavy), statistical data analysis, dietary iron requirements, and methodology to determine the biochemical indicators and coproparasitological analysis, need to be taken into account before establishing a relationship between hookworm infection and anaemia. Some of the published studies which did not find a relationship, did not satisfy these criteria (Stephenson and Holland, 1987).

Foy and Kondy (1960) felt that the estimation of numbers of worms is only the satisfactory method to establish a relationship between anaemia and hookworms burden. Probably the relationship between worm burden and egg count is non-linear because the rate of egg production per worm decreases when there are high worm burdens (Croll *et al.*, 1982; Crompton and Stephenson, 1985), day variability in eggs counts, sampling heterogeneity, and variability in technical processes (Anderson and Schad, 1985). However Layrisse and Roche (1964) found a highly significant relationship between haemoglobin levels and hookworm egg counts in some Venezuelan communities. The difference between the frequency of anaemia in the non-infected and hookworm-infected groups showed that about one-third of all anaemias were probably due to hookworm disease.

However, small hookworm burdens may be sufficient to reduce haemoglobin levels in groups in which iron losses or requirements are relatively high and reserves are low (menstruating woman and growing children) (Chemin, 1954). This situation was observed in Venezuela where a significant low haemoglobin values appeared with smaller number of eggs in menstruating women and growing children (Layrisse and Roche, 1964). Iron requirements, dietary intake of utilisable iron, diet composition, and iron stores, are crucial factors in the interpretation of these results (Stephenson and Holland, 1987).

3.2.3.2.2.1 Hookworm Infections and Anaemia in Vulnerable Populations.

Although general patterns in the population biology of hookworms indicate that infections are acquired gradually, the peak is usually reached during young adulthood. In general more adults than children will be infected, and the adults will harbour the larger worm burdens (WHO, 1994). Relatively little detail is known about the biology of hookworm infections in the female host, but the prevalence and intensity of hookworm infection are reported to be somewhat lower in females than males (WHO, 1994). Relationships of this type are almost attributed to behaviour differences in exposure to infection. Nevertheless, millions of females of reproductive age are infected with hookworm, and adolescent girls and women of childbearing age are generally more heavily infected than younger boys and girls (WHO, 1994).

Since iron deficiency and anaemia affect a significant part of the population in many countries, hookworms infections have became in an exacerbating factor of these health problems, mainly in groups with high requirements of iron. It has been seen that women in developing countries spend a major part of their reproductive lives pregnant or lactating, and iron deficiency in childbearing women increases maternal mortality, and prenatal, and perinatal infant loss, and prematurity. Many of girls enter puberty and have their first pregnancy presenting hookworm infections, and iron deficiency. Forty percent of all maternal perinatal deaths are linked with anaemia, and anaemic mothers have 30% to 45% less likely for a favourable pregnancy, and their infants have less than one-half of the normal iron reserves (WHO, 1994).

Estimations of hookworms infections in pregnant women with in different regions of the world during 1990 has been as follows: 32% in Sub Saharan Africa, 34% in Latin America and Caribbean, 22% in Middle Eastern, 42% in India, 34% in China, and 35.6% in Asia (WHO, 1994). Otherwise, the prevalence of anaemia in pregnant women in different regions of the world in 1992 was as follows: 52% in Africa, 40% in Latin America, 18% in Europe, 50% in Eastern Mediterranean, 74% in South-East Asia, Western Pacific, and 40% in Western Pacific (WHO, 1994). These estimates will provide information about what extent hookworms infections are associated with anaemia, or iron deficiency in pregnancy, and will help to establish effective iron dietary or supplementary recommendations to prevent severe anaemia, and subsequently maternal, and infant deaths (WHO, 1994).

3.2.3.2.2.2 Hookworm Infection, Anaemia, and Productivity.

The relationship between anaemia, hookworm infection, and productivity is important, especially for societies dependent on self-employed subsistence farming (Stephenson and Holland, 1987). In many developing countries adult males and females represent the heavy workload (WHO, 1994). The ability to perform a work is reduced by even a moderate anaemia in male and female rural workers. Heavy manual work is impossible once a haemoglobin level below 70 g/L. is reached because physiological compensation is inadequate and lactic acid accumulates leaving the person breathless

(Fleming, 1982). Populations suffering of potential factors associated with an unbalanced iron status will produce a negative impact on both public health and economical development. Brooks *et al.*, 1979; Latham 1983, Basta *et al.*, 1979, Gardner *et al.*, 1977, and Edgerton *et al.*, 1979 showed a decreased work capacity and productivity in anaemic workers.

In some developing countries females are the most vulnerable human group to iron deficiency, because they will be not only homemakers, agricultural workers, labourers, and water carriers, but also will spend a part of their lives either in pregnancy or lactating which demand high requirements of iron. (WHO, 1994).

3.2.4 *Strongyloides stercoralis*

Worm female measures 2 mm. This parasite lives in the small intestine, specifically the duodenal and upper jejune sections. Eggs are deposited in the intestinal wall and larvae are passed into the intestinal lumen and excreted in faeces. In appropriate environment conditions (soil, temperature, humidity) rhabditiform larvae become in the infective stage (filariform) and penetrate the human skin (no soil contact) to continue developing (direct cycle) or adult worms (females and males) undergoing for mating (indirect cycle) in the soil (Neva, 1986; Stanford *et al.*, 1999). Sometimes larva become infective (filariform) in the intestine and this process is called auto-infection probably associated with situations such as immunosupression, cancer, and malnutrition.

When larva penetrate the human skin produce lesions similar with those by hookworm infections. Damage to the intestinal wall includes oedema, bleeding, granulome and ulceration and is related with the intensity of the infection. This is may accompanied by epigastric pain, diarrhoea, chronic dysentery, nausea, vomiting and eosinophilia. (Stanford *et al.*, 1999). Fatal strongyloidiasis can be commonly associated with malnutrition, presence of other infections, immunosupression, and massive spreading of the infection (liver, lungs, pancreas, and surrounding organs) (Neva, 1986). Diagnostic is carried out by showing larvae in faeces and bronquial aspirates. Passed-larva in faeces is not regular and when infection is strongly suspected, the string test can be used. This string is wrapped in a jelly substance and ingested. The string is impregnated with duodenal secretions and larva are detected and identified by microscopic observation. Serological reactions and hypersensitivity test are not truthful (Stanford *et al.*, 1999).

3.2.5 *Hymenolepis nana.*

The worm *H. nana* averages 20 by 0.7 mm and may has as many as 200 proglottides. The small, globular scolex bears a short, retractile rostellum with a single ring of small hooks and four cup-shaped suckers. The mature trapezoidal proglottid, about four times as broad as long, has a single genital pore on its left side, three round testes, and bi-lobed ovary. In the gravid proglottid, the sacculate uterus contains 80 to 180 eggs. The oval or globular egg, 47 by 37 μm, has two membranes enclosing a hexacanth embryo with six hooklets. The inner membrane has two polar thickenings from each of which arise four to eight slender polar filaments.

The habitat of the worm is in the upper two thirds of the ileum. Its life span is several weeks. It is estimated that more than 20 million persons throughout the world are infected. Surveys reveal an incidence by countries of 0.2% to 3.7%, although in certain areas 10% of the children are infected (Neva and Brown, 1994). Transmission is dependent upon immediate contact because eggs, which are susceptible to heat and desiccation, cannot long survive outside of the host. Infection is transmitted directly from hand to mouth and less frequently by contaminated food or water and possibly by insect intermediate hosts (arthropoda). Soil is not required for egg embryonation (Shulman *et al.*, 1999). The unhygienic habits of children favour the prevalence in the younger age groups (Neva and Brown, 1994).

Ordinarily there is no material damage to the intestinal mucosa, but enteritis may be produced by heavy infections, as many as 2000 worms. Light infections produce either no symptoms or vague abdominal disturbances. Children may show lack of appetite, abdominal pain with or without diarrhoea, vomiting, and dizziness. Diagnosis is by microscopic finding of eggs in the stools (Neva and Brown, 1994; Shulman *et al.*, 1999).

3.2.6 *Enterobius vermicularis.*

The adult female worm (8.0 to 13 mm by 0.4 mm) has a cuticular alar expansion at the anterior end, a prominent esophageal bulb, and a long pointed tail. The uteri of the gravid female are distended with eggs. The male, 2 to 5 mm in length with a curved tail and a single spicule, is seldom seen. Transmission may be carried out by hand to mouth (soil is not required) from scratching the perianal areas, or from handling contamination. Inhalation of airborne eggs in dust, and rarely, retro-infection through the anus. Eggs hatch in the perianal region and the larvae migrate back into the colon (Shulman *et al.*, 1999). Heavy infections are effected by the transference of eggs from the perianal region to the hands and hence to the mouth directly or via contaminated food. Eggs have been found in dust from floors, baseboards, tables, chairs, davenports, dressers, shelves, picture frames, windowsills, toilet seats, bath-tube, bed sheets and mattresses. This shows how the infection is spread through families or groups living in the same environment. Less than 10% of eggs can survive for 2 days in a temperature of 20°C and a relative humidity of 30% to 54%. This explains why re-infection is not universal in an infected environment. Dogs and cats do not harbour *Enterobius,* but eggs may carried on their fur and serve as a source of infection.

Stoll estimated that there were 208.8 million infected persons in the world (Neva and Brown, 1994). More recent surveys have revealed infections rates from 3% to 80%. Although this parasite is more prevalent in the lower socio-economic groups, mental institutions, and orphanages, it is also common in the wealthy communities.

E. vermicularis is relatively innocuous and rarely produce serious lesions. The clinical symptoms are due to the perianal, perineal, and vaginal irritation caused by the migrations of the gravid female worm (anal region ⇔ colon) (Shulman *et al.*, 1999), and very seldom by the intestinal activities of the parasite. The local pruritus and discomfort can produce a vicious cycle of very annoying symptoms. In addition, other symptoms such as poor appetite, weight loss, hyperactivity, enuresis, insomnia, irritability, grinding of the teeth, abdominal pain, and nausea have been reported in persons with enterobiasis, but is often difficult to prove the causal relationship of pinworm (Haswell-Elkins *et al.*, 1987).

Diagnosis is made by finding the adult worms or eggs in the faeces. However, only about 5% of infected persons show eggs in the faeces. The Scotch adhesive tape swab gives the highest percentage of positive results and the highest number of eggs. A strip of sticky Scotch is applied to the perianal region, removed, and then spread on a slide for microscopic examination. Repeated examinations on consecutive days are necessary because of the irregular migrations of the gravid female worms. The swab for eggs is preferably made in the morning before bathing or defecation (Neva and Brown, 1994; Shulman, *et al.*, 1999).

3.3 Relationship Among Intensity, Prevalence, and Level of Aggregation of Intestinal Helminths.

In the case of helminth infections, the intensity of the infection is most usually defined as the number of intestinal parasitic stages per host. This can be measured on the basis of egg counts expressed as eggs per gram of stool (epg), worms per gram of stool, or eggs per day (epd), which is more difficult to measure because it involves collecting and weighing all the stools passed per day by the subject involved (Crompton *et al.*, 1989). Sometimes, intensity in egg counts have been found to be correlated with the intensity in worm burden and can be used with some confidence (Stephenson and Holland, 1987). A more reliable measure of the intensity of infection is to count all the worms passed by the subjects for a period of 48 hours after anthelminthic treatment (Crompton *et al.*, 1989). The measures of intensity take no account of larval stages undergoing tissue migrations.

There are three important reasons to understand the intensity of a helminth infection. Morbidity is expected to be related to intensity of the infection; the regulation of the parasite's population is based on intensity (Anderson, 1982); and more efficient control measures could be devised if heavily infected people can be identified to be particularly treated. However, the average worm burden is not very useful since distribution of stages is aggregated or over-dispersed which means that few individuals are harbouring most of the worms in the community (Anderson and

Gordon, 1982). However, the average worm burden can be representative when is estimated from a large sample size (Stephenson and Holland, 1987). In addition, Albonico *et al.*, (1999) reported that the number of heavy infections prevented or cured or the total years of heavy infections prevented in a population have been recently used as outcome indicators to assess the efficacy of a control programme, given the strong relationship between the intensity of the infection and morbidity (Medley *et al.*, 1993)

To understand more about intensity of helminth infections, mathematical equations have been developed involving intensity and its relationship with the prevalence of the infection and stage of aggregation (Bliss and Fisher, 1953). If it is supposed that parasites show a negative binomial distribution, the probability to be infected is proportional to **M** in the equation 1:

(Equation 1)- $$P = 1 - (1+M/k)^{-k}$$

By substitutions and algebraic manipulations the intensity, prevalence, and aggregation of helminth parasites are associated by the mathematical expression

(Equation 2) $$(N-r) - \frac{r}{[1 + M/k]^{k} - 1} = 1/k \text{ Log } (1-r/N)$$

P= Prevalence of the Infection

M= Mean Intensity of the Infection

N= Sample Size

r= Number of Successful Cases from N

k= Level of Aggregation

The numerical function $\mathbf{f_2}$ en terms of **k** has revealed that for constant values for **M,** the **k** value increases when the prevalence **P** of the infection increases (V Jose *et al.*, 1995). When **P** is constant, **k** decreases when **M** increases. When **M** is small, **k** can be large when **P** is moderated. When **P** is large, **M** is small and **k** can be larger. The **k** is non-sensible to moderated and high values for **M** but more sensible to variations for **P**.

The figure 3.1 shows the non-linear association between the **P** and **M** for different values for **k**. The moderated or high values for **M** are accompanied by a stable **P** value.

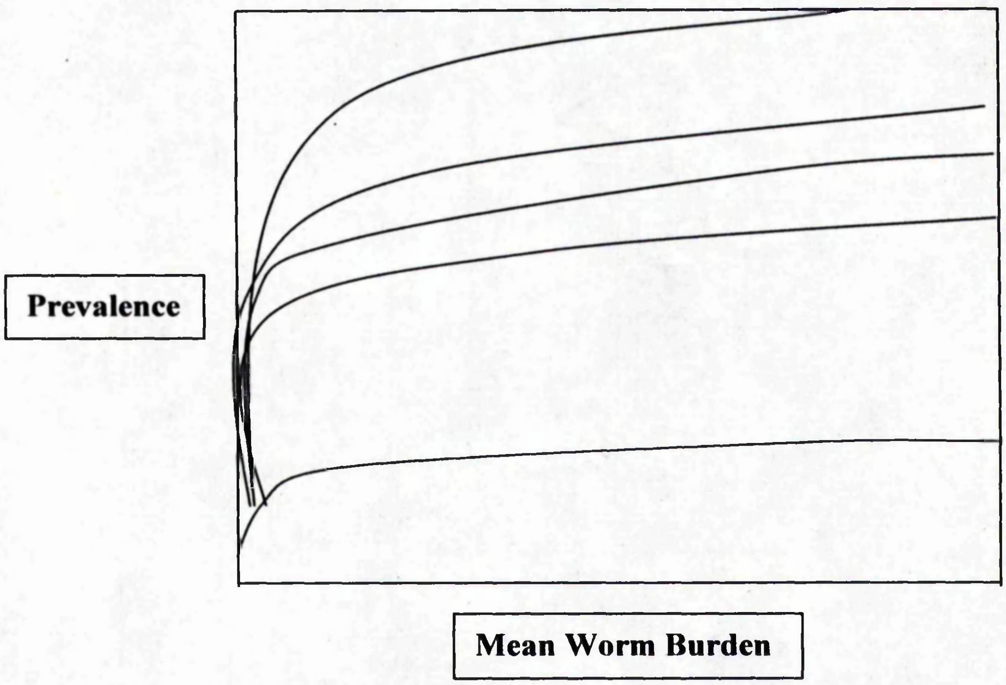

Fig 3.1 Relationship between M (worm burden) and the Prevalence (P) of the Infection

The relationship between **P** and **k** for different **M** values given by the equation 1 is shown in the fig. 3.2. The **k** and **P values** are linearly associated for small **k** values and high or moderated **M** values does not affect this relationship. For **k** values larger than 1, the association is not linear between **P** and **k**. The higher is the **P** value, the lower is the **k** value (V. Jose *et al.*, 1995)

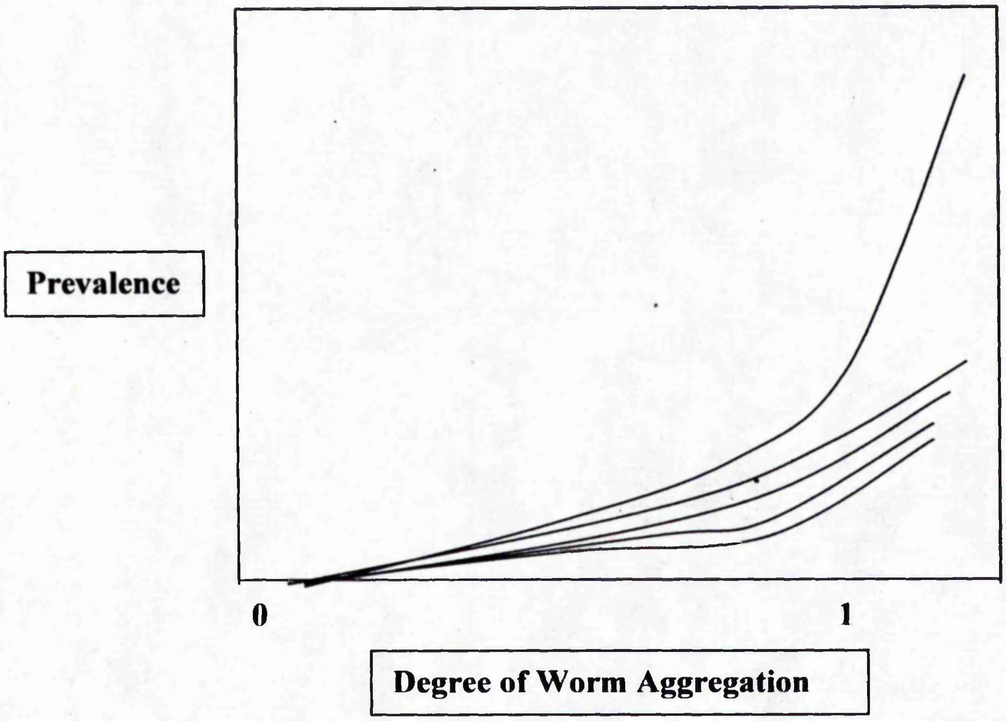

Fig 3.2 Relationship between k (worm-aggregation) and the Prevalence (P) of the Infection.

The relationship between **k** and **M** for different **P** values showed that constant **P** values are accompanied by a negative relationship between **k** and **M** (Fig. 3.3). The higher is the **P** value, the higher is **k** value for smaller **M** values (V Jose *et al.*, 1995) in some region of the plot, but after that region **k** values are independent from the variations of the **M** value (moderate)

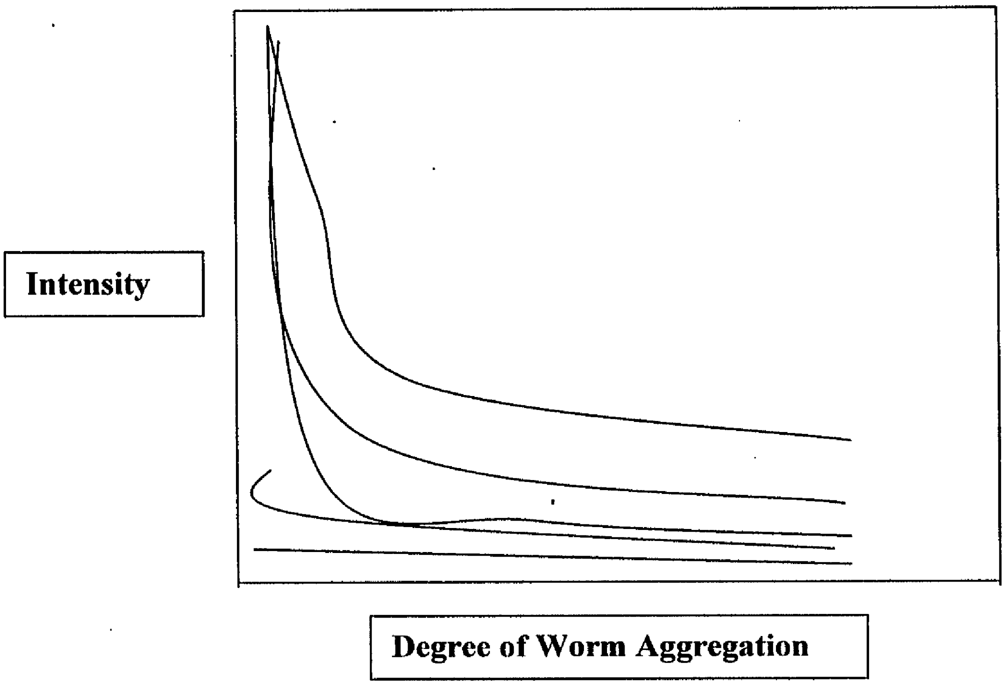

Fig 3.3 Relationship between k (worm-aggregation) and Intensity (M) of the Infection.

3.4 Brief Description of Giardiasis and Amoebiasis in Humans.

Human being is the host of seven species of luminal flagellates including five intestinal and two atrial parasites: 5 non-pathogenic and cosmopolitan species such as *Chilomastix. mesnili*, *Enteromona hominis*, *Retortamonas intestinalis, Trichomonas hominis* and *Trichomonas tenax* of the mouth, and 2 pathogenic- species *G. lamblia* and *Trichomonas vaginalis* (vagina). In addition, six species of amoebas belonging to four genera have been established as parasites of man: 4 non-pathogenic species such as, *Entamoeba coli*, *Dientamoeba fragilis*, *Endolimax nana*, and *Iodamoeba butschlii,* and 2 pathogenic species such as *Entamoeba histolytica* and *Entamoeba gingivalis* (mouth). Certain free-living protozoa (*Naegleria* and *Acanthamoeba* spp) are accidental parasites of human beings (Neva and Brown, 1994).

3.3.1 *Giardia lamblia*

Giardia lamblia is the aetiological agent of giardiasis. The trophozoite is a billaterally symmetrical, pear-shaped flagellate, 12 to 15 μm, with a broad, rounded anterior and tapering posterior extremity. The dorsal surface is convex. An ovoid, concave sucking disk occupies about three fourths of the flat ventral surface. There are two nuclei with large central karyosomes, two axonemes, two blepharoplasts, two parabasal bodies, and four pairs of flagella, although five have been shown (Neva and Brown, 1994). The ellipsoidal cyst, 9 to 12 μ, has a smooth, well-defined wall and contains two to four nuclei and many of the structures of the trophozoite. *G. lamblia* inhabits the duodenum and upper jejunum and possibly the bile ducts and gall-bladder.

Transmission is through food and water contaminated by sewage, flies, food-handlers, and by hand to mouth. Infection is more common in children than in adults, particularly in the school children aged 6-10 years probably associated with a competitive immune-response. Abdominal discomfort, severe diarrhoea accompanied by steatorrhoea, impaired absorption of vitamin A, folate, vitamin B12, carbohydrates, D-xilosa, and weight loss have been experienced from people suffering of giardiasis. (Nash *et al.*, 1987). Between 1970 and 1980, 23% of nearly 1500 tourists to the ex-Soviet Union became ill with giardiasis, experiencing severe diarrhoea (Neva and Brown, 1994). Some outbreaks of giardiasis have probably resulted from wild animals as sources of *G. lamblia* capable of infecting human beings.

Few trophozoites are passed formed stools except in diarrhoeic stools. Cyst-passing individuals are the spreaders of this infection. When cysts are ingested pass unharmed through the gastric juices and undergo excystation in the duodenum. The pre-patent period ranges from 6 to 15 days and the infections can last from 41 days as long as 4 months (Farthing, 1989).

Although *G. lamblia* is found in the stools of many children or adults without symptomatology, it is now clear that the parasite can cause diarrhoea and intestinal malabsorption. These findings have led to hypothesise that the parasite may obtain nourishment from the epithelial cells through its sucking disk. In addition, shortening of villi and inflammatory foci in the crypts and lamina propia, impaired production of disaccharidases, uptake of bile salts, and reduced activity of pancreatic lipase may explain intestinal malabsorption. Occasional parasites have been observed within mucosal cells, but it seems to occur rarely. It is still unknown how *G. lamblia* produce abnormalities in the intestinal mucosa, although mechanical damage and production of toxic factors have been speculated. The possible role of increased bacterial overgrowth in the small bowel in association with giardiasis is still controversial.

Diagnosis is usually made by finding cysts in formed stools, and trophozoites and cysts in diarrhoeic faeces. Small bowel aspirates and biopsies of symptomatic patients have shown a large number of trophozoites in the duodenum and proximal jejunum but they are invasive techniques The distinctive morphology of *G. lamblia* in saline and iodine mounts and in stained smears distinguishes it from other intestinal parasites. Concentration methods increase the chances of detection of cysts (Neva and Brown, 1994; Stanford *et al.*, 1999).

3.4.2 *Entamoeba histolytica.*

Amoebiasis is most prevalent among people living under crowded conditions and lack of sanitary facilities such as mental hospitals and migrant labour camps, and generally poor socio-economic conditions. Male homosexuals constitute a group with infection prevalence as high 25% to 35% because of oral and anal intercourse (Allason, 1986). The main source of infection is the cyst-passing chronic patients or asymptomatic carriers (chronic giardiais). The resistant cysts formed in the lumen of the large intestine pass out in the stools and are immediately infective. Acutely ill patients are not important as source of infection because they pass the non-infective trophozoite. Cysts reach human through water, and vegetables and food contaminated by flies, food-handlers, or by direct transmission.

The lesions produced by *E. histolytica* are primarily intestinal and secondarily extra-intestinal. The intestinal lesions, except for a few cases in the terminal portion of the ileum are confined to the large intestine. The habitat of the trophozoites is the wall and lumen of the colon, mainly in the caecal and sigmoidorectal regions where the colonic flow is slow. The less frequent sites are the ascending colon, rectum, sigmoid, or appendix. When extraintestinal invasion occurs (clinical dysentery or latent infections), the liver is most frequently involved, but nearly every organ in the body can be infected (lungs and brain). The pathogenic activities of *E. histolytica* depend upon the resistance of the host (immunity response, nutritional status, re-infection), virulence and invasiveness of the amoebic strain, conditions in the intestinal tract (alkalinity, physical or chemical injury of the mucosal) and intestinal location (Adams, *et al.*, 1977; Denis and Chedee, 1988; Neva and Brown, 1994; Stanford *et al.*, 1999).

Sub-clinical chronic infections may persist for years with or without exarcebations, but asymptomatic infections are more common mainly in temperate zones. The vague abdominal discomfort, weakness, and neurasthenia have been reported by some patients and the nest stage lack of specificity such as malaise, constipation, mild diarrhoea, and abdominal pain.

Acute intestinal amoebiasis has an incubation period from 1 to 14 weeks and is characterised by severe dysentery with numerous small stools containing blood, mucus, with abdominal pain and fever, dehydration, toxemia, and prostration. However chronic amoebiasis is characterised by recurrent attacks of dysentery with abdominal pain and constipation, weight loss and weakness.

The final diagnosis of amoebiasis depend upon the identification of the parasite in the faeces or tissues. In addition serological tests can be applied but sometimes are not truthful because the presence of antibodies is not concomitant with the presence of *E. histolytica* (Denis and Cheede, 1988). If amoeba is inhabiting the intestinal lumen serological tests may be negative but they are positive at the time tissue invasion is present (Shulman *et al.*, 1999). Presence of trophozoites is a most important requirement than cysts to confirm a final diagnosis because *E. histolytica* cysts are usually misdiagnosed with non-pathogenic amoebas, leucocytes, or other elements observed in stools (Shulman *et al.*, 1999) The clinical diagnosis of intestinal amoebiasis requires differentiation from other types of dysentery and intestinal diseases, and that of hepatic abscess from viral hepatitis and bacterial infections

(Neva and Brown, 1994).

3.4.3 Other Non-pathogenic Intestinal Protozoa.

C. mesnili, E. coli, D fragilis, E. nana, and *I. butschlii* are also intestinal protozoan of human being, but no pathological condition has been associated with them. However, estimations of their prevalence in a survey is valuable to provide evidence about the levels of faecalism, alimentary habits, eduçation, and environmental sanitation prevailing in a population because their transmission is similar with that from other pathogenic intestinal protozoan species (Neva and Brown, 1994).

CHAPTER 4.

IRON DEFICIENCY AND ANAEMIA.

4.1 A Brief Description

Iron deficiency is the state in which the content of iron in the body is lower than normal. The term iron depletion has been applied to the earliest stage of iron deficiency, in which iron storage is decreased or becomes absent but where serum iron concentration, blood haemoglobin and haematocrit levels are normal. Iron deficiency anaemia is the more advanced stage, and is characterised by decreased or absent iron stores, low serum iron concentration, low transferrin saturation, and low haemoglobin concentration or haemotocrit value (Dallman *et al.*, 1993).

4.2 Risk Factors Leading to Iron Deficiency and Anaemia.

It was mentioned in Chapter 3 that trichuriasis and hookworm infections are potential infections which have been found to be associated with an imbalanced iron status. However, other multiple factors can also be found favouring the prevalence of iron deficiency and anaemia in world-wide.

4.2.1 Malabsorption of Iron.

Intestinal malabsorption of iron is an uncommon cause of iron deficiency except after gastrointestinal surgery and malabsorption syndromes. Patients who have gastric resection, rapid gastro-jejunal transit, and partial digestion of food due to the presence of anastomosis, develop iron deficiency anaemia years later (Fairbanks and Beutler, 1977).

4.2.2 Bleeding by Pathological and Infectious Processes.

4.2.2.1 Gastrointestinal Tract.

In adult men and women, iron deficiency may result from chronic bleeding from the gastrointestinal tract from diverse causes. In adults, the commonest causes of gastrointestinal bleeding are peptic ulcer, hiatal hernia, gastritis, and neoplasms (Fairbanks and Beutler, 1977). Malignancy has been found to cause gastrointestinal bleeding in a low percentage (Beveridge *et al.*, 1965). Benign neoplasms (leiomyomas, adenomas), esophageal or gastric varices may lead to iron deficiency anaemia. In hereditary haemorrhagic telangiectasia lesions occur on fingertips, nasal septum, tongue, oral, and pharingeal mucose, palms and soles, and other epithelial and cutaneous surfaces throughout the body. These findings are more commonly observed in the elderly

Medications such as adrenocortical steroids, indomethacin, and phenylbutazone may also cause bleeding by inducing gastric or duodenal ulcers. Use of enteric-coated medications containing potassium chloride has led to serious bleeding from enteric ulceration. Gastritis due to alcohol ingestion may also cause significant blood loss. Chronic blood loss is the cause of anaemia in rheumatoid arthritis (probably as result of salicylate or steroid therapy), ulcerative colitis, and regional enteritis. Haemorrhoids may lead to severe iron deficiency anaemia.

Anaemia which follows subtotal gastrectomy has usually been attributed to reduced absorption of dietary iron (Moeschlin and Schmid, 1964), but also gastrointestinal bleeding may also be a contributory factor (Kimber *et al.*, 1967).

Gastrointestinal bleeding may also be associated in the development of iron deficiency anaemia in infants. There is a substance in fresh cow's milk which unless inactivated by heating, may induce gastrointestinal bleeding in infants, probably on the basis of hypersensitivity or allergy (Wilson and Lahey, 1965; Lahey and Wilson, 1966; Heiner *et al.*, 1964; Woodruff *et al.*, 1972). However, since most infant-feeding formulas derived from cow's milk are subjected to heat treatment at some stage, this factor may rarely play an important role. Intrinsic lesions of the gastrointestinal tract, listed above, may cause bleeding in infants and in older adult males anaemia is rarely caused by dietary iron deficiency alone. It is also well documented that not only deficient dietary iron can cause iron deficiency but also the presence of some dietary factors which influence iron bioavailability (Dokkum, 1992).

4.2.2.5.1 Anaemia by Vitamin Deficiency.

Chronic deficiency of vitamin A produces an anaemia similar to that observed with iron deficiency. Both mean corpuscular volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) are reduced and anisocytosis and poikilocytosis may be present (Dallman *et al.* 1993). Serum iron levels are low, but iron stores in the liver and bone marrow are increased. Administration of iron does not correct significantly the anaemia but simultaneous treatment with vitamin A produces better haematologic response than does administration of vitamin A or iron supplementation alone.

Isolated nutritional deficiencies of members of the vitamin B group (vitamin B6, riboflavin, pantothenic acid, and niacin) with exception of folic acid and vitamin B12 are apparently very uncommon in humans. Deficiency of these vitamins induced experimentally in animals is commonly associated with haematological abnormalities (Dallman, *et al.*, 1993). Vitamin B6 includes pyridoxal, pyridoxine, and pyridoxamine. They act as a coenzyme in the descarboxylation and transamination of amino-acids and in the synthesis of δ- aminolevulinic acid, the porphyrin precursors. Vitamin B6 deficiency has been associated with hypochronic and microcytic anaemia, and patients with sideroblastic anaemia have responded to administration of vitamin B6.

In addition, riboflavin deficiency result in a decrease of the red cell glutathione reductase activity because this enzyme requires flavin-adenine dinucleotide for activation. Reduced activity of these enzyme induced by riboflavin deficiency has no been associated with haemolytic anaemia, but human volunteers have developed red cell aplasia, and erythroid precursors are evident prior to the development of aplasia.

Anaemia in vitamin B12 deficiency is characterised by the large and immature red blood cells indicative of slow DNA synthesis (Dallman, *et al.*, 1993). It is also present in folate deficiency. Vitamin B12 is needed to free folate and without it can not help to manufacture red blood cells. In addition, vitamin B12 requires an intrinsic factor for intestinal absorption. This factor (specific binding proteins) is synthesised in the stomach and its absence impairs intestinal absorption of vitamin B12 and the anaemia resulting is known pernicious anaemia (Dallman, *et al.*, 1993).

Folate deficiency may result not only from an inadequate intake, but also from impaired absorption or an unusual metabolic need for the vitamin. There is an impaired cell division and protein synthesis, and replacement of red blood cells and gastrointestinal tract cells is slow as a consequence of the low activity of tetrahydrofolate and dihydrofolate coenzymes. Anaemia is characterised to be macrocytic and normochromic (Dallman, *et al.*, 1993).

In addition, it is unclear whether vitamin C has a direct role in haemapoiesis, or anaemia observed by deficiency of vitamin C (scurvy) is a result of the interaction of ascorbic with folate and iron metabolism, because attempts to induce anaemia in human volunteers by restriction of vitamin C have been unsuccessful. Anaemia observed in subjects with scurvy may be normocytic, macrocytic or hypochromic, and the marrow may be hypocellular, normocellular or hypercellular. A prompt haematologic response is observed when vitamin C is administered. Vitamin C is required for the maintenance of folate reductase. An impairment of this enzyme results in an inability to form tetrahydrofolic acid the active metabolic form of folate, which results in the appearance of megaloblastic anaemia. In these circumstances vitamin C will produce a haematologic response if folate is present to interact with vitamin C (Dallman, *et al.*, 1993).

Vitamin E (α-tocopherol) is a fat soluble vitamin that appears to serve as an antioxidant in humans. Nutritional deficiency of this vitamin is extremely uncommon because of the widespread occurrence of α-tocopherol in food. Haematologic manifestation of deficiency of vitamin E in humans is limited to the neonatal period and to pathologic states associated with chronic fat malabsorption. Low birth weight infants will develop haemolytic anaemia in 4 to 6 weeks of age when they are fed with a diet rich in polyunsaturated fatty acids and inadequate in vitamin E. Anaemia is associated with morphologic alteration of the erythrocyte and thrombocytosis. Treatment with vitamin E produces an increase in the haemoglobin level, a decrease in the reticulocyte count, normalisation of the red cell life span, and disappearance of the thrombocytosis (Dallman, *et al.*, 1993).

Myelofibrosis and anaemia have been reported with deficiency of vitamin D and both are reversible with vitamin D therapy. Deficiency of this vitamin is very uncommon (Dallman, *et al.*, 1993).

4.2.2.6 Valence State and pH.

Ferrous iron is better absorbed than ferric iron due to the fact that ferric iron precipitates more readily than the ferrous iron which means that ferric compounds are generally less soluble at the pH of the intestinal contents. Both oxidised and reduced forms of iron are soluble at the low pH of the stomach, but at high pH in the duodenum they precipitate unless iron form soluble iron-chelates (Dokkum, 1992).

4.2.2.7 Iron Complexes with Nutritional Inhibitors and Stimulators.

Animal proteins containing amino-acids cysten and histidine which seem to increase the availability of iron to be absorbed as compared with vegetable protein which cause a reduction of soluble iron and/or soluble chelates. Ascorbic acid is the best enhancer of non-haeme iron absorption. Ascorbic acid may reduce ferric iron to ferrous iron increasing its intestinal absorption or ferric iron may form a ferric-ascorbate complex at the low pH in the stomach. This iron-chelate remains stable and soluble at the higher pH in the duodenum facilitating its absorption. Fructuose, lactose, and citric acid have also been associated with enhanced iron availability for absorption through the formation of soluble chelates. Phytate and dietary fiber are claimed to be inhibitors of iron absorption. The possible effect of phytate on the availability of iron is influenced strongly by the presence of other divalent metals which may form insoluble fe-metal-phytate precipitates. Oxalic acid may form insoluble fe-oxalate complexes reducing the availability of iron to be absorbed. Polyphenols are believed to have a strong inhibitory effect on the availability of iron for absorption apparently by formation of insoluble complexes. There is also evidence that intra-metal interaction can influence iron bioavailability, during post-absorption stage (mucosal cell level). It is assumed that an excess of iron intake may stimulate the synthesis of thioneins (low molecules weight), sulfur rich protein in the mucosal cell. Both Zinc and Cooper may bind to thioneins forming metallo-thioneins, but Cooper binds more strongly than Zinc. This result in an impaired transport of Cooper across the cell and reduced levels of Cu in circulation due to the Cu-thionein complex is released into the lumen by desquamation of the cell. Thus hypocupraemia impairs iron absorption inducing anaemia (Dokkum, 1992).

4.2.2.8 Iron Bioavailability and Processing.

Food processing (heat, milling, fermentation, enzymes) may contribute to either enhanced or reduced iron availability. New compounds may be formed, environment may be altered and new external factors may be introduced. Heating food and storing cooked food may easily destroy iron absorption stimulators (ascorbic acid) which decrease iron availability for absorption. Prolonged storage of canned products may increase the iron content of food. Overcooking of meat reduce haeme-iron content. This means that bioavailability of iron in processed or cooked food may not reflect the iron bioavailability of the original or supplemented food iron (Dokkum, 1992)

4.3 Iron Metabolism and its Biochemical Indicators.

The metabolism of iron has been divided into three stages according to the reductions on its biochemical indicators as a consequence of continue iron shortage.

4.3.1 Iron Depletion (Stage 1)

The first biochemical indicator of iron metabolism to be affected by a shortage of iron is the serum ferritin level (Fig. 4.1). Total Iron Binding Capacity (TIBC), the transport molecule for iron (transferrin) is increased. The serum ferritin reflects the size of iron stores except under some exceptions (pathological and/or inflammatory disease, infection processes such as malaria, and severe malnutrition as observed in kwashiorkor, marasmic kwashiorkor, and marasmus) where serum ferritin may be within the normal or increased range. Values of 10 to 20 ng/ml. should be regarded as presumptive (iron depletion) but not diagnostic of iron deficiency, and values of less than 10 ng/ml. are characteristic of iron deficiency anaemia. TIBC is a measure of the total capacity of the transferrin molecule to bind plasma iron and value is expressed in percentage. Thus, if serum ferritin levels are reduced (iron storage), the number of binding sites in the transferrin molecule increase as well (TIBC). Serum iron, transferrin saturation, haemoglobin, free erythrocytic protoporphyrin, and erythrocytic morphological appearance are normal (Fig. 4.1) (Dallman, *et al.*, 1993).

4.3.2 Iron Deficiency (Stage 2)

At this stage high values for TIBC remain but values for serum iron, serum ferritin, and transferrin saturation are depleted. Free erythrocytic protoporphyrin (FEP) is also increased as a consequence of reduced synthesis of haeme (the iron-holding part of the haemoglobin) due to lack of iron, although the same can result from conditions such as lead poisoning, and sideroblastic anaemias and/or inflammatory or malignant processes. Serum ferritin and serum iron concentrations are usually low. The transferrin is normally present to bind 250 to 450mg iron/100 ml of serum. Since normal serum iron concentration is about 100 mg per 100ml, transferrin molecule is about one third saturated with iron (one third of the binding sites are occupied). At this stage transferrin is saturated with less one third with iron, but haemoglobin and erythrocytic morphological features remain normal (Fig. 4.1)(Dallman, *et al.*, 1993).

4.3.3 Iron Deficiency Anaemia (Stage 3)

At this stage degree of iron deficiency is serious and all indicators of iron metabolism are altered. TIBC and free erythrocytic protoporphyrin continue remaining high, and the rest of indicators are depleted (serum ferritin, serum iron, transferrin saturation). Haemoglobin concentration decreases and microcytosis and hypochromia appear. The changes in erythrocyte volume and haemoglobin content are expressed quantitatively in the erythrocyte corpuscular indices (Fig. 4.1)(Dallman, *et al.*, 1993).

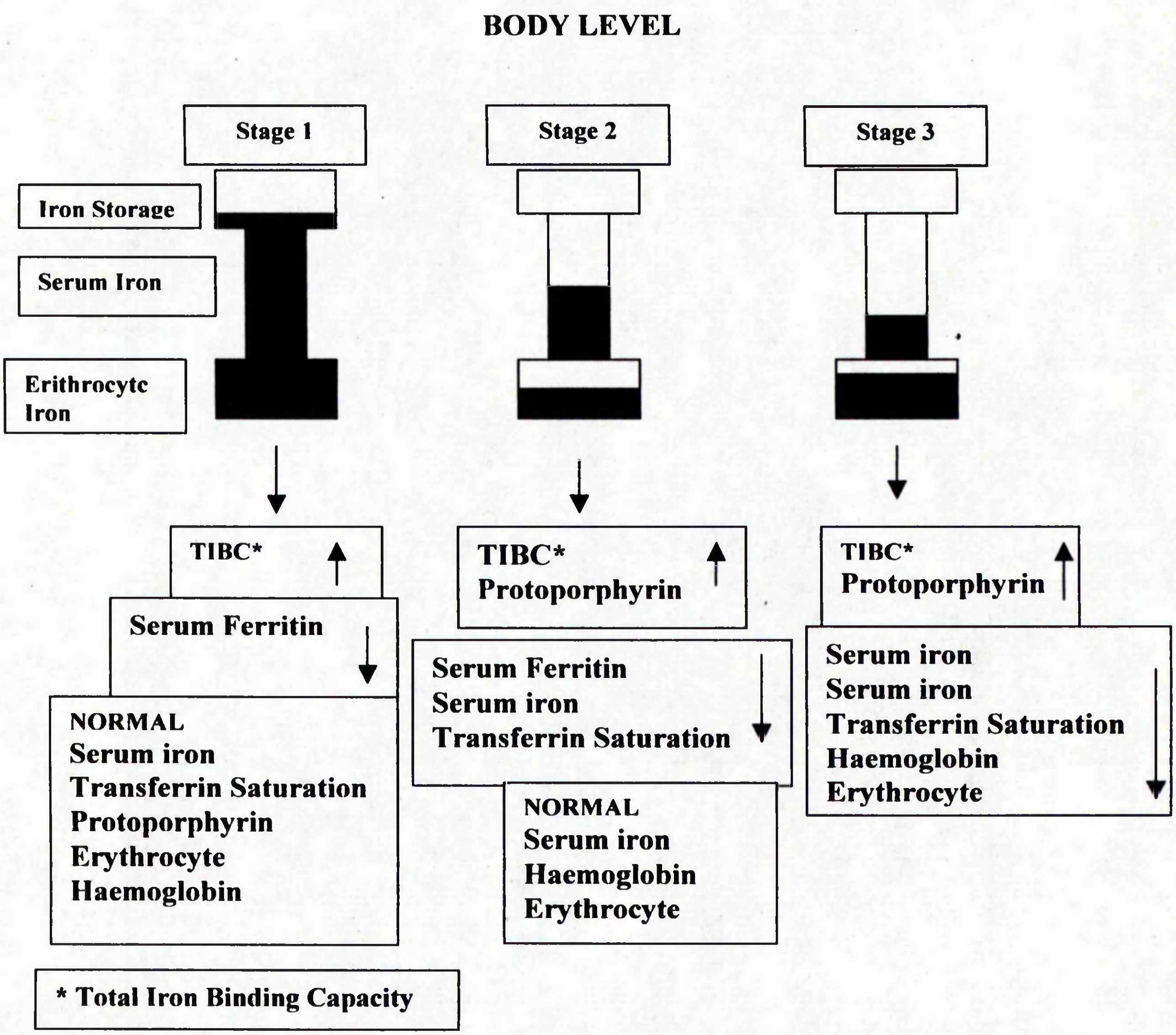

Fig 4.1 Biochemical Indicators of Iron Status

CHAPTER 5

MATERIALS AND METHODS

5.1 Aims of the Project

5.1.1 General Objective:

To evaluate the current prevalence of intestinal parasitic infections in school children from Mexican sub-urban and rural communities after seven years of the national administration of albendazole.

5.1.2 Particular Objectives:

1. To evaluate the prevalence of intestinal parasitic infections in school children from Northern sub-urban and rural communities in school children (State of Sinaloa) communities after seven years of the national administration of albendazole.

2. To evaluate the prevalence of intestinal parasitic infections in school children from Southern sub-urban and rural communities in school children (State of Oaxaca) communities after seven years of the national administration of albendazole.

3. To determine the levels of intensity of helminth infections in school children from sub-urban and rural communities of the States of Sinaloa and Oaxaca after seven years of the national administration of albendazole.

4. To detect and identify the risk factors associated with the transmission of intestinal parasitic infections in school age children from the States of Sinaloa and Oaxaca after seven years of the national administration of albendazole.

5. To contribute in the re-organisation of appropriate strategies by the health authorities removing every factor which is interfering in the development of the

components of the national deworming campaign in school children from rural communities of the States of Sinaloa and Oaxaca.

5.1.3 Hypothesis

1. There is a high prevalence of intestinal parasitic infections in school children age 6 to 10 years from rural communities of the States of Sinaloa and Oaxaca.

2. The prevalence of intestinal protozoan infections is higher in school children aged 6-10 years from rural communities of Sinaloa than those in school children aged 6-10 years from rural communities of Oaxaca.

3. The prevalence of intestinal hekminth infections is higher in school children aged 6-10 years from rural communities of Oaxaca than those in school children aged 6-10 years from rural communities of Sinaloa.

4. There is a reduced prevalence of ascariasis in the school children aged 6-10 years from rural communities of the States of Sinaloa and Oaxaca.

5. Trichuriasis is still a persistent helminth infection in school age children aged 6-10 years from rural communities of the States of Sinaloa and Oaxaca.

6. The demographic, socio-economic, health and nutritional conditions of the school children aged 6-10 years old from sub-urban and rural communities of the States of Sinaloa and Oaxaca are influencing factors in the prevalence and levels of intensity of intestinal parasitic infections.

5.2 Participant Members and Institutions.

The present project was carried out by mutual agreement of collaboration among the University of Glasgow, Centre of Research and Development (CIAD A.C), Autonomous University of Sinaloa and the Technological Institute of Oaxaca and represented by their research members as follows:

David Crompton, Professor and Head of the WHO Collaborating Centre for Soil-Transmitted Helminthiases at the University of Glasgow. Scotland U.K. Expertise in epidemiological research on parasitic infection, particularly helminth infections. He provided academic scientific supervision on the parasitological component of the project, and financial support.

Stephen Phillips, Professor and Head of the Division of Infection and Immunity of the University of Glasgow, Glasgow Scotland U.K. Expertise in epidemiological research on Malaria. He provided academic scientific supervision on the parasitological component of the project, working area, and financial support.

Mauro Valencia, Current scientific researcher and Head of the Department of Human Nutrition of the Centre of Research in Food and Development (CIAD A.C) located in the city of Hermosillo, Sonora in the Northwest of Mexico. Expertise in epidemiological work in human nutritional studies. He provided scientific academic supervision on the nutritional status component of the project, working area, equipment infrastructure, and financial support. Also, laboratory installations were provided by CIAD.A.C unity of Culiacan Sinaloa.

Sylvia P. Diaz Camacho, scientific researcher and Head of the Department of Public Health in the Faculty of the Biological Sciences of the University of Sinaloa. She provided scientific academic supervision on the parasitological component of the project, working area, technical, and equipment support.

Aracely Triana T. scientific researcher and member of the academic staff of the Division of Chemical Engineering and Biochemical in the Technological Institute of Oaxaca. She provided academic supervision on nutritional and dietary studies of the project, working area, and technical and equipment support.

Luis Quihui Cota, member of the technical staff of the Department of Human Nutrition in the Centre of Research in Food and Development A.C. Currently a PhD student in the Division of Infection and Immunity of the University of Glasgow Scotland U.K. He was the responsible to establish formal and mutual agreement of collaboration among the participant institutions, to obtain financial support from the appropriate Mexican funding organisation, to set up suitable laboratory conditions in the study sites, to recruit the physician assistant, technical and field workers, and to carry out the required training of personnel. He was also in charge of the organisation of recognising studies (to get familiar with local people, customs, cooperation, local diet, etc), the quality and maintenance of the used equipment and methodologies, sample collection, sample preservation, analysis (faecal microscopic examinations, fresh blood smears, haematic and iron biochemical indicators determination in blood and serum samples, and indoxil potassium-sulphate determination in urine samples), disposing of the biological material, total supervision of the field-work, collection and analysis of data.

5.3 Ethical Considerations

The proposal for the present project was submitted and approved by the Ethical Committees of the Centre of Research and Food and Development A.C., the Faculty of the Biological Sciences and Department of the Public Health of the Autonomous University of Sinaloa, the Regional Head of the Public Health Ministry of the State of Sinaloa, and the University of Glasgow. In addition, the responsible investigator ensured that a written consent to the study was given and signed by the parents of the children, explaining the nature and reason of the study (appendix 4). A form with the written results and signed by the responsible laboratory worker and the principal investigator was personally given to the parents of the children. Also an explanation about their significance was offered (appendix 4). All children who refused or felt uncomfortable about taking part in the study conditions were excluded even when their parents wished their participation. Finally, at the end of the examination of each child, where the results indicated that medical attention was required, this was provided immediately.

5.4 Study Sites and Selection Conditions.

5.4.1 The State of Sinaloa

The State of Sinaloa is one of 32 Mexican States and is located in the Northwest of Mexico and bordering on the Northwest with Sonora, Northeast with Chihuahua and Durango, and Southeast with Nayarit (appendix 1 and 3). It occupies 2.9% of the Mexican land mass. The sinaloense population originates from a combination of indigenous groups (pimas, seris, apaches, coras, tarascos, yaquis, mayos, tarahumaras and acaxes as original inhabitants) and immigrant European and Asiatic population (Spanish, English, Chinese and Japanese) (El Estado de Sinaloa, 1997)

The State of Sinaloa has 18 municipalities, and the municipality of Culiacan is located in the middle region of the State (appendix 3). The city of Culiacan was founded on September 29th in 1531 by the Spanish Nuno Beltran de Guzman. In 1823 the antique Villa de San Miguel de Culiacan was considered a city and officially named the capital of the State of Sinaloa.

The demographic census in 1995 reported a total population for the State of Sinaloa of 2.4 million inhabitants, and a total population of 696,262 inhabitants for the municipality of Culiacan. 505,518 inhabitants were established in urban and semi-urban settings of the city of Culiacan and 190,744 inhabitants were distributed in different rural communities within the municipality. Its population live by agriculture, trade, livestock, fishing and industrial activities (Anuario Estadistico del Estado de Sinaloa, 1996)

The municipality of Culiacan has a tropical climate, a mean annual temperature about 25.5°C., mean annual lowest temperature about 24.5°C, mean summer-season temperature about 38°C. (June-October), and mean annual humidity of 60-80% (Anuario Estadistico del Estado de Sinaloa, 1996). The environmental conditions prevailing in this area favour the transmission of parasitic infections. An epidemiological study carried out in the State of Sinaloa and involving 958 subjects (Diaz *et al.*, 1994), reported a prevalence of 25-30% of trichuriasis, 20-25% of ascariasis, 40% of hymenolepiasis, 45% of giardiasis, and hookworm infections and strongyloidiasis less than 5% in a sub-urban population aged 0-14 years. The parasitic prevalence observed in the population ≥15 years was lower. The same authors reported also a mean prevalence of 20-25% for ascariasis, 20-25% for trichuriasis, 40-45% for hymenolepiasis, and hookworm infection and strongyloidiasis lower than 10% in a rural population aged 0-14 years. The prevalence of intestinal parasitic infections observed in the population ≥15 years was lower.

5.4.1.2 Selected Rural Communities within the Municipality of Culiacan.

Before starting the pilot study official meetings with personnel from Health Services of different Mexican Health Organisms (Institute Mexicano del Seguro Social, Instituto de Servicios de Seguridad Social de Trabajadores del Estado de Sinaloa, Hospital Civil de Culiacan and Secretaria de Educacion Publica) were carried out in order to explain the objectives of the project.

The Ministry of Public Health provided complete information about the National Deworming Campaign established in Mexico in October 1993 This information included dose, route, and times of albendazole administration per year, target population, criterion of selection of municipalities in the State of Sinaloa, health institutions, operational plan of activities, responsible personnel in charge of the health workers in the field work, and dates of albendazole administration.

An agreement of mutual support with the personnel in charge of the deworming campaign was established. Negotiated terms included no action taken by the health authorities in school children of the selected rural communities but avoiding the treatment was delayed not longer 3 weeks. It was ensured all children (recruited or not) received the appropriate treatment at the end of the work.

5.4.1.3 Selection of the Study Sites (appendix 3).

The information required to select the study sites was as follows:

a). Presence of intestinal parasitic infections from records of the Mexican Health Ministry from 1992-1997 b). Presence of intestinal parasitic infections from records of the Public Health Department of the University of Sinaloa from 1987-1996.

c). Rural communities which were not visited in the last year and/or six months by the control programme.

d). Evidence of *Ascaris* worm expulsion in children given by the parents in the last year (personal interview).

e). Lack of Health Services (Health Ministry of Sinaloa).

f). Study sites particularly closer to the sample processing site (Anuario Estadístico del Estado de Sinaloa, 1996).

g). Lack of basic public services and housing conditions (Anuario Estadístico del Estado de Sinaloa, 1996).

h). High school population (Secretary of Public Education, 1997).

5.4.1.4 Brief Description of the Selected Study Sites (appendix 3)

a). **Imala** is a rural community located 25 Km Northeast of the city of Culiacan. Imala has total population of 321 (Anuario Estadístico del Estado de Sinaloa, 1996). According to the Ministry of Public Education (Secretaría de Educación Pública, 1998), its primary school "Escuadron 201" had a roll of 45 children aged 6-10 years from 1997 to 1998.

b). **Las Puentes** is a rural community 45 Km Southwest of the city of Culiacan. It has a total population of 928 and 87 children aged 6-10 years were enrolled in its primary school "Adolfo Lopez Mateos" from 1997 to 1998. (Secretaría de Educación Pública, 1998).

c). **El Treinta** is a rural community located 30 Km. South of the city of Culiacan. It has a total population of 180, and 40 children aged 6-10 years were enrolled in its primary school from 1997-1998. (Secretaría de Educación Pública, 1998).

d). **Pueblo Nuevo** is another rural community located 33 Km South of the city of Culiacan. It has a total population of 565, and 61 children aged 6-10 years were enrolled in its primary school "Adolfo Lopez Mateos" from 1997 to 1998. (Secretaría de Educación Pública, 1998)

e). **Doroteo Arango** is a rural community located 55 Km Southeast of the city of Culiacan. It has total population of 167 and 30 children aged 6-10 years were enrolled in its primary school from 1997 to 1998. (Secretaría de Educación Pública, 1998).

f). **El Paraiso** is a rural community located 58Km Southeast of the city of Culiacan. It has a total population of 158 and 30 children aged 6-10 years were enrolled in its primary school from 1997 to 1998. (Secretaría de Educación Pública, 1998).

g). **El Higueral** is a rural community located 60 Km Southeast of the city of Culiacan. It has a total population of 1721 and 215 children aged 6-10 years were enrolled in its primary school "Lazaro Cardenas" Ford-38 from 1997 to 1998. (Secretaría de Educación Pública, 1999).

5.4.2. The State of Oaxaca.

The State of Oaxaca is located in the Southwest of Mexico (appendix 3). It has borders with the States of Puebla and Veracruz in the North, Pacific Ocean in the South, Chiapas in the East, and Guerrero in the West (Ysunza, 1996).

The capital of the State of Oaxaca is Oaxaca. In the demographic census in 1995 the State of Oaxaca has a total population of 3,228,895 inhabitants (12.45% infant and pre-school children 0-4 years, 13.5% school children 5-9 years, 11.1% adolescent 10-14 years, and 63% equal to or older than 15 years) INEGI, 1995). In addition Oaxaca is the Mexican entity with the largest number of ethnic groups (Ysunza, 1996) with well defined cultural characteristics and traditions.

The State of Oaxaca covers an area of 93,367 Km^2, and is divided into 570 municipalities. Also, with 3 mountainous range (Sierra Madre Oriental, Sierra Madre

Occidental, y Sierra Madre del Sur) the State of Oaxaca has the roughest terrain in the country. According to geographical and ecological differences the state of Oaxaca has been divided into 7 regions: Central Valley, Itsmo, Alto Papaloapan and Tuxtepec, Mountainous range, Canada, and Mixteca (Ysunza, 1996).

5.4.2.1 Public Health in the State of Oaxaca.

The State of Oaxaca is one of the Mexican States with the most difficult of public health problems. It has the highest rates of mortality and morbidity associated with respiratory infections and diarrhoea (with no defined aetiological causes), intestinal parasitic infections and malnutrition (Thesis Martinez, 1992). Because of the rough geographical terrain the state of Oaxaca has numerous groups of small populations with deficient health services. It has been reported that 500,000 people from Oaxaca do not have access to public or private health (Ysunza *et al.*, 1996).

Records from the Public Health Ministry (Secretaría de Salud del Estado de Oaxaca, 2000) have shown that intestinal parasitic infections were the first and second cause of mortality in pre-school and school children respectively. Also, they were the most important cause of morbidity in the general population. A mean prevalence of 18%, 9.6%, and 8.8% for ascariasis, hookworms, and trichuriasis respectively, was estimated from limited epidemiological surveys carried out in the State of Oaxaca during 1955-1965 and 1976-1985 (Quihui, 1995, unpublished information). In addition, a technical report from the Mexican control programme in 1993 (Epidemiología de las Helmintiasis Intestinales en México. Bases para su Control, 1993) reported a prevalence of 24%, 17.3%, and 9.6% for ascariasis, trichuriasis, and hookworm infection respectively in the Region of Itsmo. Another study carried out in a rural population with access to the Mexican Institute of Social Security reported a prevalence of 64%, 23.7% and 8.3% for amoebiasis, giardiasis, and ascariasis respectively (Martínez, 1992). Although these estimates were generated by limited and sometimes inaccurate information, it is not in doubt that intestinal parasitic infections represent a significant general problem of public health in the State of Oaxaca.

Also, evidence presented by the XI Demographic Census of Population and Housing, 1990 (Epidemiología de las Helmintiasis Intestinales en México. Bases para su Control, 1993), showed that the State of Oaxaca presented the largest Mexican percentage of illiterates in the population (27.5%) and the poorest housing conditions (51.4% of houses with bare earth floor, 41.9% without clean water supplies, and 70% without sewage) (appendix 2). In addition, the indigenous population represents 40% of the total Oaxaqueña population with highest levels of poverty (Ysunza, *et al.*, 1996). The rate of malnutrition in pre-school children is higher than that for the Mexican mean average (68-75%) (Ysunza, *et al.*, 1996). According to the nutritional indicators of height/age, and weight/age, a high percentage of malnutrition in school age children was reported by the last two Mexican Food Surveys in 1989 and 1996 in the South of Mexico including Oaxaca. Wyatt and Triana, (200), reported that 80% of pre-school children from a rural population of low socio-economic level presented malnutrition by the indicator of weight/height (Wyatt and Triana, 2000).

Low socio-economic conditions, lack of basic infrastructure (sanitation and water supplies), poor public health and education services, and malnutrition, are risk factors for the flourishing of intestinal parasitic infections, and possible predictors of the degree of the negative effects on the general health of the population.

5.4.2.2 Selected Study Sites in the State of Oaxaca. (appendix 3).

Before starting the study, official meetings with personnel from Health Services of different Mexican Health Organisations (Instituto Mexicano del Seguro Social, Instituto de Servicios de Seguridad Social de Trabajadores del Estado de Oaxaca, Hospital Civil de Oaxaca, and Secretaria de Educacion Publica) were carried out in order to explain the objectives of the project.

Complete information was obtained (Ministry of Public Health of the State of Oaxaca, 1999) about the activities of the National Deworming Campaign in the state of Oaxaca (dose, route, and times of albendazole administration each year, target population, criterion of selection, health institutions involved, operational plan of activities, personnel in charge of the health groups in the field work, dates of albendazole administration, and municipalities not included for albendazole administration in 1999.

A thorough search for information about current and past intestinal parasitic infections in the state of Oaxaca was carried out. Places and people visited included the Secretary of Public Health, Mexican Institute of Social Security, Civil Hospital, Pediatric Hospital, Science Research Institutes (Centro de Capacitacion Integral para Promotores Comunitarios, CECIPROC), National Institute for Indigenas), Institutes for Social and Health Assistance (Helping Hands centre, Centro Dioscesano de Pastorales Indigenas, Grupo Cuchiteco), and Library of the University of Oaxaca.

5.4.2.3 Selection of the Study Sites.

The information required to select the study sites was as follows:

a). Communities not included by the control programme in 1999.

b). Presence of intestinal parasitic infections from records of the Mexican Health Ministry from 1995-1999 (Secretaría de Salud, 2000)

c). Presence of intestinal parasitic infections in the State of Oaxaca (published and unpublished) in 1992, 1993, 1998, and 1999. (Martínez, 1992; Navarrete, *et al.*, 1993; Soriano, 1998; LESPO, 2000)

d). Evidence of *Ascaris* worm expulsion in children during 1998-1999 given by the parents (1998-1999) (personal interview).

e). Lack of Health Services (INEGI, 1995).

f). Study sites particularly closer to the sample processing site (INEGI, 1995).

g). Lack of basic public services and housing conditions (INEGI, 1995).

h). High school population (Secretaría de Educación Pública, 1999).

5.4.2.4 Brief Description of the Study Sites (appendix 3).

a). The **city of Oaxaca** is 1600 meters above sea level, and has a warm and humid climate, an annual mean temperature of 17°C, the highest temperature is about 32 °C (March-June), and annual mean rainfall of 751 mm^3 (INEGI, 1995). **Lomas de San Jacinto** is a shanty town located in the Northwest of the city of Oaxaca. Its first houses appeared in 1990, and still lack the basic services (electric energy, clean water supply, and sanitation) (Reyes, 1999). Its primary school "Lazaro Cardenas" had a total of 285 children aged 6-10 years enrolled during 1998-1999 (Secretaría de Educación Pública, 1999).

b). **Rancho La Era** is a small rural community of the municipality of Etla in the Central Region. It is located 20 Km Northwest of the City of Oaxaca. La Era has approximately 300 inhabitants (INEGI, 1995). Climatic conditions are similar to those for the city of Oaxaca (INNEGI, 1995). It is borders Guadalupe Hidalgo and Santiago de Etla in the North, San Jose and San Andres Ixtlahuaca in the South, San Pablo Etla in East, and San Felipe Tejalapa in the West (INEGI, 1995). Its primary school "Juan Jacobo Rousseau" had a population of 49 children aged 6-10 years enrolled during 1998-1999 (Secretaría de Educación Pública, 1999).

c). **La Lobera de Ejutla**.is a rural community of the Municipality of Ejutla de Crespo and located 60 Km. Southwest of the city of Oaxaca It is borders Ocotlan Morelos in the North, Zimatlan de Alvarez in the East, Sola de Vega in the West, and Miahuatlan in the Southwest. It has a total population of 386 (52% male and 47.9% female). 31% of the population is aged 6-14 years (INEGI, 1995). Its primary school "Miguel Hidalgo" had 49 children aged 6-10 years enrolled during 1998-1999 (Secretaría de Educación Pública, 1999).

d). **Pluma Hidalgo** is a rural community located 203 Km. Southwest of the city of Oaxaca. It has a humid and warm climate, a mean annual temperature of 20°C. and a mean annual rainfall of 2692.3 mm^3. It is borders the municipalities of San Pedro El

Alto in the North, Candelaria Loxicha in the West, San Pedro Pochutla in the South, San Mateo Pinas in the East, and Santa Maria Huatulco in the Southwest (INEGI, 1995). It has a total population of 477 (49% males and 50.9% females). 28% of the population are children aged 6-14 years (INEGI, 1995). Its primary school "Evolucion Social" had 102 children aged 6-10 years enrolled during 1998-1999 (Secretaría de Educación Pública, 1999).

e). **Santa Maria de Magdalena** is a rural community of the municipality of Pluma Hidalgo and located 215 Km. Southwest of the city of Oaxaca. It has a warm and humid climate, a mean annual temperature of 28°C., and a mean annual rainfall of 2692.3 mm^3. It has a total population of 369 (49.32% male and 50.6 female), and 24.6% are children aged 6-14 years (INEGI, 1995). Its primary school "Vicente Guerrero" had 55 children aged 6-10 years enrolled during 1998-1999 (Secretaría de Educación Pública, 1999)

5.5 Procedure for Collection and Analysis of Biological Samples

5.5.1 Stool Collection.

A current register of children aged 6-10 years was provided by the head teacher of the primary schools visited. Meetings with parents and mothers were arranged to explain the reason for the project.

Children were asked to collect three serial faecal samples. To achieve this a written list with the names of the children was prepared, and clean plastic containers (pressure cap, vol. 10 ml), properly labelled (name of the child, primary class, class list number, number of sample, and code number of the community) were delivered to the child, (a container for each collected sample). It was ensured that stool samples were collected from three different days. Stool samples contaminated with water, urine, or other material were rejected. Ice-boxes were used to keep the stools and protect the integrity of the parasites in the stools during the periods of sampling and transportation.

Most of the samples were immediately analysed, but when it was not possible, the remaining samples were keep at 4-7°C until analysed (within 24 hours).

5.5.1.1 Coproparasitological Methodology

a). Ritchie formalin-ether (qualitative concentration method).

1 g of faeces was emulsified in normal saline and filtered. The filtered solution was centrifuged 1500 g for one minute. This step was repeated until the supernatant was clear. 10 ml. of 10% formalin were added to re-suspend the sediment and it was allowed to stand for 10 minutes. Then 5 ml of ether was added, shaken vigorously (10 seconds), and centrifuged 1500 g for five minutes. The supernatant fluid was discarded and the sediment examined (Markell and Voge, 1976). Lugol solution (Iodo 5g, Potassium Iodine 10 g., and distilled water 100 ml.) was used to stain the parasites. 10x and 40x objectives were used to detect and identify the parasites present in the sediment.

b). Faust technique (qualitative concentration method).

A faecal suspension (1ml) was prepared in approximately 10 to 15 times its volume of tap water. This suspension was centrifuged for 5 min at approximately 1500 g and the supernatant was discarded. This step was repeated three times to remove any debris material which could interfere during the microscopic observation. 3 ml. of zinc-sulphate solution (Zinc Sulphate 350 g., distilled water 1000 ml., at density 1.180) was used to re-suspend the sediment; this suspension was centrifuged again at 1500 g for another five minutes. After centrifugation, material floating on the surface was removed with a wire loop and placed onto a slide with a drop of iodine solution and covered with a coverslip. Microscopic observation was carried out using the 10x and 40x objectives (Markell and Voge, 1976).

c). The Kato Katz Thick Smear Technique (qualitative and quantitative)

Using a template with a calibrated hole, 50 mg of sifted faeces was placed onto a slide. The template was removed and the whole faecal sample remained on the slide. A square piece of cellophane (25x30mm) previously plunged (1 week) in a glycerol-malachite green solution (malachite green 3%, glycerine 100 ml., distilled water 100 ml.) was used to cover the sample. A low pressure was exerted in order that the faecal sample was spread on the slide. After 30 minutes at 37^0C the prepared sample was observed under the microscope (40x). Eggs onto the total surface of the slide were counted to obtain the number of eggs per gram of faeces (Markell and Voge, 1976).

5.5.1.2 Reliability of Parasite Detection and Identification

The Faust technique has good efficiency in qualitative detection of protozoan cysts (*G. lamblia, E. histolytica E. coli, Endolimax nana, I. butschlii,* and *C. mesnili*), eggs of some helminth species (hookworms, *A. lumbricoides, T. trichiura, H. nana*), some larvae of helminths (hookworms and *Strongyloides*). However, it is not satisfactory for detection of the eggs of *Schistosoma*, operculate eggs of *Fasciola*, or *Diphyllobothrium*. These parasites have not been reported in the selected study sites. The flotation technique has been considered superior to sedimentation (Ritchie technique) for concentrating cysts and eggs (Neva and Brown, 1994).

The Kato Katz thick smear technique is known to be very reliable and highly sensitive for quantitative estimation of *T. trichiura* and *A. lumbricoides*. There is a probability greater than 99% that a single thick smear containing 50 mg. of faeces can detect an infection with 100 eggs per gram of faeces (Martin and Beaver, 1968). The multiplication factor of 20 was used to convert egg to counts per gram of faeces. However, although the examination of 50 mg of stool sample can detect all infections of 50 or more eggs per gram of faeces, very light infections are likely to be missed (Stephenson and Holland, 1987)

5.5.2 Assessment of the Iron Status.

5.5.2.1 Procedure for Blood Collection

The study site selected for the assessment of the possible association between trichuriasis and biochemical indicators of iron status in school children was the rural community of El Higueral in the municipality of Culiacan, Sinaloa, Mexico.

All the non-infected and *T. trichuria* infected school children recruited were subjected to measurements of biochemical indicators of iron status. Before blood collection formal meetings were held with the head teachers and parents, who had their children participated in this part of the project. They were usually arranged for the day before the blood collection day. A full explanation about the procedures for blood collection was offered (child in healthy and fasting status, at the time of blood collection, collection of 3-hour-urine sample, confirmation of date, time, and blood collection took place, signed consent form (appendix 4), and preparation of a written list with the children's names.

Children and parents arrived at 7: 30 am in the primary school "Lazaro Cardenas" Ford 38. A classroom was selected as a blood collection site. The child was placed in comfortable conditions (sitting down and arm on a flat surface). Using the vacutainer system (2 tubes previously labelled with child's name, primary class, and code number), blood was drawn (10 ml) from the cubital vein (no anaesthetic), applying a tourniquet *in situ* for less than one minute to avoid local haemoconcentration (Baker and Ramachandran, 1984).

3 ml. of blood in 1 ml. of anticoagulant heparin were collected for haemoglobin, haematocrit, red blood cell count, and red blood cell indices and these were promptly analysed. 7 ml. were allowed to clot and centrifuged to separate the serum fraction which was stored at -20°C for further iron, ferritin, transferrin saturation, and total iron binding capacity determinations. Alcohol and cotton was used to disinfect the venepuncture area.

5.5.2.2 Haematological Determinations

a). Haemoglobin.

Iron is the essential component of the haemoglobin (Hb) molecule which is the oxygen-carrying pigment of the red cells and is composed of a globin (protein) and 4 molecules of haeme. Determination of Hb was carried out by expelling 0.02 ml. of the blood sample into Drabkin's solution (5 ml.). The ferrous ions (Fe-II) of the haemoglobin are oxidised to the ferric state (Fe-III) by potassium ferric-cyanide (potassium cyanide 0.050 g., potassium ferricyanide 0.200g, and 0.140 g. dihydrogen potassium phosphate, and distilled water 1000 ml.) to form haemiglobin (methemoglobin or ferrihaemoglobin). In turn, haemiglobin reacts with the cyanide ions (CN^-) provided by potassium cyanide to form cyanmethaemiglobin. This technique can measure all forms of haemoglobin (oxyhaemoglobin, methaemoglobin, carboxihaemoglobin) with the exception of sulfhaemoglobin (Gibson, 1990)

The absorbance of cyanmethaemiglobin at 540 nm (Coleman spectrophotometer UV-VIS, USA was used) is proportional to its concentration (Law of Lambert-Beer). The haemoglobin concentration of the blood sample was calculated comparing the measured absorbance of the sample against a standard curve. The standard curve was prepared and re-checked every month using certified commercially available (Merk) cyanmethaemiglobin standards. The standards had to be prepared every 10 days when storage conditions were not appropriate (fridge in bad conditions or presence of contaminant particles in the solution) (manufacturer recommendations), and the equivalent haemoglobin content was obtained by multiplying the stated cyanmethaemiglobin content (800mg/Liter) with the dilution factor used in processing the blood sample (1:251). Dilutions (200,100,50,25,0) were prepared and absorbance values of standards were plotted (ordinary graph paper) on the Y axis against the haemoglobin concentration in g/L on the X axis (Baker and Ramachandran, 1984).

b). Haematocrit

Haematocrit is a measure of the ratio of the volume of the red cells to the volume of the whole blood sample. The micromethod was used to determine the haematocrit. Fifty µl of a thoroughly mixed blood sample were drawn into heparinized capillary tube sealed with clay (5-7 mm in depth) and centrifuged (10,000 g) 5 minutes in a special centrifuge holding up to 24 hours capillary samples in numbered positions. Using a special reading device (haematocrimeter), the bottom of the packed cell column was lined up to the 0 mark, and reading was taken. The grayish-red layer of leukocytes at the interface between red cells and plasma was excluded from the measurement. Haematocrit was calculated from the ratio of the length of red cell column (mm) to the length of the whole sample (red cells and plasma) and result was expressed as a percentage (Dacie and Lewis, 1991)

c). Red Blood Cells Counts

Twenty µl of blood were diluted (1:200) in 4 ml. of formal-citrate solution (10 ml of 40% formalin made up to 1 litre with 32 g/l trisodium citrate), into a micropipette (Dacie and Lewis, 1991). The diluted blood was mixed in mechanical mixer (2 min). A Neubauer chamber with the cover-glass in position (special thick and flat glass) was filled with the suspension in one action (no fluid into the surrounding channel). The chamber was left not more than 2 minutes (to avoid drying) for the cells to settle. The number of blood red cells per Liter was calculated by No. Cells counted/Volume counted (µl) x dilution (200) x 10^6.

d). Red Blood Cell Indices.

The red cell indices are derived from the values of haemoglobin, haematocrit, and red cell counts. They provide information about the different types of anaemia. Mean Cell Volume (MCV) is a measure of the average size of the red blood cells and it was calculated from the ratio of haematocrit (volume fraction)/ red blood cells counts/L, and result was expressed in fL (Dacie and Lewis, 1991).

The mean cell haemoglobin concentration (MCHC) is the mean concentration of haemoglobin in the red blood cells and was calculated from the ratio of haemoglobin (g/L)/ haematocrit (volume fraction) and expressed in g/L (Dacie and Lewis, 1991).

The mean cell haemoglobin (MCH) refers to the mean haemoglobin content of the individual red blood cells. It is derived from the ratio of haemoglobin (g/L)/red blood cells counts (10^{12}/L) and expressed in pg (Dacie and Lewis, 1991).

5.5.2.2.1 Haematological Cut-Off Values For Children Aged 6-10 years

The haematological cut-off points in individuals residing at sea level derived from the World Health Organisation, (1972), NHANES II (Pilch and Senti, 1984), Gibson, 1990, and International Nutritional Anaemia Consultative Group, 1990, are as follows:

Haemoglobin 12g/dl (120 g/L)
Haematocrit mean 40% (limit lower 35%)
Mean Red Blood Cells $5 \times 10^{12} \pm 1 \times 10^{12}$ (Liter)
Mean Cell Volume 80 ± 6 (fl).
Mean Cell Haemoglobin 24 ±2.5 (pg)
Mean Cell Haemoglobin Concentration 300 ± 25 (g/L).

5.5.2.2.2 Reliability of the Haematological Determinations.

Haemoglobin concentration can be affected by diurnal variation (tends to be lower by up to 1.0 g/dl in the evening than in the morning), recent drink, body position, exercise (alterations of plasma volume), and recent food (lipaemia). Blood samples from all children aged 6-10 years were drawn at 7:30 in the morning and haematological measurements were performed on the same day. Children were allowed to rest for at least 15 minutes before blood collection and ice-boxes were used to transport the fresh blood samples from the collection site to the processing site. Lipaemia can falsely elevate the haemoglobin value (as much as 3g/dl) (Baker and Ramachandran, 1985) because of turbidity. This was avoided by using blood samples from fasting children.

MCV is less affected by sampling errors than haemoglobin, because red cell size is unaffected by the tissue fluids

Knowledge of inherent and possible technical errors led us to establish rules of strict training and proper standardisation and calibration of the material and equipment used to obtain accuracy and precision with the minimal error in the results. All measurements were carried out in duplicate, and every 10 days accuracy and precision were monitored by making extra-measurements on one sample labelled and distributed randomly among other specimens. The mean of these values should not deviate for more than ±2SD (Baker and Ramachandran, 1984). The haematological methodologies used in this project have been recommended by the International Committee for Standardisation in Haematology and by the International Nutritional Anaemia Consultative Group for prevalence surveys in developing countries (Baker and Ramachandran, 1984).

5.5.2.2.3 Biological and Pathological Factors Influencing Haematological Determinations.

Haematocrit values decrease when haemoglobin production is impaired. Some cases of moderate iron deficiency and marginal values of haemoglobin have been associated with normal haematocrit values (Graitcer ***et al.***, 1981). Haematocrit is affected by all factors influencing haemoglobin concentrations (age, sex, and capillary or venous blood). Haemoglobin and haematocrit decrease during the third stage of iron deficiency (see Chapter 4). Haemoglobin values also decrease in chronic infections and inflammations, haemorrhage, protein-energy deficiency, thalassaemia minor, vitamin B-12 or folate deficiency, haemoglobinopathies, pregnancy, and overhydration or acute plasma volume expansion (Chapter 4). In contrast, elevated haemoglobin values occur in polycythaemia and haemoconcentration caused by dehydration (Gibson, 1990).

Median haemoglobin values increase slightly during the first ten years of childhood, and during the second decade of life (puberty) the haemoglobin concentration is 2 g/dl higher for young men than that in women (Yip, *et al.*, 1984).

A low value for MCV is a relatively specific index for severe iron deficiency anaemia. Normal values (normocytic) of MCV have been associated with anaemia of chronic disease, thalassaemia minor, and lead poisoning. High values (macrocytic) have been associated with anaemia caused by vitamin B-12 or folate deficiency. Red blood cells are generally larger at birth than adulthood and gradually decrease in size during childhood. MCV values are slightly higher in young females than in males.

MCHC is less affected by age (Matoth *et al.*, 1971). Low MCHC values (hypochromic) are observed in iron deficiency anaemia. Normal (normochromic) values of MCHC can be observed during anaemia of chronic disease or deficiency of vitamin B-12 or folate.

The MCH changes progressively throughout life in similar way to MCV but values are slightly higher in young adult males than in young adult females (Gibson, 1990). MCH is low in iron deficiency anaemia, and high during anaemia caused by deficiency of vitamin-B-12 or folate and normal in anaemia of chronic disease.

5.5.2.3 Determination of the Biochemical Indicators of Iron

Serum iron, total iron binding capacity, transferrin saturation, and ferritin were included as biochemical indicators to assess the iron status in *T. trichiura*-infected rural school children in the State of Sinaloa. The relationship among these indicators and trichuriasis was studied to detect any possible influence of *T. trichiura* on the iron status.

a). Serum Iron.

Iron in the sample (0.5 ml) was released from transferrin by acetic acid pH 4.3 (0.5 M) and reduced to the ferrous ion by hydroxylamine (0.3 M) and thioglycolate (25 mmol/L). The ferrous iron was complexed with FerroZine Iron Reagent (0.5 mmol/L) and the monitored change in absorbance at 560 nm was directly proportional to the concentration of iron in the sample. The SYNCHRON CX Systems (composed by a microcomputer which controls automatically a carrousel with hold samples micro-cuvettes, a spectrophotometer UV-VIS to measure changes in absorbance, a screen and printer to show results) aliquoted the appropriate sample and reagent volumes into a cuvette (1:8) and calculated the iron concentration (Synchron CX, Systems, 1998).

Transferrin- $(Fe^{3+})_2$ + Acetic Acid ⟶ Tranferrin $+2Fe^{3+}$+Hydroxylamine +Thioglycolate ⟶ Fe^{2+}+ 3FerroZine ⟶ Fe^{2+} $(FerroZine)_3$ read at 560nm
expressed in iron concentration

b). Total iron Binding Capacity (TIBC).

To 0.5 ml of serum (transferrin) was added 1 ml of ferric ammonium sulfate (0.09 mmol/L) and transferrin was completely saturated by shaking the solution vigorously (2-3 seconds) and incubated 5-7 minutes at room temperature. The mixture was poured into a magnesium carbonate column until the entire mixture passed through (8 minutes). The effluent was collected for TIBC analysis and the column discarded. Any iron not bound to transferrin is absorbed by the magnesium carbonate (300 mg) and removed by centrifugation (1500 g) for ten minutes at room temperature (Synchron CX, Systems, 1998).

The iron in the sample (0.5 ml) was released from transferrin by acetic acid pH 4.3 (0.5 M) and reduced to the ferrous state by hydroxylamine (0.3 M) and thioglycolate (25 mmol/L). The ferrous iron was complexed with FerroZine Iron Reagent (0.5 mmol/L) and the change in absorbance at 560 nm was directly proportional to the total concentration of iron bound transferrin (TIBC)(Beckman Synchron Systems, 1997).

Transferrin $(Fe^{3+})_2$ (unsaturated)+Fe^{3+}(excess) ⟶ Transferrin $(Fe^{3+})_2$ (saturated)+Fe^{3+}(excess)+$MgCO_3$ ⟶ Transferrin$(Fe^{3+})_2$saturated (supernantant)+$MgCO_3/Fe^{3+}$(precipitate) ⟶ Transferrin $(Fe^{3+})_2$ Acetic Acid

⟶ Transferrin $+2Fe^{3+}$+Hydroxylamine +Thioglycolate ⟶

Fe^{2+}+ 3 FerroZine ⟶ $Fe^{2+}$$(FerroZine)_3$ read at 560nm ⟶

expressed in iron concentration

c). Transferrin Saturation

Transferrin is the principal plasma protein for the transportation of iron, after the catabolism of haemoglobin, to haematopoietic tissue. Transferrin is a glycoprotein which (MW=80,000) bounds to one or two atoms of iron, although rarely are both sites occupied. It is synthesised in the liver and to a small extent in reticulo-endothelial cells, testes, and ovaries (Gibson, 1990).

Determination of serum iron and total iron-binding capacity were performed simultaneously, and transferrin saturation was calculated as follows:

Transferrin Saturation (%)= Serum Iron (μg/dl)/TIBC (μg/dl) (100%).

d). Serum Ferritin

The ferritin molecule consists of a protein shell (MW 450,000) and a core of iron. High concentrations are found in liver cells, splen, and bone marrow. Ferritin serves as storage to provide iron to the body when necessary, protecting against the toxic effects of excess of iron, and to maintain the mobilisation of iron for erythropoiesis (Coat-A-Coat Ferritin IRMA, 1999; Gibson, 1990).

Ten μl of serum sample, 10 μl of control (T), and 10 μl of each calibrator (9 marked from A= (NSB)= 0; B= 5; C= 25; D 100; E= 200; F= 500; G= 1,000; to H ("MB")=2000 ng/mL), were put into the labelled tubes coated with anti-ferritin murine monoclonal antibody. Calibrator A is the non-specific binding counts, and calibrator H is the percent of maximum binding (%B/MB). 200 μl of ferritin assay buffer were added to all tubes (except to T tubes). The mixture was shaken for 30 minutes on a rack shaker and emptied thoroughly. 2 ml. buffered wash solution were added to each tube and tubes emptied thoroughly. All residual droplets were removed by striking the tubes sharply on absorbant paper. 100 μl of ^{125}I Ferritin Ab were added to every tube, shaken for 30 minutes on a rack shaker, and emptied thoroughly. 2 ml of buffered wash solution were added to each tube, and emptied thoroughly (this wash step was repeated). All residual droplets were removed. Counts (1 minute) were determined in a gamma counter (Coat-A-Coat Ferritin IRMA, 1999).

During the procedure, ferritin was captured by monoclonal anti-ferritin antibodies immobilised on the inside surface of the polystyrene tube and the radio-labelled polyclonal anti-ferritin tracer. Unbound I^{125}-labelled anti-ferritin antibody is removed by decanting the reaction mixture and washing the tube reducing non-specific bindings to a very low level and ensuring excellent low-end precision. The ferritin concentration was directly proportional to the radioactivity (measured by a gamma counter) present in the tube after the wash step.

Results were calculated from a log-log representation of the calibration curve using the different calibrators A= (NSB)= 0; B= 5; C= 25; D 100; E= 200; F= 500; G= 1,000; and H ("MB")=2000 ng/mL. Net counts of each pair of tubes were calculated by subtracting the average of counts per minute (CPM) from the average of non-specific binding (NSB) tubes (calibrator A). Then the resulting percent binding of each tube was calculated by the ratio Net Counts/Net MB Counts (calibrator H) X 100. Using a log-log graph paper, the Percent Bound was plotted versus concentration for each calibrator (A-H). Concentration for control and unknowns were estimated from the calibration curve by interpolation.

The calibrator T was used as optional total counts for quality control. T is the Total Counts per minute, and its %NSB and %MB were calculated as follows:

%NSB= Average NSB Counts (calibrator A)/Total Counts X 100.

%MB= Net MB Counts (calibrator H)/Total Counts X 100

The results were expressed in µg/L (Coat-A-Coat Ferritin IRMA, 1999).

5.5.2.3.1 Cut-Off Values for Biochemical Indicators of Iron in Children Aged 6-10 years

The cut-off points for biochemical indicators of iron in individuals residing at sea level have been derived from the World Health Organisation, (1972), NHANES II (Pilch and Senti, 1984), (Gibson, 1990), and International Nutritional Anaemia Consultative Group (Baker and Ramachandran, 1984).

Serum Iron 50-120 µg/dl

Total Iron Binding Capacity 250-400 µg/dl

Transferrin saturation<16%, >70% (Iron overload)

Ferritin <12µg/L

5.5.2.3.2 Reliability of the Biochemical Assay for Iron.

Serum iron and TIBC concentrations were quantified by a Beckman SYNCHRON CX Clinical System (CA 92621-6209)(1998). This equipment automatically aliquoted the appropriate sample and reagents. All reagents, calibrators kit, and material were managed as recommended by the manufacturer (storage conditions, stability and expiration times for maximum performance, glassware material free of iron or copper trace), and equipment was operated by a trained technician.

The Beckman SYNCHRON CX System had an accuracy of one standard deviation equal to 4.55 μg/ml or a coefficient of variation of 3.25% for iron and 13 μg/ml (1DS) or a coefficient of variation 5.2% for TIBC. Controls (high and low) were analysed daily, or after a new calibration, new lot of reagents, and after maintenance of the equipment.

Plasma or whole blood (EDTA or heparin) interfere with this method, and serum samples free of red blood cells haemolysate, bilirubin, lipaemia, magnesium or copper were used.

Serum ferritin concentration was quantified by a Beckman Gamma Counter (Coat-A-Count Ferrintin IRMA, 1999). The antibodies used were raised against human liver ferritin and were selected to react with both liver and spleen ferritin. The Ferritin Ab-coated-tube (containing less than 20 microcuries of radioactive ^{125}I-polyclonal anti-ferritin) methodology offered speed, convenience, and negligible non-specific binding.

The protein based calibrators (FEI3-9, X) with ferritin values from 0, 5, 25, 100, 200, 500, 1000, and 2,000 (ng/ml), ^{125}I Ferritin Ab (IFE2), Ferritin Assay Buffer (FEAB), and Buffered Wash Solution Concentrate (2TSBW, 4TSBW), were managed as recommended by the manufacturer (storage conditions, stability and expiration times for maximum performance). Control or serum pools with low and high concentrations of ferritin levels were routinely assayed as unknowns for monitoring inter-assay precision.

The micropipette and automatic pipettor diluters delivered the exact amounts of samples and calibrators were evaluated before they were used. The equipment was operated by a trained technician.

The assay was standardised in terms of the World Health Organisation's Second International Standard for Ferritin (2nd IS 80/578). The assay can detect as little as 0.5 ng/ml, and its ferritin values have been compared against a well-established immunoradiometric assay (ELISA) for ferritin (accuracy). Crossreactivity to human heart ferritin is less than 1% (specificity). (Coat-A-Coat Ferritin IRMA, 1999). At the end of the assay ^{125}I Ferritin remaining was disposed in a pit for dangerous biological and reactive material located in the Centre of Research and Food Development A.C.

The frozen serum samples for ferritin quantification were allowed to thaw at room temperature, and repeated thawing and freezing was avoided. No fasting conditions, bilirubin, or haemolysis has any clinically significant effect on the Coat-A-Count Ferritin IRMA procedure, but anticoagulant (EDTA or heparin) may interfere.

5.5.2.3.3 Biological and Pathological Factors Influencing the Biochemical Indicators of Iron Status.

Serum iron, TIBC, transferrin saturation, and ferritin in combination with the haematological values are very useful to differentiate between nutritional deficiencies of iron and iron deficits from chronic infections, inflammation, or chronic neoplastic diseases. The serum iron and TIBC reflect the transit of iron from the reticulo-endothelial system to bone marrow. The serum iron content is the measure of the number of atoms of iron bound to the iron transport protein transferrin. Serum iron levels rise during childhood, but TIBC falls. Values for both indices are highest in young adults and decline with age (Gibson, 1990). The gender does not have an important effect on serum iron or TIBC levels during the first decade of life. However the NHANES II (Pilch and Senti, 1984) showed a tendency for serum iron levels to be higher in males than females after puberty. Decreased serum iron levels, rise of TIBC values, and low transferrin saturation appear during the second stage of iron deficiency (Gibson, 1990).

Conditions such as haemochromatosis, haemolytic anaemia, acute liver disease, excessive absorption of iron from the gut, transfusions, iron therapy, iron overload can occur. It results in an increased serum iron levels, low TIBC, and elevated transferrin saturation (>70%)(NHANES II). Infection, inflammation, and malignancy produce low serum iron levels, low TIBC values (used to distinguish true iron deficiency), and low transferrin saturation. Such trends arise from the defects in the low releasing of iron from the reticulo-endothelial system and subsequent reduced transport of iron from these stores to transferrin resulting in a shortage of iron in bone marrow. Although iron stores are adequate, serum iron levels are decreased and the body does not respond by increasing absorption of iron from the diet. Consequently, transferrin synthesis is not increased and TIBC remains low (Gibson, 1990)

Vitamin B-12, folic acid deficiency, certain drugs and toxins may be associated with decreased erythropoiesis. In these circumstances iron serum levels are normal or slightly above normal (decreased iron uptake for haemoglobin synthesis), TIBC is normal or low, transferrin saturation may be low, and serum ferritin levels may be normal or slightly above normal limits. However, when there is increased erythropoiesis in response to iron therapy, blood transfusion, or vitamin 12 or folic acid therapy, serum iron levels may decrease, while TIBC is normal or high (trend similar to that for iron deficiency), and serum ferritin level is normal and will decline slowly as iron storage is used for haemoglobin synthesis.

Biological variation of serum iron can be very high (coefficient of variation 30%) perhaps as a result of the large diurnal effects (Dallman, 1984). Serum iron levels tend to be elevated in the morning and low in the afternoon or evening (Wiltink *et al.*, 1973). As a result measurements of serum iron should be determined on fasting blood samples in the morning and thereby effects of recent dietary intake and diurnal variation can be minimised. TIBC is less affected by biological variation, especially diurnal effects than serum iron, but is more susceptible to analytical errors.

Ferritin is normally present in human plasma and gives a satisfactory index of body iron stores showed by its positive correlation with stainable iron in bone marrow, liver biopsy, phlebotomy, iron therapy, and repeated transfusions (Jacobs *et al.*, 1972; Kimber *et al.*, 1983). Serum ferritin falls in iron deficiency before haemoglobin and serum iron, but does not reflect the severity of iron deficiency, because several factors affect the concentration of serum ferritin. In conditions of frank iron-deficiency with microcytic and hypochromic anaemia, serum ferritin levels are very low, reflecting exhaustion of iron storage. On the other hand serum ferritin with serum iron and TIBC provide information about iron overload (Gibson, 1990). Infection, inflammation, and neoplastic diseases produce an increased rate of ferritin synthesis in the reticulo-endothelial system reflected in a high concentration of serum ferritin. In acute or chronic liver disease, serum ferritin levels are very high, probably the result of the release of ferritin of the damaged liver cells. Such damage can be observed in children with protein-energy malnutrition (Wickramasinghe, *et al.*, 1985). Also, high ferritin levels are observed in leukaemia and Hodgkin's disease and may be associated with increased deposition of iron of the reticulo-endothelial cells, or leukaemic cells and damaged cells with high levels of ferritin (Worwood, 1979).

At birth, serum ferritin concentrations are relatively high and during the first two months continue increasing because of iron released from foetal red cells and decreased erythropoiesis. After that, levels fall throughout infancy and childhood. Adult and elderly men have higher levels of ferritin than children and postmenopausal women. Women have relatively constant levels of ferritin but they increase gradually after menopause, while in men they rise progressively after adolescence (Cook *et al.*, 1976).

Pilon *et al.*, (1981), reported an average intra-subject day to day coefficient of variation of 14.5% for serum ferritin compared with 28.5% for serum iron, although analytical variation for ferritin assay is higher than that for serum iron assay.

5.6 Measurements used as Exclusion Criteria.

To assess the influence of *T. trichiura* on the iron status of rural school children from Sinaloa, measurements to separate any possible effect on iron balance resulting from trichuriasis from those associated with malaria (*P.vivax*) and/or bacterial overgrowth in the small intestine, were included in the study.

a) Urinary Indoxil Potassium Sulfate (indican).

The technique of Sharlit, modified by Meiklejohn and Cohen (1942) (Bryan, 1965), was used to determine urinary indican. 0.5 ml. of a 3-hour-urine sample was diluted to a volume of 5ml. using distilled water. Then, 0.5 ml. of a 1% potassium per-sulfate solution, 0.5 ml. of 1% thymol solution in 95% ethyl alcohol, 5ml.of 25% trichloroacetic acid, and 5 ml. of concentrated HCl, were added. The solution was placed at 45°C for 45 minutes and subsequently cooled at room temperature. The solution was centrifuged (1500 g) 10 min and the supernatant fluid discarded. The coloured remaining complex (indigo) was diluted with 3 ml. glacial acetic acid. The solution was spectrophotometrically read at 540 nm and the indican concentration was calculated against a standard curve.

The standard curve for urinary indican was prepared and re-checked every two weeks using a certified standard commercially available from Sigma (100 mg). Dilutions of the standard (20, 18, 16, 14, 12, 10, 8, 6, 4, 2, 0 mg/dl) were prepared and absorbance values were plotted on the Y axis against the indican concentration in mg/dl on the X axis on ordinary graph paper. The indican concentration in the 3-hour urine sample was expressed per milligram of urinary creatinine.

b). Urinary Creatinine.

Urinary creatinine was also determined by diluting 0.1 ml. of 3-hour urine sample to 0.9 ml. with distilled water. 0.5 ml. 10% NaOH solution and 3 ml. concentrated picric acid were added (Jaffe reaction) (Richterich and Colombo, 1981). After 20 min. the solution was spectrophotometrically read at 520 nm. at room temperature (20-37°C). The concentration of urinary creatinine was calculated by using a creatinine standard solution from Merk and the law of Lambert-Beer: Creatinine unknown=Aunk/Astd [Creatinine standard (1g/dl)] (dilution 10). Standard and urine samples were routinely measured together.

The 3-hour urine samples for indican and creatinine analysis were collected on the blood collection day. Explanation and distribution of labelled (name of the child, community, primary class, and collection time) and clean plastic containers were carried out the day before the collection. Analyses were carried out on the collection day. However when it was not possible, urine samples were stored at -20°C and analysed latter (urine samples have shown consistent results for urinary indican and creatinine after 2 weeks at -20°C storage)

c). Thick film for malaria detection.

As malaria (*P. vivax*) has been detected in the study site, a drop of fresh blood was placed onto a slide. A second slide was used as spreader to obtain the blood film and methanol to fix it onto the slide. A rapid staining (Giemsa's stain) assured dehaemoglobinisation and diagnosis of malaria parasitaemia. Preparations of the film were carried out on the blood collection day (before and after albendazole treatment) and properly transported for microscopic observation.

5.6.1 Reliability of the Measurements used as Exclusion Criteria.

Because there is evidence that bacterial overgrowth in the small intestine is associated with megaloblastic anaemia or steatorrhoea as a result of the malabsorption of vitamin B_{12} in the distal ileum or degradation of bile salts respectively (Bullen *et al*, 1978; Tabaqchali and Booth, 1966), a indirect measurement to detect bacterial overgrowth in the small intestine was included to assess the relationship between trichuriasis and iron status of school children in the State of Sinaloa.

Indoxil potassium sulphate (indican) is the major urinary metabolite of indole in mammals (Bryan, 1965). Indican is derived from the bacterial decomposition of tryptophan to indole which is mostly re-absorbed in the intestinal tract and converted to indican (in liver) and finally excreted in urine (Wells, 1918). Therefore, it is expected that changes in the excreted urinary indican are derived from high counts of bacterial (clostridia, bacteroides, enterobacteria, bifidobacteria) (Hamilton, *et al.*, 1970), in the small intestine or rich diets in protein with high content of tryptophan (Asatoor, *et al.*, 1963; Fordtran *et al.*, 1964).

Because it has been found difficult and unreliable to collect 24 hour urine samples in children during field work, other authors (Robert, *et al.*, 1975; Vega-Franco, 1986) have found more simple and reliable the 3 hour urine samples to determine indican expressed per equivalent of creatinine, because the urinary excretion of the latter in 3 hours is not significantly different with that excreted in 24 hours (Robert, *et al.*, 1975).

Creatine is synthesised from arginine and glycine (pancreas, liver, and kidney) and passes into the plasma and is taken up by muscle cells and phosphorylated to form creatine phosphate, analogous to adenosine triphosphate (ATP). By muscular contraction creatine phosphate is converted into mechanical energy, but it also undergoes to spontaneous decomposition to creatinine which is excreted in urine. Creatinine excretion is not significantly affected by diet (Folin, 1905 in Richterich and Colombo, 1981), but a significant elevation is observed in muscular atrophy, muscular dystrophy, amputations, strong physical exercise, and severe kidney disease (Richterich and Colombo, 1981). Children presenting these pathological profiles were excluded from the study.

The Jaffe reaction was selected to determine urinary creatinine. Although, it has been shown that this reaction is influenced by more than 50 different chromogenic substances, it is possible to improve its specificity (reducing the interference of pseudo-creatinines) by optimal reaction conditions, and appropriate concentrations of picric acid and sodium hydroxide (Helger *et al.*, 1974).

It has been found that concentrations higher than 0.18 mg of Indican/mg of creatinine have been associated with bacterial overgrowth in giardiasis and diarrhoea (Vega, *et al.*, 1986). Unfortunately, because indican is an indirect measurement to detect bacterial overgrowth, it is not possible to distinguish children with high counts of bacteria in the small intestine from those on diets rich in protein with high content of tryptophan.

Finally, thick films (fresh preparations) for malaria detection were screened by trained people on the blood collection day.

5.7 Anthropometric Evaluation

a). Measurement of Height

The measurements of height were carried out in the primary schools attended by the children. A stadiometer (2.05 ± 0.0005 m), Holtain Ltd, UK, was used and placed on a flat surface and calibrated before each measurement session. The child was measured without shoes and wearing light clothing. His/her body was stood straight with the head in the horizontal plane with the floor (Frankfurt plane), feet together and tip of the feet and passes into the plasma and is taken up by muscle cells and phosphorylated to form creatine phosphate, analogous to adenosine triphosphate (ATP). By muscular contraction creatine phosphate is converted into mechanical energy, but it also undergoes to spontaneous decomposition to creatinine which is excreted in urine. Creatinine excretion is not significantly affected by diet (Folin, 1905 in Richterich and Colombo, 1981), but a significant elevation is observed in muscular atrophy, muscular dystrophy, amputations, strong physical exercise, and severe kidney disease (Richterich and Colombo, 1981). Children presenting these pathological profiles were excluded from the study.

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The measurements of height were carried out in the primary schools attended by the children. A stadiometer (2.05 ± 0.0005 m), Holtain Ltd, UK, was used and placed on a flat surface and calibrated before each measurement session. The child was measured without shoes and wearing light clothing. His/her body was stood straight with the head in the horizontal plane with the floor (Frankfurt plane), feet together and tip of the feet separated, knees straight, and heel, buttocks, shoulders, and head in contact with the vertical surface of the stadiometer (Gibson, 1990). Arms were hanging loosely at the sides with the palms facing the thighs. When the required position was reached, the head-bar of the stadiometer was lowered until it touched the crown of the head and the hair was compressed. The height reading was taken to the nearest millimeter, taking the lower reading when it fell between two values. All measurements were carried out in a private place to reduce environmental disturbance (distractions of the measured subject) (Jordan, 1988).

b). Measurement of Weight

The measurements of weight were also carried out in the primary schools and a digital electronic balance with capacity 0-150 ±0.05Kg (AND FV-150 KA1, A&D Co. LTD, Japan) was used for this purpose. The balance was placed on a hard flat surface and checked for zero-balance and calibrated with a set of weights before each measurement. The child stood unassisted in the centre of the platform and was asked to look straight ahead and relaxed Gibson, 1990).

5.7.1 Reliability of the Anthropometric Measurements as Indicators of Nutritional Status and Technical Influencing Factors.

5.7.2 Indicators of the Nutritional Status.

When data on weight, height, and age are combined, it provides indicators of the nutritional status at individual or population levels (Table 5.1). The indicators most widely used to evaluate the nutritional status in children are Weight/Height (W/H), Weight/Age (W/A), and Height/Age (H/A) ratios. These indicators are recommended by the World Health Organisation to detect problems of malnutrition by deficiency or excess and they give evidence of the severity of malnutrition of our study population in respect to a reference population (Ulijaszek, 1993).

The weight/age ratio is used to identify a current protein-energy imbalance by deficiency or excess in children aged between 6 months and 7 years (Gibson, 1990). The weight changes rapidly when dietary or environmental factors also change (Jordan, 1979).

The weight/height ratio is also a sensitive indicator of current protein-energy imbalance (Gibson, 1990). This indicator is relatively independent of age between 1 and 10 years (Romero, 1974).

The height/age ratio is an indicator of stunting or chronic malnutrition as a result of long periods of disease and poor energy intake (Dibley *et al.*, 1987). Stunting is a very common condition in developing countries and reflects the difficult biological, environmental, and socio-economic conditions of children and adults (Martorell, 1993). A low height/age ratio can represent a short period of reduced growth in children at early stage in life or a longer period at older life-stages.

Table 5.1 Interpretation of the Nutritional Status on Basis of the Combination of H/A, W/A, and W/H Indicators (WHO, 1986).

Group	Indicators			Interpretation
	W/H	W/A	H/A	
1		Low	Low	Normally Fed with Past of Malnutrition
2	Normal	Normal	Normal	Normal
3		High	High	Tall, Normally nourished
1		Low	High	Current Malnutrition
2	Low	Low	Normal	Current Malnutrtion
3		Normal	High	Current Malnutrition
1		High	Low	Obese
2	High	Normal	Low	Current Overfed with Past Malnutrtion
3		High	Normal	Overfed but not Necessarily Obese

5.7.3 System of anthropometric Reference

The World Health Organisation recommended in 1978 the use of the United Sates National Centre for Health Statistics (NCHS) reference growth data to monitor the nutritional status in children, to evaluate the nutritional status of a population, and to assess the effectiveness of nutritional intervention programmes (Gibson, 1990).

The NCHS reference data meet most of the required criteria suggested by the International Union of Nutritional Sciences (well-nourished population, a representative population of 200 individual for each age and sex, cross-sectional sample for cross-sectional comparisons, sampling procedures well defined and reproducible, use of appropriate equipment and study-design, repeated calibrations, and raw data available for public reviewing) (IUNS, 1972).

Nutritional data from children aged 2 to 18 were compiled by the NCHS from three different sources: values collected during the Health Examination Survey (HES) Cycle 1 (1963-1965) for ages 6 to 11; HES Cycle 2 (1966-1970) for ages 12 to 17; and the First National Health and Examination Survey NHANES I (1971-1974) for ages 2 to 17 (Gibson, 1990).

5.7.3.1. Standard Deviation Scores

The use of standard deviation scores was recommended by Waterlow in 1977 to ..y nutritional status into low, moderate, or severe by evaluating W/H, W/A, and H/A indicators in units of standard deviation for a study population in respect of the NCHS population recommended by WHO.

Although, these indicators can be expressed in percentiles in relation to the median (-2SD-+2SD= 3rd centile-97th centile, -1.19SD-+1.19SD=12th centile-80th centile, and 0SD=50th centile), the results in SD score permits parametric statistical comparisons (mean, standard deviation, and standard error), and the percentile system does not (non-lineal scale) (Jelliffe, 1989; Vazquez, 1996).

The Standard Deviation (SD) Score is calculated by using the following formula: Z= Weight or Height – Median Value (NCHS)/Median Value (NCHS)- 1DS below or above the Median Value (Dustin, *et al.*, 1979).

5.7.3.2 Reference Data for Children and Cut Off Points to Define Severity of Malnutrition.

The reference data for the weight and height of children are published in charts by the National Centre for Health Statistics covering children and adolescents up to 18 years old. (Dustin, 1979. The reference limits used included the cut off points for strict cover (-2SD-+1SD) most usually applied in nutritional surveys, although it will depend on the study objectives and available resources. Strict-cover is used to identify and to treat only severe malnourished population. Cut off points for wide-cover (-1SD-+1SD) are used to identify and treat moderate and severe malnourished population (Ponce, 1996).

Often SD scores <–1 are indicating Low Malnutrition; <-2.0 Moderate Malnutrition; and <-3 Severe Malnutrition. For Normal >-1 - <+1, and Overweight ≥ +1 - <+2.

5.7.3.3 Reliability of the Height and Weight Measurements

The anthropometric evaluation is a very important source of information on the nutritional status of the population. Height (measure of the skeletal bony tissue) and weight (body mass) are considered among the major measurements included in the anthropometric evaluation to assess nutritional status, because they are relatively economic to carry out, objective, understandable, give numerically graded results, and information about adequate growth, protein-energy deficiency and obesity (Jelliffe, and Jelliffe, 1989).

On the other hand, some confounding factors may be present during the anthropometric measurements:

a). Inaccuracy of the measurements.

b). The precise ages of children are may not be known.

c). The nutritional diagnostic is limited (anthropometric measurements give information about the nutritional status of the individual, but they do not provide information about the cause of the malnutrition

d). Selection of the appropriate reference data e). Growth can be influenced by internal (genetic diseases such as endocrine malfunction, obstetrical such as low birth weight, and sex of the individual) and external factors (diet, environment such altitude and climate, drugs, diseases such as infections, congenital, chronic illness and emotional deprivation) (Jelliffe, and Jelliffe, 1989).

The anthropometric measurements in this study were carried out by a team of 3 trained workers. Their duties were as follows: a person who calibrated the equipment before each measurement session, a person who measured the height and weight proportions, and a person who registered the weight and height on the data form. The duties within the group were changed regularly and measurements on the same child were carried out to verify reproducibility among the members of the team.

Because undressing poses problems of modesty, minimum clothing was retained. Also, it may be noted that some variation in weight can occur because of recent meal, defaecation and micturition. Theoretically, weighing should be done before a meal or with an empty bladder but these precautions are impossible to carry out in field-work (Gibson, 1990).

In addition, because precise age was a very important factor, birth certificates were asked for each child. Ages were calculated in months from the birth-date to the day at which measurements were carried out.

Numbers of months = (Number of years)(12 months) + Number of Months after the birth-date. (A month was added if number of remaining days were higher than 15 days).

The anthropometric results obtained were compared with those of the reference growth data of the NCHS derived from different sources. The values for weight, height, and weight/height from birth to 36 months were taken from a longitudinal survey by the Fels Research Institute (Yellow, Spring Ohio) between 1960 and 1975, and a cross-sectional survey from 2 to 18 years (National Health Examination Survey). The sample included at least 200 well-nourished individuals in each age and sex group with full growth potential which made it large and representative, from different economic and ethnic groups in the U.S. (Jelliffe and Jelliffe, 1989; Gibson, 1990). In addition, measurements of weight, height, arm circumference, and skinfolds, were included, and comparison among them in different countries or regions in the world at different times can be done to detect protein-energy deficiency or obesity. However, despite their usefulness and advantages, the NCHS data has limitations (Jelliffe and Jelliffe, 1989), because they were compiled from a longitudinal survey (0-3 years), and a cross-sectional study (2-18 years), and hence there is an overlap from 2 to 3 years. In addition there is an incomplete documentation on whether height was measured in children 2-3 years of age (Jelliffe and Jelliffe, 1989).

Some data were derived from obese children (Jelliffe and Jelliffe, 1989) and differences between the main genetic groups in the U.S. may have been blurred by combining all the information into one set of figures. However, it has been shown that growth development seems to be more influenced by the socio-economic factors than genetic factors. The largest differences in growth have been observed between populations of developed and developing countries, and between high and low income groups from the same country (Ulijaszek, 1993). In addition, no anthropometric data are available for all basic measurements from a single population for all age groups (Jelliffe and jelliffe, 1989). However, there is not an absolute global standard of excellence for all genetic groups (Jelliffe and Jelliffe, 1989), but the reference data of the NCSH met most of the criteria suggested by the International Union of Nutritional Sciences for ideal reference data (Gibson, 1990).

Currently, there are other reference data (Metropolitan Life Insurance Society of Actuaries, and Harvard, US), based on samples from economicly biased groups, but no control in the quality of the measurements, small number of children, limited genetic representativeness, out of datedness, unavailability of original raw data, and lack of arm circumference and triceps measurements. Other data have been collected from populations of European ancestry but they could be wrongly interpreted reflecting unintended ethnocentric anthropometric imperialism or racial superiority (Jelliffe and Jelliffe, 1989).

In addition, other reference data from different less developed countries (India, Bangladesh, and Hong Kong), where there are few well nourished children, ethnic and/or genetic are the predominant factors affecting growth potential, and energy intakes are unrealistic (Jelliffe and Jelliffe, 1989). Also, these were derived from measurements of well-fed, medically, and socially protected children of the elite or children attending

Young Child Clinics (excluding those with acute or chronic disease, and including those with clinical malnutrition)

5.8 Evaluation of Dietary Intake.

24-hour recall method repeated twice and separated at least 1 month between interviews was used to assess the dietary intake of the children the previous 24 hours (Sànjur and Rodriguez, 1997). The recall was given to the child in presence of his/her mother and conducted by personal interview using a structured questionnaire (appendix 5), food models, traditional dishes and utensils.

The amount of each reported food item consumed was estimated by using the pre-recorded weight and volumes on the food models, dishes, and utensils. The estimated amounts were put in a codified nutrient data base programme (in grams) using dictionary based on nutrient composition tables from different sources (Ortega *et al*, 2000).

The composition of the reported diet was calculated in terms of the total energy in kilocalories or kilojoules, the percent of total of energy contributed by fat, carbohydrates, and proteins. In addition other nutrients such as Fe, Cu, Zn, B1, B2, Niacin, vitamin E, PB6, cobalamin, pantothenic acid, Na, K, Ca, Mg, P, vitamin A, vitamin C, folic acid, and selenium were also reported.

5.8.1 Recommended Dietary Allowances for Children 6-10 Years.

The calculated amounts for each particular nutrient in the reported diets for the recruited school children in this study were compared with those recommended as a daily allowances in children aged 6 to 10 years (Table 5.2)(Recommended Dietary Allowances, 1989).

Table 5.2 The allowances expressed as average daily intakes (Food and Nutrition Board, National Academy of Sciences, National Research Council, and Recommended Dietary Allowances, 1989)

Macronutrients: Energy Intake Energy (Kcal) 2000 **Contribution to Energy Intake (%)** Protein 14-18 (280-360 Kcal) Fat 30-36 (600-720 Kcal) Carbohydrates 50 (1000 kcal) **Intakes (g)** Protein 28 Fat 60-72. Carbohydrates 250	**Water Soluble Vitamins:** Vitamin C (mg) 45 Thiamin (mg) 1.0 Riboflavin (mg) 1.2 Niacin (mg) 13 Vitamin B_6 (mg) 1.4 Folate (μg) 100 Vitamin B_{12} (μg) 1.4 Biotin (μg) 30 Pantothenic Acid (mg) 4-5
Minerals Calcium (mg) 800 Phosphorus (mg) 800 Iron (mg) 10 Zinc (mg) 10 Selenium (μg) 30	**Fat Soluble Vitamins** Vitamin A (μg) 700 Vitamin D (μg) 10 Vitamin E (mg) 7

5.8.2 Reliability of the Dietary Evaluation.

There is no ideal method for collecting dietary information (Sanjur and Rodríguez, 1997), but there are preferred methodologies for defined purposes. To estimate quantity of food consumed, food records or food weighting methods are possible choices, and to obtain information about present or past food intakes repeated food recalls, food frequencies, and diet histories can be applied. To obtain qualitative dietary profiles from large groups, multiple and non-consecutive 24-hour recall is the best choice (Sanjur and Rodríguez, 1997).

The 24-hour recall was applied in this study because of its logistical simplicity, number and particular features of the sampled population. This method has been criticised because it does not provide a reliable estimate of the usual intakes of individuals. However, a recent Consensus Workshop on Dietary Assessment sponsored by the National Centre for Health Statistics of the US Department of Health and Human Services concluded that the 24-hour recall is the best suited for most nutrition monitoring needs (Sanjur and Rodriguez, 1997).

The 24-hour recall method is based upon foods and drinks eaten by the individuals during the previous 24 hours. This method is inexpensive, easy to obtain, non-obtrusive, quick (in one day), covers large number of subjects, high co-operation, and used with illiterates. However, it is not representative of the usual intake, relies heavily on memory, it is not accurate with quantities, and involves considerable probing and enquiry.

Although, Todd *et al.* (1983) have shown that other parameters such as day of the week effect, training and interviewer effect, do not contribute significantly to the total variance of this method, considerations about these parameters were taken into account:

a). Training sessions among the members of the interview team were conducted before starting the study. Sessions included visits to the most popular centres of foods in the study site, familiarity with local traditional foods, and dishes and utensils, availability and costs, searching of food models similar to those found in the study site, labelling the visual food models with the appropriate weights and sizes using scales portables, appropriate form to conduct the interview (questions included time periods during the day (morning, noon, evening, night, school time) and movements chronologically backward in the time (appendix 5), and how to use and to fill the food questionnaire (appendix 5), and management of the nutritional data base programme.

b). The 24-hour recall was applied a minimum of twice and separated 1 month by interviews to minimise the variation in intake from day-to-day that apparently is the major component of variance for this method. A third interview was carried when unusual information about daily intake was observed. The interview included the presence of the child and the person responsible (mother/father or another family member) in a quiet place to avoid outside influences. There is evidence that combined responses by parents and children give more accurate information than that by the parent alone (Eck, 1989; Thompson, 1994).

c). The use of food, dishes, and utensils model helped to stimulate the memory of the responders and minimise biases by memory effects (missing information).

d). Effects by personal traits between the interviewer and responders (socio-economic status, ethnic background, rural/urban origin, language barrier, familiarity degree) were minimised by interviewers who lived in the study sites. Because the study involved rural diets which were very monotonous in their composition day to day variability may did not contribute significantly to the variance in the reliability of the results, but seasonal variation may be significantly become some of the studied populations subsisted from agricultural crops. To minimise errors related to information about mixed dishes it was necessary to identify the major nutrient contributions derived from the core foods.

Calculated nutrients from the reported diet in this study were compared with the Recommended Dietary Allowances (RDA, 1989). The RDA's have been prepared by the Food and Nutrition Board since 1941. The first edition was published in 1943, and has been revised periodically to incorporate new scientific knowledge and interpretations (RDA's, 1989). The RDA's are intended to reflect scientific nutrient allowances for the maintenance of good health and to evaluate the adequacy of diets of groups of people (Recommended Dietary Allowances, 1989).

The RDA's are based on individuals subjected at low or deficient levels of nutrients, followed by correction of the deficit with measured amounts of the nutrient, nutrient balance studies (nutrient status in relation to intake), biochemical measurements of tissue saturation or molecular function in relation to nutrient intake, nutrient intakes of fully breastfed infants or apparently healthy people from their food supply, nutritional status of populations in relation to intake, and in some cases extrapolation of data from animal experiments (Recommended Dietary Allowances, 1989).

Some factors have been introduced to compensate for physiological variation for absorption of the nutrient, incomplete utilisation and variation in requirements among individual, and different bioavailability among food sources (Recommended Dietary Allowances, 1989).

5.9 Collection of Particular, Health, and Socio-economic Information by Structured Questionnaires.

Each child included in the study was asked for particular information and subjected to health and socio-economic evaluation in presence of his/her parent or primary caregiver (appendix 5).

a). Particular information included complete name, address, birth-date (confirmed by birth certificate), birth-place, how long they have lived in the current community, religion, name of the primary school, and primary class attended (appendix 5). To facilitate identification and registration code numbers were assigned for each child and community.

b). The Health Status Study was carried out in 2 parts by a local physician:

The first part included an indirect inquiring to search information about diseases suffered by the child or other members of the family (respiratory and parasitic infections, allergies, previous surgeries, other health problems related to organs such as heart, liver, or kidney), and intake of substances (drugs, vitamin supplement or injections, and medication). The second part included a physical examination (check up) (lungs, skin, nose, throat, ears, and eyes) to search for current clinical symptomatology and signs (respiratory infections, asthma, cough, allergy, fever, headache, abdominal pain, and diarrhoea). A binary code (0,1) was used to collect qualitative health data (appendix 5) by home daily visits for one week.

c). The socio-economic status was evaluated by the Method of Mendez (1978) taking into consideration living conditions, family members, and services available, and family income was evaluated by the method of Camberos (1993).

Living conditions involved house size, quality of its construction material, house services (clean water, sewage, electricity), and number of family members. House size and number of family members were arranged in ordinal scale (units) and for house basic services was assigned a nominal scale (0,1). The construction material was arranged in ordinal scale agree to the quality and local-cost levels.

The economic status included total income of the family members (parents or primary caregivers) (ordinal scale), economic activity and education (parents or primary caregivers)(ordinal scale), and economic support from other sources (government) (nominal scale)(appendix 5). Other questions involving access to medical attention (ordinal scale), knowledge about the National Deworming Campaign (nominal scale) were also included. The difference among choices in ordinal scale was the unit. The nominal scale denoted presence or absence.

5.9.1 Reliability for Collection of Particular, Health, and Socio-Economic Information.

Before starting the study, the interview team carried out some visits to the study sites to observe the co-operation of the people, external features of houses, to find out about the cost of the most common construction material used in houses, and to identify the visited centres of health.

Also, training sessions were carried out to conduct the interview appropriately, to adapt questionnaires to the study site (housing-conditions), and how to use and to fill the questionnaires.

A local physician (Sinaloa) in active service and professional background for several years was given an explanation about the reasons for the study and clinical information should be collected. The physician determined the current health status of the child by filling the questionnaire for health status. All children presenting high fever and abdominal pain at the time of the physical exploration were properly treated. Also children with visible disability or under medical treatment were excluded from the

5.10 Albendazole Selected as Anthelmintic for the Treatment of Trichuris Infected School Children during the Assessment of the Possible Association Trichuriasis and Iron Status.

Albendazole was introduced in 1977 as a broad-spectrum oral anthelmintic in Australia (WHO, 1996). It was used for pinworm infection, ascariasis, trichuriasis, strongyloidiasis, hookworm infections, although effectiveness varies by species. It was approved for human use in 1982.

After oral administration albendazole is absorbed and metabolised to albendazole sulfoxide and to a lesser extent to other metabolites. Its half-life is 8-9 hours and their metabolites are excreted in the urine and faeces (bile).

Albendazole blocks glucose uptake by larval and adult stages of susceptible parasites depleting the glycogen stores and decrease the formation of ATP. The parasite is immobilised and dies. Albendazole has larvicidal effects in *N. americanus* and ovicidal effects in ascariasis, ancylostomiasis, and trichuriasis.

Mild transient epigastric distress, diarrhoea, headache, dizziness, lassitude, and insomnia have reported in a low percentage of patients. Rare allergic phenomena (oedema, rash, urticaria), abnormal liver function and leucopenia (only higher dosages and prolonged treatment) have been reported occasionally (WHO, 1996).

The safety of albendazole has not been established in children younger 2 years old and pregnant women. The drug has been shown to be teratogenic and embryotoxic in some animal species (rabbits and rats) at least during the first trimester (Katzung, 1989).

Albendazole was selected in this study as anthelmintic drug because is currently used for the Mexican Deworming Campaign in school children and it has a recognised background in large scale control programmes directed to reduce morbidity as a result of the prevalence and intensity of nematode infections in a variety of countries with diverse terrain, climate, demography and cultures (Israel, Japan, South Korea, Sri Lanka). In addition, albendazole along with levamisole, pyrantel, and mebendazole has been included in the recently revised essential drug list of WHO (1996) to be used against soil-transmitted nematodes (WHO, 1996).

5.10.1 Administration of Albendazole for Trichuris Infected School Children.

Albendazole was administered in a single dose (400 mg) to Trichuris infected school children. When necessary, treatment was repeated again for two consecutive days until ensure complete deworming. Egg counts were carried out to assess the efficacy of the treatment in Cure Rate (CR) and Egg Reduction Rate (ERR). CR is the proportion of patients treated who are egg-negative on one follow-up examination, and ERR to the percentage fall in egg counts after treatment based an a follow-up examination (WHO, 1996) (Stephenson and Holland, 1987). The arithmetic means of the untransformed counts were used to estimate the per cent egg reduction rate. A placebo was administered to uninfected school children in a similar way with that for albendazole. Worm recovering was carried out but it was not taken into account to estimate levels of intensity in worm reduction counts before and after treatment because collected information was not reliable.

Per Cent Cure Rate = Initial Prevalence - Final Prevalence/Initial Prevalence X 100
Per Cent Egg Reduction Rate = Initial Mean epg - Final Mean epg/Initial Mean epg X 100.

5.11 Statistical Methodology

The confidence intervals considered for the analysis of the results were determined by the 95% (z=-1.96 and z= +1.96), the level of significance to reject the null hypothesis or to accept the alternative hypothesis was α= 0.05, in a two tailed test (Dawson-Saunders and Trapp, 1994). Depending of the variance of a non-normally distributed sample, **Logarithmic** or **square root** transformations were used and the **Arcsine** transformation for proportions.(Fowler and Cohen, 1992). The results obtained were analysed by the following statistical tests: the **Chi-Square test** was used to compare two or more proportions (prevalence of intestinal parasitic infections), the **Spearman's Rank Correlation** (non-parametric) to measure the linear relationship between two variables measured on a numerical scale (prevalence, levels of intensity, biochemical indicators of iron status, anthropometric measurements, and dietary records), the **Logistic Regression** to predict a nominal or categorical outcome (independent variables include both numerical and nominal measures and the outcome variable is binary, or dichotomous having only two values), **Analysis of Covariance (ANCOVA)** to compare the means of the same variable controlling for the influence of a confounding variable (dependent variable is numerical and the independent measures are grouping variables on a nominal scale), the parametric **z-test** (more than 30 measurements), **t-test** to compare the means of two independent groups,), the **Paired t-test** to compare two measurements on each subject treated as a single measure, or the non-parametric **Mann-Whitney.rank sum-test** (independent groups) or **Wilcoxon rank-sum test** (paired groups)) to compare the medians of two groups, the **Analysis of Variance** (parametric **ANOVA**) to compare the means of three or more groups, the **odds ratio** to estimate the relationship between two nominal variables (Dawson-Saunders and Trapp, 1994).

All above analysis were developed using the Statistical Software for Windows 95, Number crunchier statistical systems (NCSS)

CHAPTER 6

PREVALENCE OF INTESTINAL PARASITIC INFECTIONS IN SCHOOL CHILDREN OF THE STATES OF SINALOA AND OAXACA.

6.1 Introduction.

Intestinal parasitic infections are still considered to be a serious public health problem in Mexico (Secretaria de Salud, 1993). Presently intestinal parasitic infections are not a significant problem in developed countries, but in developing countries such as Mexico a complex of factors favouring their transmission is still present (Flores *et al.*, 1983; Garcia *et al.*, 1987). The accelerated social and industrial development of Mexico and the inappropriate distribution of economic resources have resulted in a large number of sub-urban and rural communities with less than 500 each inhabitants living in conditions of poverty. Although any human population is at risk of intestinal parasitic infections, there are conditions that particularly favour their presence (Lara *et al.*, 1990). The environment is a very important factor for the survival of the parasites. The lack of basic services (clean water supply, sanitation, electric energy, health and education) increases the risk for the spreading of the different intestinal parasitic infections in the population. Other risk factors such as health and nutritional status, behaviour and cultural patterns of the population play a very important role in the prevalence and persistence of intestinal parasitic infections. Infants, pre-school, and school children are the age groups most affected by these kind of infections in Mexico (Martuscelli, 1987) and a negative impact in the economic development at individual and community levels is not surprising.

Mexico is a large country and officially has been divided into 32 states (INEGI, 1995). The present study evaluated the prevalence of intestinal parasitic infections in the Mexican States of Sinaloa (Northwest) and Oaxaca (Southeast). These States are very different in geographical, socio-economic, demographic, cultural, and ecological characteristics.

Information from the 11th General Census of Population and Housing (INEGI, 1990) revealed that Oaxaca is one of the poorest States in Mexico (appendix 2). Oaxaca was the second State with the highest percentages of illiteracy (27.5%) and houses lacking clean piped water (41.9%), and first with the highest percentage of houses lacking a sanitation system (70.1%) and with a floor of bare-earth (51.4%) (Table 6.1). In contrast, Sinaloa showed lower percentage of illiteracy (9.8%) and houses lacking clean piped water (20%), sanitation system (45.1%) and with floor of bare earth (22.8%).

Table 6.1 The Six Mexican States with the Highest and Lowest Levels in Illiteracy and Households Conditions According to the XI General Census of Population and Housing Conditions (INEGI, 1990).

Rank	State	Total Population (thousand)	Illiteracy (%)	Households with bare earth floor (%)	Households without piped water (%)	Households without sewage (%)
Mexican States with the Lowest Education and Housing Conditions						
1°	Oaxaca	3019.6	27.5	51.4	41.9	70.1
2°	Chiapas	3210.5	30	48.7	41.6	58.8
3°	Guerrero	2620.6	26.8	46.9	43.1	62.4
Mexican States with the Highest Education and Housing Conditions						
1°	Distrito Federal	8235.7	4.0	2.1	3.7	6.2
2°	Nuevo Leon	3098.7	4.6	6.0	7.1	19.1
3°	Baja California Norte	1660.9	4.7	7.8	9.5	33.2

In addition, the Fourth National Survey of Nutrition in the Mexican Rural Environment (1996) showed that the Mexican prevalence of malnutrition measured as the ratio weight/age in pre-school children was 42.7% (Avila-Curiel *et al.*, 1998). Oaxaca is one of the States with the highest national prevalence of mild (31.29%), moderate (18.65%), and severe (4.59%) malnutrition according to the ratio weight/age. However, a lower prevalence of mild (19.1%), moderate (5.33%), and severe (1.95%) malnutrition for the same nutritional indicator were observed in Sinaloa (appendix 6). A similar pattern was observed for the indicator of height/age. Oaxaca showed a higher prevalence of mild (23.93%), moderate (24.06%), and severe (22.88%) malnutrition than those observed in the State of Sinaloa (21.46%, 9.39%, and 4.32% for mild, moderate, and severe malnutrition respectively) (appendix 6). Therefore, the information provided by the Mexican economic census and Mexican nutritional survey have shown that life conditions in Sinaloa are better than those in Oaxaca.

The intestinal parasitic infections (ascariasis, trichuriasis, hymenolepiasis, giardiasis, and amoebiasis) are the most common problems of public health in Sinaloa and Oaxaca according to reports from the Ministry of Public Health (Secretaria de Salud de Sinaloa, 1998; Secretaria de Salud de Oaxaca, 2000), few published parasitological studies (Navarrete *et al.*, 1993; Diaz, *et al.*, 1994), and unpublished information (Soriano, 1998; LESPO, 2000).

The problem of intestinal parasitic infections stimulated linked efforts between health authorities and the different public health institutions (Centre of Health, Mexican Institute of Social Security, Security Institute for Employees of the State, Paediatric and Civil Hospitals) to establish of a National Deworming Campaign in 1993 directed at Mexican school children (Secretaria de Salud, 1993).

After 7 years of active operations, it was important to introduce this study in different communities of the States of Sinaloa and Oaxaca to monitor and evaluate the trends in prevalence of the different intestinal parasitic infections, and to investigate their interrelationship to socio-economic and nutritional factors in their populations. The information obtained will provide help to the health authorities to assess the efficacy of the current control programme in school children of Sinaloa and Oaxaca. It will also allow decisions and priorities to be taken to ensure the optimal use of the human and economic resources to benefit the most affected Mexican school children populations.

6.2 Prevalence of Intestinal Parasitic Infections in the States of Sinaloa and Oaxaca.

The coproparasitological analysis were carried out of 1476 stool samples collected from a total of 492 (240 females and 252 males) school children (41.5% from 1185 officially enrolled children) from 1 sub-urban and 11 rural communities of the States of Sinaloa (n=341) and Oaxaca (n=151). The school children with intestinal parasites were assigned to the following categories: parasitic infection group (with the presence at least one parasite species), multiple infection group (with the presence of two or more parasite species), helminth group (with intestinal helminths only), protozoan group (with intestinal protozoan species only), and groups with the presence of a particular species of parasite. Also, the term pathogenic intestinal parasite was assigned to those parasites associated with human health problems in the literature.

The results revealed a high general prevalence of intestinal parasitic infections (75%), multiple infections (53.9%), protozoan infections (67.5%), and helminth infections (37%) in the school children from both States (Fig 6.1). Statistical comparison (Chi-square) among the prevalence of the different intestinal parasitic infections showed that the prevalence of protozoan species such as *G. lamblia* (26%), *E. coli* (49%), and *E. nana* (40%) was found to be significantly higher than those for the rest of each parasite species detected in this study ($p<0.05$). In contrast, a low prevalence of *A. lumbricoides* (4%), *T. trichiura* (11.8%), and *E. histolytica* was observed (2.3%). Hookworm infections and *S. stercoralis* were not detected in school children from the States of Sinaloa and Oaxaca.

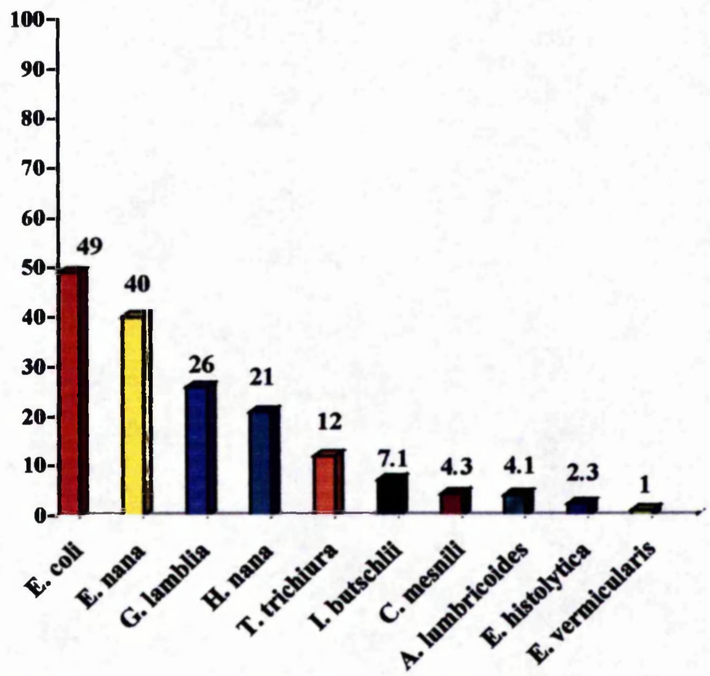


Fig 6.1 The Prevalence of Intestinal Parasitic Infections in 492 School-Children from the States of Sinaloa and Oaxaca.

6.2.1 General Prevalence of Intestinal Parasitic Infections in School Children from Rural Communities of the State of Sinaloa, Mexico.

From 341 school children aged 6 to 10 years enrolled in the primary schools of 7 rural communities (Imala, El Treinta, Pueblo Nuevo, Doroteo Arango, El Paraiso, Las Puentes, El Higueral) visited in the State of Sinaloa, 53.5% (341) (167 females and 174 males) participated in this study. A total of 1023 stool samples were collected (3 stools per child) and analysed to estimate the prevalence of the different intestinal parasitic infections per each community (appendix 7), and the prevalence average in the State of Sinaloa (Fig. 6.2).

High prevalences of parasitic, multiple, and protozoan infections were observed (76.7%, 53.9%, and 70.4% respectively) (Fig 6.2). Statistical comparison (Chi-square) among the prevalence of the different intestinal parasitic infections showed that the prevalence of protozoan infections (70.4%) was higher than that for helminth infections (31.7%) ($X^2 = 28.9$, $p<0.001$).

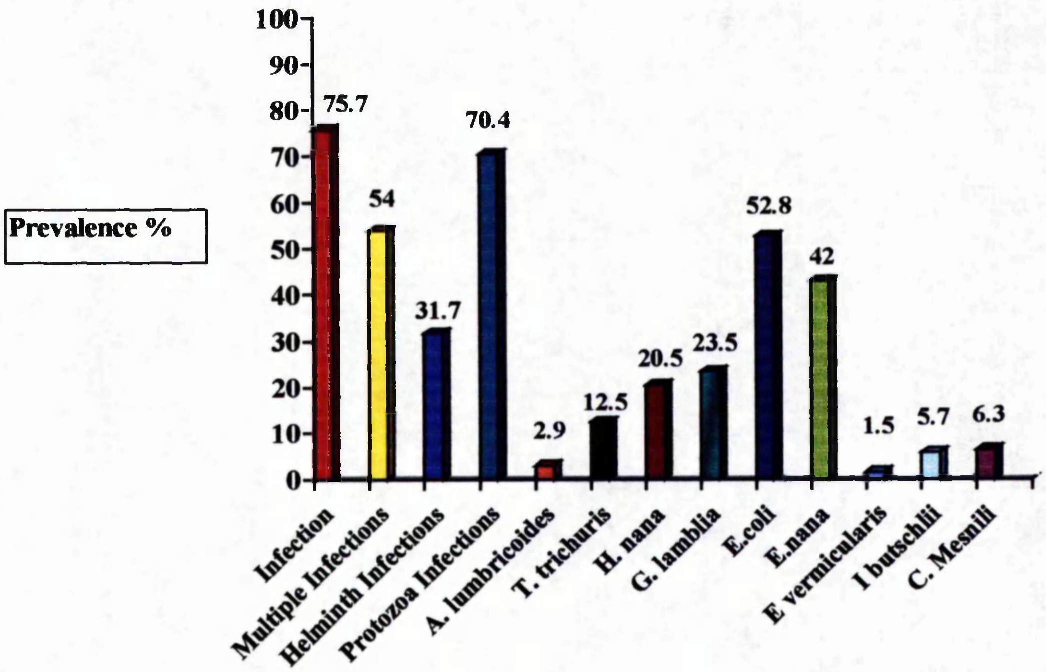


Fig 6.2 The Prevalence of Intestinal Parasitic Infections in 341 School-Children from the State of Sinaloa.

E. coli, and *E. nana* were found to be the intestinal parasites (protozoan) with the highest prevalences (52.8%, and 42% respectively) ($p<0.05$) in the State of Sinaloa. *E. coli* and *E. nana* contributed significantly to the prevalence of protozoan infections ($X^2=13.1$ and $X^2= 3.9$ respectively, df=1, $p<0.05$).

The prevalence of ascariasis was significantly lower than those showed by the rest of the species of parasites identified (chi-square, $p<0.05$) (Fig. 6.2) and the prevalence of trichuriasis was lower than *E. coli* and *E. nana* ($X^2= 34.9$ and $X^2= 21.3$ respectively, $p<0.001$). The prevalences of *E. vermicularis* (1.5%), *I. butschlii* (5.7%), and *C. mesnili* (6.3%) were also found to be low.

6.2.2 General Prevalence of Intestinal Parasitic Infections in School Children from Suburban and Rural Communities in the State of Oaxaca, Mexico.

A total of 453 stool samples from 151 school children aged 6 to 10 years enrolled in the primary schools of 1 suburban and 4 rural communities (Lomas de San Jacinto, Rancho la Era, La Lobera, Pluma Hidalgo, and Sta. Maria. de Magdalena) in the State of Oaxaca, 28% (n=151) (66 females and 85 males) were collected (3 stools per child) and analysed to estimate the prevalence of the different parasitic infections per each community (appendix 8) and the prevalence average in the State of Oaxaca (Fig. 6.3). High prevalences of parasitic infections, multiple infections, helminth infections, and protozoan infections were observed (73.5, 53.6, 49 and 60.9% respectively) (Fig 6.3). No difference was found between the prevalence of protozoan infections (60.9%) and helminth infections (49%) ($X^2=2.01$, $p= 0.1556$) in the State of Oaxaca. *G. lamblia*, *E. coli*, and *E. nana* were found to be the intestinal parasites (protozoan) with the highest prevalences (31.1%, 40.4%, and 35.1% respectively) in Oaxaca ($X^2=2122.79$, $p<0.05$). *G. lamblia*, *E. coli* and *E. nana* contributed significantly to the prevalence of protozoan infections ($X^2= 7.4$, $X^2=2.3$, and $X^2= 0.92$ respectively), (df=1, $p<0.05$).

A. lumbricoides showed a lower prevalence than the rest of the species of parasites (Chi-square, $p<0.05$) except against *T. trichiura*, and *E. histolytica* ($X^2= 0.6114$ and $X^2= 0.0$, respectively, $p>0.05$). The prevalence of *T. trichiura* was significantly lower than those showed by *H. nana*, *G. lamblia*, *E. coli*, and *E. nana* ($p<0.05$) (Fig. 6.3). *E. vermicularis* and *C. mesnili* were not detected in school children from Oaxaca. *H. nana* showed the highest prevalence (21.9%) among the helminth species (*A. lumbricoides* and *T.*

trichiura)($p<0.05$) (Fig 6.3). A low prevalence of *E. histolytica* (7.3%) was observed in school children from Oaxaca. 62.3% of the school children presented pathogenic parasites (*A. lumbricoides*, *T. trichiura*, *H. nana*, *E. histolytica*, and *G. lamblia*).

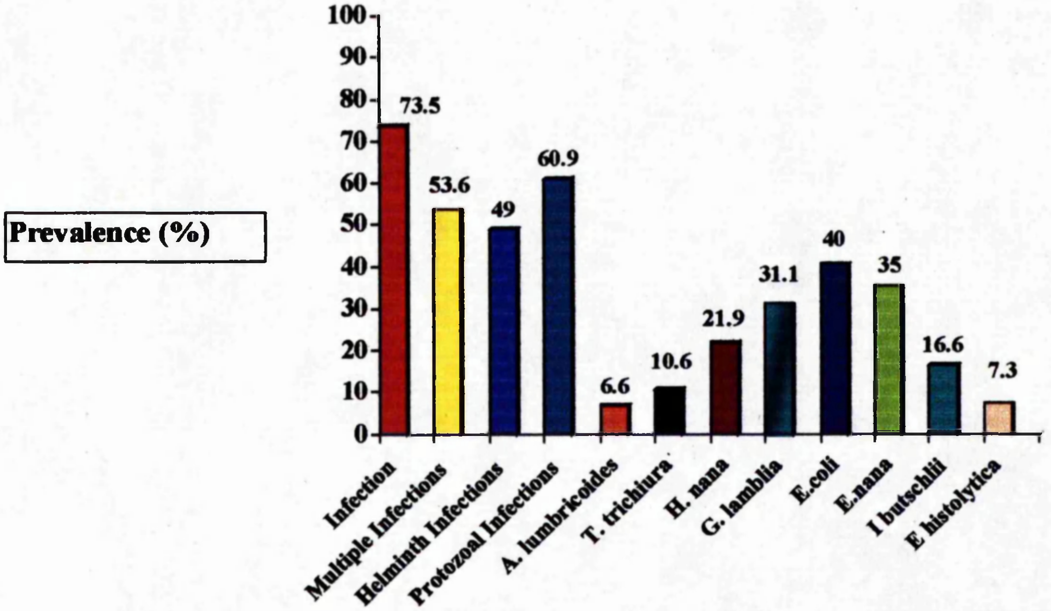


Fig.6.3 The prevalence of Intestinal Parasitic Infections in 151 School-Children from the State of Oaxaca.

6.3 Comparison of Intestinal Parasitic Infections between the States of Sinaloa and Oaxaca.

The statistical comparison (Chi-square) of the prevalence of the different intestinal parasitic infections evaluated from 492 school children between the States of Sinaloa (n=341) and Oaxaca (n=151) was as follows:

High prevalences of intestinal parasitic infections and multiple infections were found in the States of Sinaloa (75.6% and 54% respectively) and Oaxaca (73.5% and 53.6%

respectively). A significant higher prevalence of helminth infections (X^2= 6.02, p<0.01) was found in Oaxaca than that in Sinaloa. No statistical difference was observed in the prevalences of protozoan infections (X^2= 1.41, p=0.2340) or multiple infections (X^2= 0.02, p=0.8873) between Sinaloa and Oaxaca. In addition, no difference was found in the prevalences of the different species between both States, excepting for *I. butschlii* (it was higher in Oaxaca than Sonora, X^2= 10.31, p= 0.0031). *E. vermicularis* and *C. mesnili* were found only in Sinaloa (1.4% and 6.1% respectively), and *E. histolytica* in Oaxaca (8%), although in low prevalence.

6.3.1 Brief Analysis about the Trends of Intestinal Parasitic Infections in the Different Communities of Sinaloa and Oaxaca.

Sta. Maria de Magdalena, Oaxaca (92.3%), Pluma Hidalgo, Oaxaca (90.9%), and Imala, Sinaloa (84.2%) showed high intestinal parasitic infections in comparison with El Paraiso, Sinaloa (54.5%), Las Puentes, Sinaloa (66.1%), La Era, Oaxaca (63.6%), and Lomas de San Jacinto, Oaxaca (60%) (p<0.05) (appendixes 7 and 8). The lowest prevalence of intestinal parasitic infections was found in the communities of El Paraiso, Sinaloa (54.6%) and Lomas de San Jacinto, Oaxaca (60%). The prevalence of helminth infections was found to be low in the communities of Doroteo Arango, Sinaloa (17.4%) and El Paraiso, Sinaloa (18.2%), and high in Sta. Maria de Magdalena, Oaxaca and Pluma Hidalgo, Oaxaca. Sta. Maria de Magdalena, Oaxaca showed a high prevalence of protozoan infections and the opposite was found in Lomas de San Jacinto, Oaxaca. The presence of *A. lumbricoides* and *T. trichiura* was important in Sta. Maria de Magdalena, Oaxaca, Pluma Hidalgo, Oaxaca, and El Higueral, Sinaloa, *G. lamblia* in Sta. Maria de Magdalena, Oaxaca, and Pluma Hidalgo, Oaxaca, and *H. histolytica* Sta. Maria de Magdalena, Oaxaca, and La Era, Oaxaca.

6.4 The Prevalence of Intestinal Parasitic Infections in School Children in the Communities of the States of Sinaloa and Oaxaca by Gender and Age Classes.

Statistical analysis (Chi-square) showed no significant difference in the prevalence of the different intestinal parasitic infections between 240 (48.8%) school age females and 252 (51.2%) males from the States of Sinaloa and Oaxaca (infection X^2=0.24, p=0.6242; multiple infection X^2= 0.3216, p=0.57; protozoan X^2=1.10, p=0.3; and helminths X^2= 0.0, p=1.0).

In addition, a total of 174 (51.02%) girls and 167 (48.9%) boys aged 6-10 years from rural communities in the State of Sinaloa were recruited for parasitological analysis. Statistical comparisons (Chi-square) of the prevalence of parasitic infections, multiple infections, helminth infections, and protozoan infections between genders showed no statistical difference (X^2= 0.10, p=0.7422; X^2=0.72, p=0.0.39; X^2= 0.0, p=1.0; X^2= 1.16, p=0.28, t=1.5518 respectively, p>0.05). No difference was found in the prevalence of the different species of parasites between genders per each rural community in Sinaloa.

A total of 66 (43.7%) girls and 85 (56.3%) boys aged 6-10 years from 1 sub-urban and 4 rural communities were recruited in the State of Oaxaca. Statistical comparisons (Chi-square) of the prevalence of parasitic, multiple, helminth, and protozoan infections between genders showed also no statistical difference (X^2=0.92, p=0.33; X^2=0.0, p=1.0; X^2= 0.98, p=0.32; X^2=0.33, p=0.56 respectively, p>0.05). No difference was found in the prevalence of the different species of parasites between genders per each community in Oaxaca.

6.4.1 Prevalence of Intestinal Parasitic Infections by Age Classes in the States of Sinaloa and Oaxaca.

Analysis of the general prevalence of intestinal parasitic infections by age classes in the States of Sinaloa and Oaxaca showed the following tendencies: the prevalence of parasitic, multiple, and protozoan infections showed a decrease with age (Fig. 6.4). The prevalence of helminth infections remained constant and low throughout all ages. *A. lumbricoides* remained low for each class age, while *T. trichiura* increased slightly with age, and *H. nana* decreased slightly. *G. lamblia* prevalence also remained constant with age, but *E. coli* and *E. nana* decreased slightly although their prevalence remained high. The prevalence of *E. histolytica*, *I. butschlii*, *C. mesnili*, and *E. vermicularis* remained low.

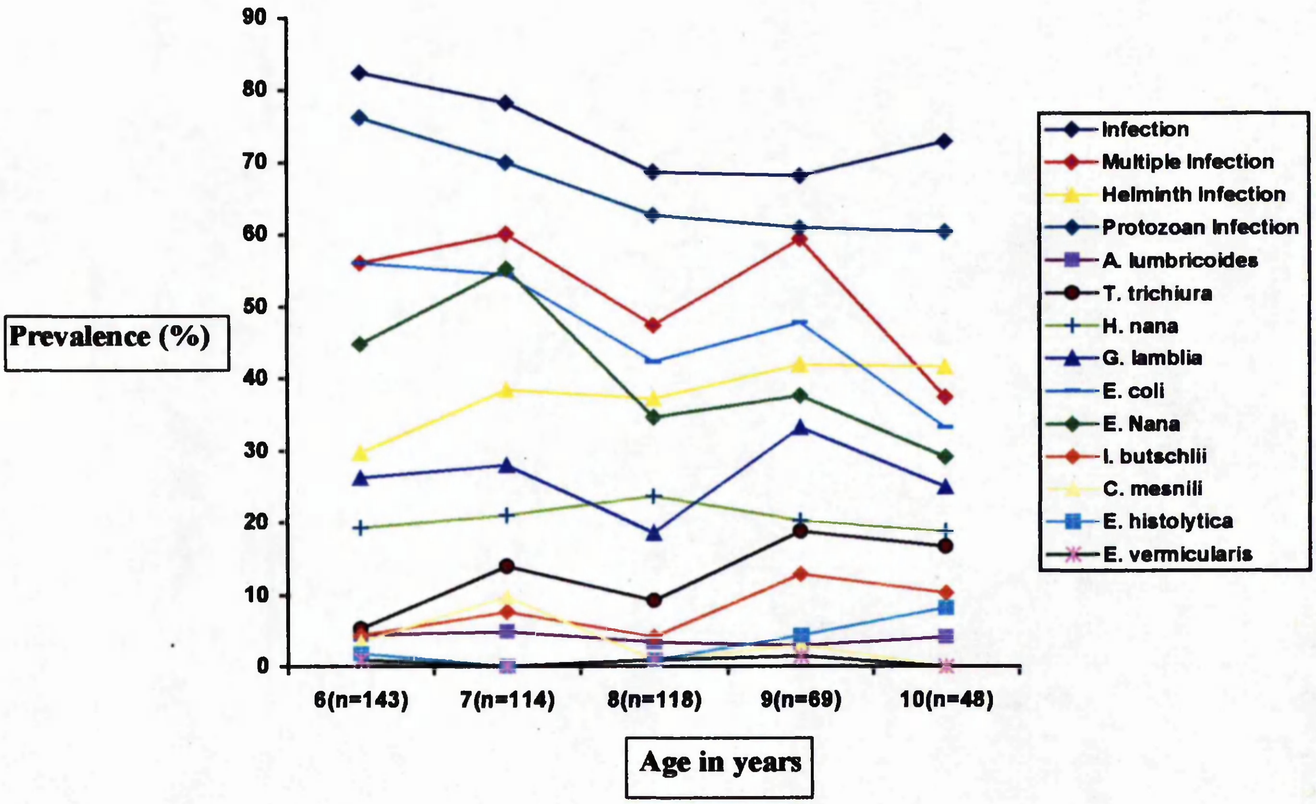


Fig. 6.4 The General Prevalence of Intestinal Parasitic Infections by Age Classes in 492 School Children from the States of Sinaloa and Oaxaca.

Analysis of the prevalence of intestinal parasitic infections by age classes in Sinaloa showed the following tendencies: the prevalence of parasitic infections, multiple infections, and protozoan infections showed a decrease with age (Fig. 6.5). The prevalence of helminth infections remained constant but low with age. *A. lumbricoides* remained low for each age class, but *T. trichiura* increased with age, and *H. nana* remained stable. *G. lamblia* also remained stable with age, but *E. coli* and *E. nana* decreased with age although their prevalence remained high. The prevalence of *I. butschlii*, *C. mesnili*, and *Enterobius vermicularis* remained low throughout the age range.

High peaks of parasitic infections, protozoan infections, *E. coli*, and *C. mesnili* were observed in the 6 and 7 age-groups. In addition, a peak of *T. trichiura* was observed in the 10 age-group. There were few children in some age classes when results were analysed per each community.

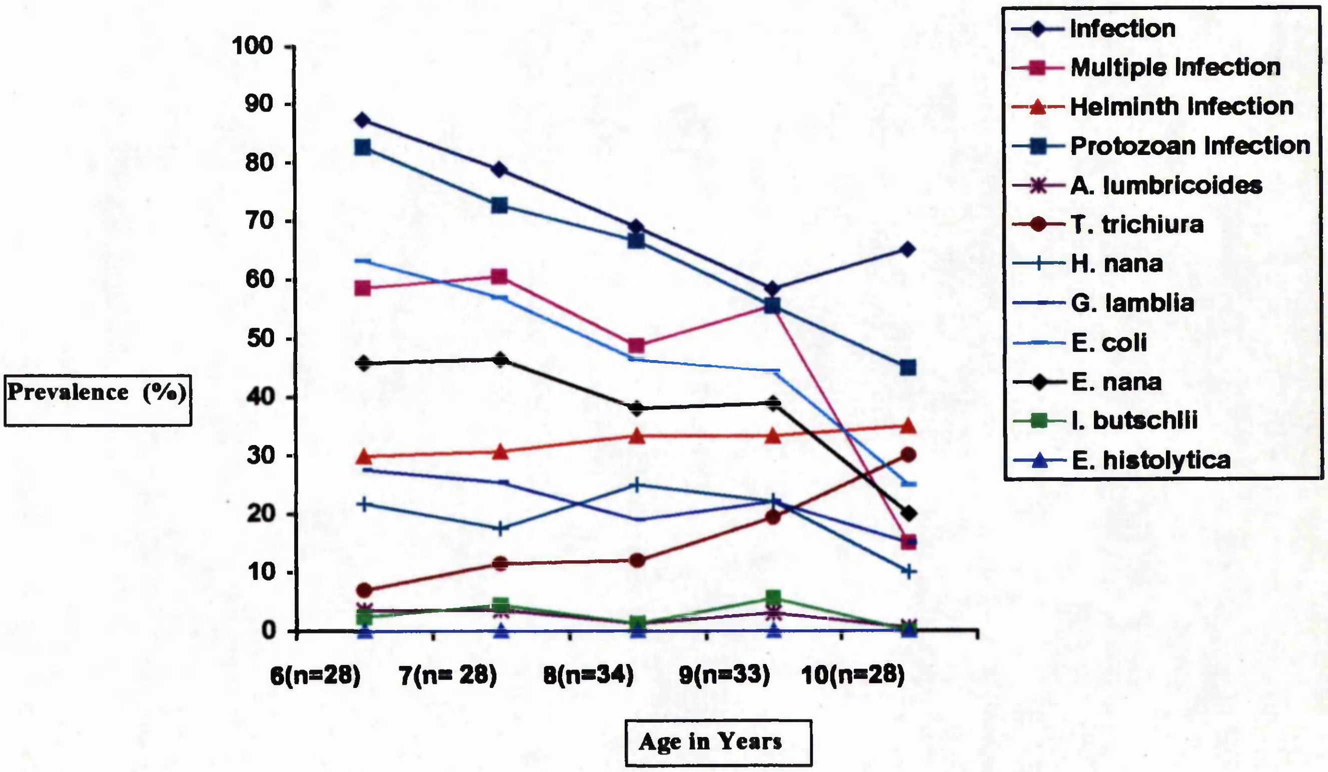


Fig 6.5 The prevalence of Intestinal Parasitic Infections by Age Classes in 341 Rural School Children from the State of Sinaloa.

Analysis of the prevalence of intestinal parasitic infections by age classes in Oaxaca showed the following tendencies: the prevalences of parasitic, multiple, protozoan, and helminth infections remained stable and high through the age range (Fig. 6.6). *A. lumbricoides* remained very low all the time, and *T. trichiura* decreased with age. *G. lamblia*, *E. coli*, *E. nana*, *H. nana* and *I. butschlii* remained constant and high with age. The prevalence of *Entamoeba histolytica* was found to be constant and low throughout the age classes. A higher peak of helminth infections and *T. trichiura* was observed in the 7 year age-group (Fig. 6.6). There were few children in some age classes when results were analysed by community.

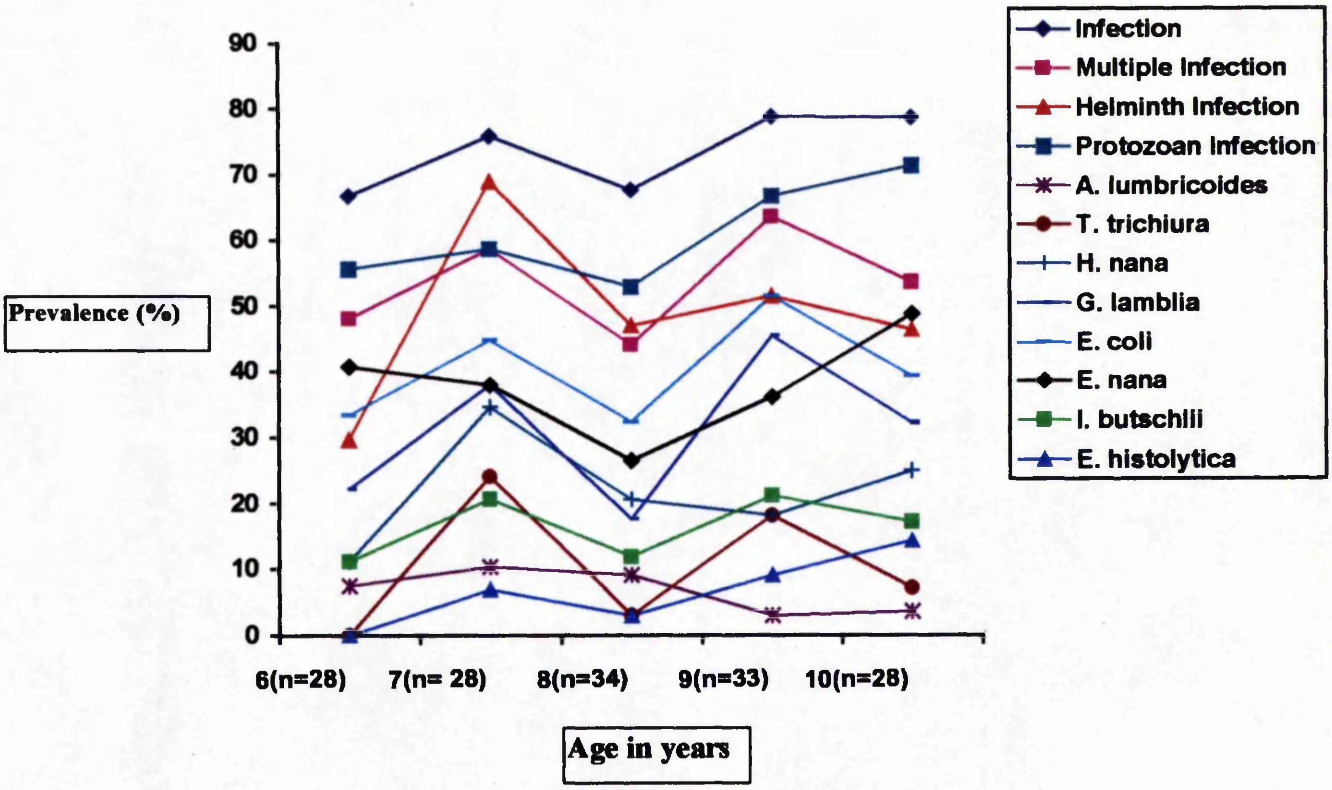


Fig. 6.6 The Prevalence of Intestinal Parasitic Infections by Age Classes in 151 School Children from the State of Oaxaca

The statistical comparison (Chi-square) of the prevalence of the different intestinal parasitic infections in the 6-year-old school children between Sinaloa and Oaxaca showed a higher prevalence of intestinal parasitic infections (X^2= 11.1, p=0.0008), protozoan infections (X^2= 15.6, p=0.0000), *E. coli* (X^2=16.8, p=0.000) in 6-year-old school children (n=87) from Sinaloa than those found in school children of the same age (n=27) from Oaxaca.. A significant higher prevalence of helminth infections (X^2= 27.4, p=0.0000), *H. nana* (X^2=6.7, p=0.009), and *I. butschlii* (X^2=10.6, p=0.001) was found in 7-year-old school children from Oaxaca (n=29) than those found in children of the same age from

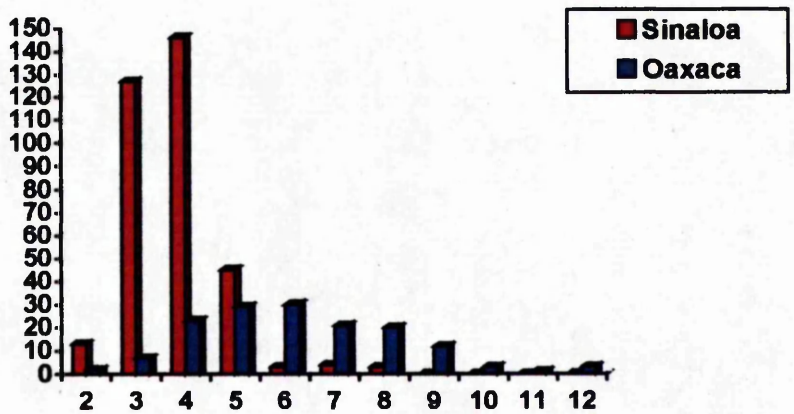
Sinaloa (n=114) (df= 141, p<0.05). The prevalence of *I. butschlii* (X^2=7.2, p=0.007) was higher in 8-year-old school children from Oaxaca (n=34) than those from Sinaloa (n= 84). The comparison also showed a significant higher prevalence of *G. lamblia* (X^2=7.12, p=0.007) in 9-year-old school children from Oaxaca (n=33) than those from Sinaloa. Finally, a higher prevalence of multiple infections (X^2= 30.5, p=0.0000) and *T. trichiura* (X^2= 16, p=0.0000) was found in 10-year-old school children from Oaxaca (n=28) than those from Sinaloa (n= 20).

6.5 Association between the Number of Family Members and the Intensity of Intestinal Helminth Infections in School Children from the States of Sinaloa and Oaxaca.

An association between the levels of intensity of helminths infections estimated in eggs per gram of faeces (epg) and the number of family members was analysed.

The frequency for the number of family members of the recruited school children from Sinaloa and Oaxaca (mean= 4.5, median=4, SD=1.75, range=2-12) is shown in the Fig. 6.7.

Number of Cases



Number of Family Members

Fig 6.7 Frequency of the Number of Family Members of 492 School Children in the States of Sinaloa and Oaxaca.

The levels of intensity of, ***H. nana***, ***A. lumbricoides***, and ***T. trichiura*** were estimated indirectly by counting the number of eggs per gram of faeces (epg). Epg counts were carried out in 186 (37.8%) of 492 school children of the States of Sinaloa and Oaxaca. The general results were as follows: 72 school children presented epg for *H. nana* ranging from 20 to 520 (mean 114.4, SD= 84.8), 29 with epg for *A. lumbricoides* ranging from 20 to 9200 (mean=532.8, SD=1818.6), and 85 with epg for *T. trichiura* ranging from 20 to 900 (mean= 149.4, SD=192.2). Logistic Regression analysis showed no association between the numbers of family members and levels of intensity of *H. nana* and *T. trichiura* (p>0.05).

Egg counts were carried out on 107 (31%) of 341 rural school children from Sinaloa, and the results obtained were as follows: 44 school-children presented epg for *H. nana* ranging from 40 to 300 (mean 104.6, SD= 56.5), 12 children with epg for *A. lumbricoides* ranging from 40 to 9200 (mean= 1023.3, SD= 2590.1), and 51 children with epg for *T. trichiura* ranging from 40 to 800 (mean= 129.31, SD= 148.84) (Fig 6.8). Most of the children with epg for *H. nana* were from Pueblo Nuevo, and El Higueral (12 and 14 respectively), and most of the children with epg for *A. lumbricoides* and *T. trichiura* from El Higueral (8 and 40 respectively). Logistic Regression analysis showed no relationship between the number of family members and the levels of intensity of *H. nana, A. lumbricoides*, and *T. trichiura* in the children from Sinaloa (p>0.05)

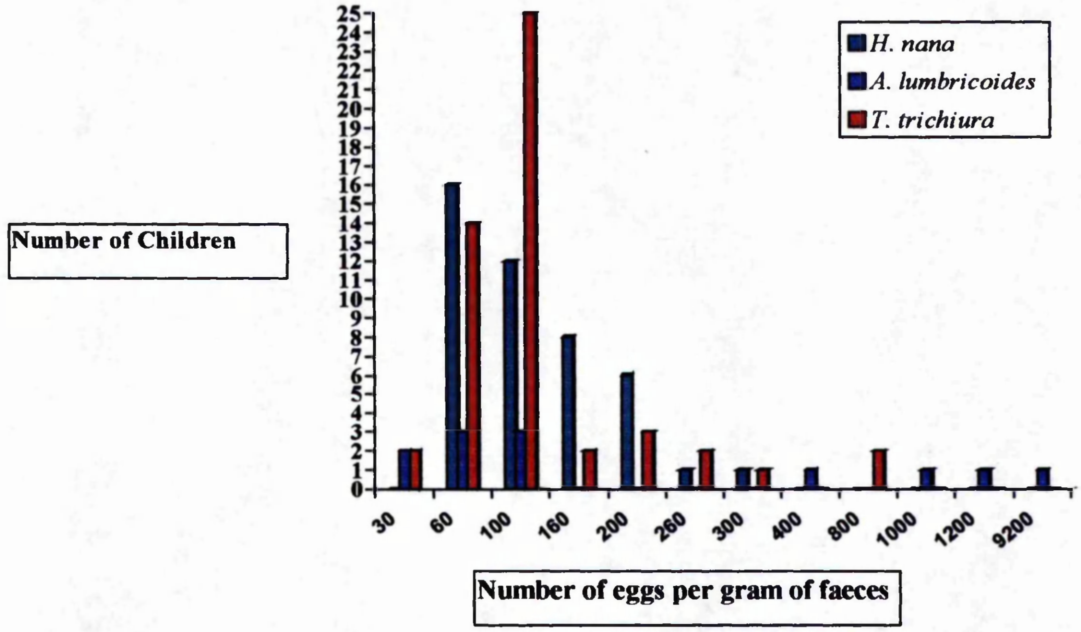


Fig 6.8 Distribution of the Number of Eggs per Gram of Faeces for *H. nana*, *A. lumbricoides*, and *T. trichiura* in 107 Rural School Children from the State of Sinaloa.

Epg counts were carried out on 79 (52.3%) from 151 school children from Oaxaca, and the results were as follows: 28 school-children presented epg for *H. nana* ranging from 40 to 800 (mean=152.72, SD= 150.73), 17 children with epg for *A. lumbricoides* ranging from 40 to 8000 (mean=920, SD= 952.6), and 34 children with epg for *T. trichiura* ranging from 40 to 1000 (mean= 183.66, SD= 248.60) (Fig 6.9). Most of the children harbouring eggs for *A. lumbricoides* and *T. trichiura* were from Pluma Hidalgo (7 and 9 respectively) and Sta. Maria de Magdalena (13 and 13 respectively). Children harbouring eggs for *H. nana* were uniformly distributed in all the communities studied. Logistic Regression analysis showed that number of family members was an influencing factor in the levels of intensity of *T. trichiura* (RC= 0.2178, SE= 0.1047, R^2= 0.030, df=1, p=0.030). The same analysis by community in Oaxaca showed no association between the number of family members and the levels of intensity of *H. nana*, *A. lumbricoides*, and *T. trichiura* (p>0.05).

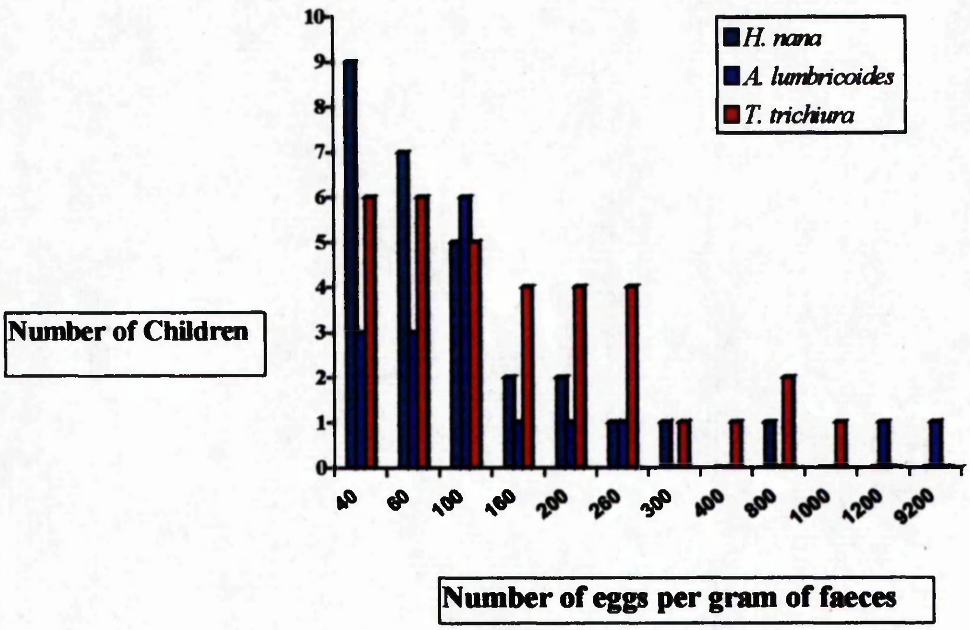
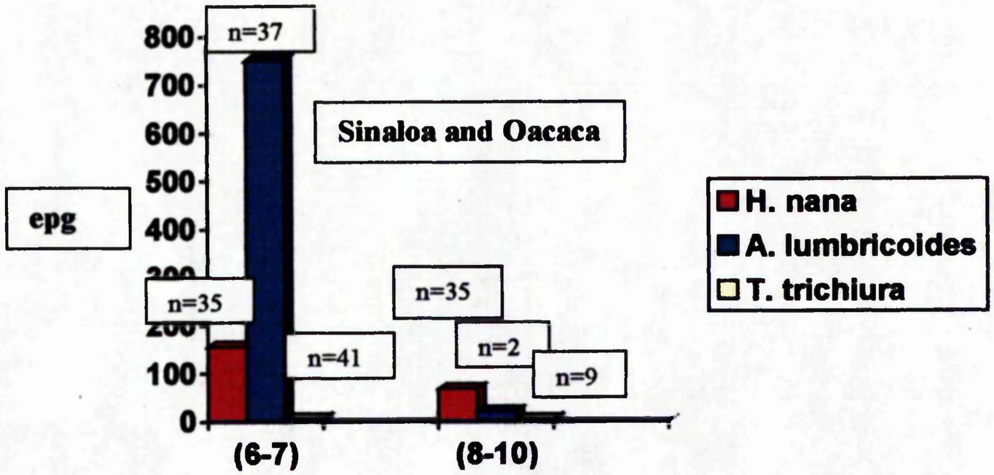


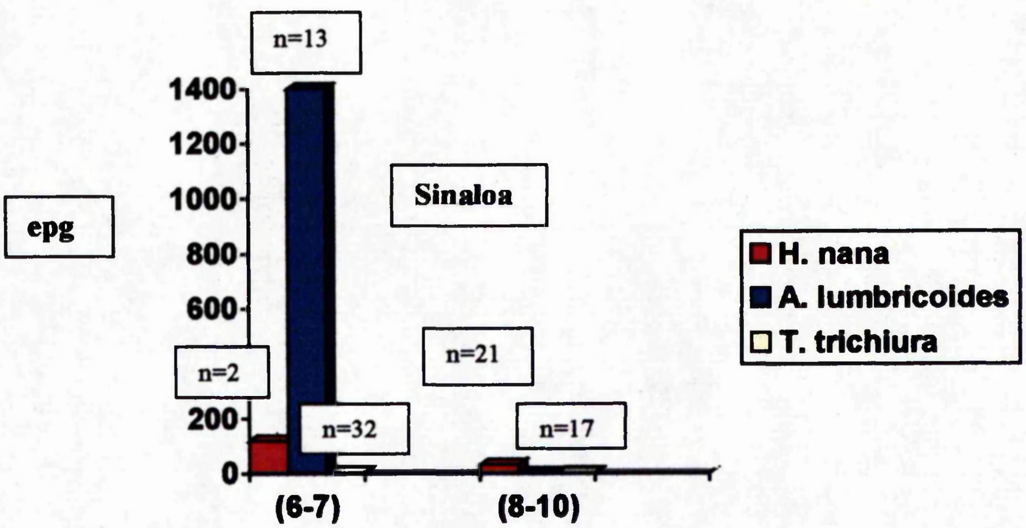
Fig 6.9 Distribution of the Number of Eggs per Gram of Faeces for *H. nana*, *A. lumbricoides*, and *T. trichiura* in 79 School Children from the State of Oaxaca.

Statistical comparison (Mann-Whitney U) showed no significant difference in the epg means of *A. lumbricoides* ($Z=2.3050$, $p>0.05$), *T. trichiura* ($Z=1.2989$, $p>0.05$), *and H. nana* ($Z=1.7025$, $p>0.05$ between the school children from the States of Sinaloa and Oaxaca.

6.5.1 Levels of Intensity of *H. nana*, *A. lumbricoides*, and *T. trichiura* and Age Classes in School Children of the States of Sinaloa and Oaxaca.

The analysis of the distribution of epg in our population showed that the younger children tend to harbour the higher epg means than the older children (Fig. 6.10, 6.11, 6.12).


Sinaloa and Oacaca
epg
800
700
600
500
400
100
0
n=37
n=35
n=41
n=35
n=2
n=9
(6-7)
(8-10)
H. nana
A. lumbricoides
T. trichiura


Sinaloa
epg
1400
1200
1000
800
600
200
0
n=13
n=2
n=32
n=21
n=17
(6-7)
(8-10)
H. nana
A. lumbricoides
T. trichiura

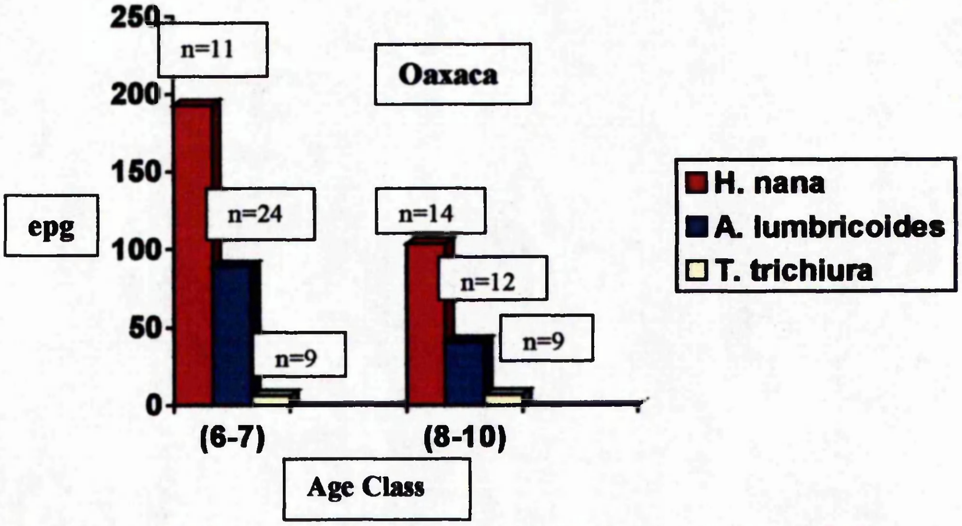


Fig 6.10, 6.11, 6.12 Means of Eggs per Gram of Faeces for *H. nana*, *A. lumbricoides*, and *T. trichiura* in 186 School Children by each Class from the States of Sinaloa and Oaxaca.

6.6 DISCUSSION AND CONCLUSSIONS.

6.6.1 Current Trends of Intestinal Parasitic Infections in School Children in the States of Sinaloa and Oaxaca.

This study has provided evidence that intestinal parasitic infections are still highly prevalent in school children from the States of Sinaloa and Oaxaca after seven years of an active National deworming campaign. The school children from Sinaloa and Oaxaca are highly susceptible to intestinal parasitic infections. More than 50% of the sampled population were infected with intestinal parasites and more than 50% harbouring more than one intestinal parasites species. However, children from Sinaloa were found to be more susceptible to protozoan infections than helminth infections. School children from Oaxaca are at the same risk to protozoan and helminth infections. Statistical comparisons showed that school children from Oaxaca are currently more affected by helminth infections than those from Sinaloa. Protozoan infections are equally present in both States and *G. lamblia* is still an important protagonist as pathogenic protozoan species.

The school children from Sta. Maria de Magdalena and Pluma Hidalgo of Oaxaca were found to be the most affected by these infections. Otherwise, the school children from

Imala showed the highest prevalence of intestinal parasitic infections in Sinaloa, meanwhile Doroteo Arango and Paraiso showed the lowest prevalence of intestinal parasitic infections, multiple infections, helminth infections in the same State.

The school children of the sub-urban community of Lomas de San Jacinto Oaxaca showed the lowest prevalence of intestinal parasitic infections, protozoan infections, and multiple infections among all communities in this study. The distance from the city of Oaxaca and the rough geographical terrain to gain access to the rural communities of Pluma Hidalgo and Sta. Maria de Magdalena probably influenced in the observed poor medical attention, lack of medicines, poor surveillance in the albendazole distribution and education programmes provided by the health personnel of the National deworming campaign, low socio-economic conditions, and high drop out of school children from the primary school to join their parents in agricultural labour activities (20% in agreement with records from teachers from Pluma Hidalgo and Sta. Maria de Magdalena). All these combined factors probably favour the development and high transmission of intestinal parasitic infections in their populations. This pattern was not observed in Sinaloa. The communities of Doroteo Arango and Paraiso are located further away than other communities studied from the city of Culiacan, but their school populations showed the lowest prevalence of intestinal parasitic infections among all communities of Sinaloa. The access to all communities of Sinaloa in this study was not difficult because they are established on plain terrain. Probably the combined operations of the current national deworming campaign and the smaller populations of Doroteo Arango and Paraiso may explain the low prevalence of these infections in those communities. The higher prevalences of *T. trichiura* and *A. lumbricoides* in El Higueral in comparison with those observed in the rest of the communities are probably the result of the re-infection event derived from the frequent defecation by children in small streams around this community in spite of the operations of the National deworming campaign.

Our visits to all communities provided evidence that the infrastructure and personnel for health services are distributed according to regional levels of development favouring the largest urban areas and increasing the detriment of the poorest areas of the whole country.

6.6.1.1 Previous and Current Intestinal Parasitic Infections in the States of Sinaloa and Oaxaca.

The comparative analysis from the few earlier epidemiological studies in Sinaloa and those found in this study has revealed that current prevalences of *G. lamblia, E. coli, E. nana, I. butschlii, T. trichuria, A. lumbricoides, E. vermicularis*, and *H. nana* in school children continue to be similar to those in the general population from different rural and sub-urban communities found by Diaz *et al.* from 1987 to 1994 (El Salado, Navolato, Batallon, Argentina, Villa Juarez, and the sub-urban communities El Mirador y Toledo of the city of Culiacan Sinaloa) (appendix 9). However, although *A. lumbricoides* has apparently had a low prevalence with time, it may have an important presence in isolated areas of the State of Sinaloa such as those found in the rural community of Villa Juarez (20.2%), and the sub-urban community El Mirador (17.8%) in the city of Culiacan in 1994. Hookworm infections, *S. stercoralis*, and *E. histolytica* were reported at low levels in those studies, but these infections were not detected in our study population, probably because of the different environmental conditions prevailing between our study sites and those from the reported sites and the different age-ranges of the study populations.

Comparisons between previous epidemiological studies in Oaxaca and those from the present study revealed that the prevalences of *G. lamblia, E. coli, E. nana,* and *I. butschlii* are currently higher than those reported in the general population by Navarrete *et al.* (1993), Soriano, (1998), and LESPO (1999) (appendix 10). Although the prevalence of *T. trichiura* showed no significant change, the prevalence of *A. lumbricoides* has shown a decrease with time in Oaxaca, probably as a result of the current national deworming campaign. Hookworm infections and *E. vermicularis* were also reported in those earlier studies, but these infections were not detected in the present study. Probable explanatory factors include the different environmental conditions and age-ranges of the populations examined between the present study compared with those from the previous epidemiological studies, the inappropriate technique to detect eggs of *E. vermicularis* (the technique of Graham using the scotch tape is the appropriate method) or the success of the national deworming campaign may have resulted in the absence of hookworm infections and *E. vermicularis* in Oaxaca in this study.

These comparisons have shown that protozoan infections have maintained high levels in Sinaloa and increasing levels in Oaxaca, with exception of *A. lumbricoides* which has maintained low levels in Sinaloa and an important decrease with time in Oaxaca.

Certainly, the observed trends for ascariasis are a consequence of the successful of the National deworming campaign which is currently administering albendazole to Mexican school children.

6.6.2 Current Levels of Intensities of *H. nana*, *A. lumbricoides*, and *T. trichiura* in School Children of the States of Sinaloa and Oaxaca.

The most convenient means of estimating the level of intensity of infections is quantifying the number of worms expelled after treatment. However, because most of school children in this study defaecated in open areas, it was difficult to obtain reliable data about the number of worms expelled. Instead, the level of intensities of *H. nana*, *A. lumbricoides*, and *T .trichiura* were estimated by quantifying the number of eggs per gram of faeces on the assumption that this is directly proportional to the number of worms in the intestine, although several sources of variation such as production of eggs by females, intra-individual variation of eggs counts day to day, and fecundity of female dependent on worm density make egg counts a relatively unreliable estimate of the intensity of infection. However, the epg mean for large groups can be representative of the mean worm burdens.

Results showed no difference in the levels of intensities of *H. nana*, *A. lumbricoides*, and *T. trichiura* in school children from Sinaloa and those in school children from Oaxaca. It was also observed that eggs of *H. nana*, *A. lumbricoides*, and *T. trichiura* were concentrated in children living in the communities of Pueblo Nuevo and El Higueral in the State of Sinaloa. However, the risk of harbouring a higher number of eggs for *A. lumbricoides* and *T. trichiura* was only associated with the higher prevalence of these infections found in the rural community of El Higueral. The prevalence and level of the intensity of *H. nana* had no relationship with location.

Eggs of *A. lumbricoides* and *T. trichiura* were mainly concentrated in school children living in the communities of Pluma Hidalgo and Sta. Maria de Magdalena in Oaxaca. The risk of harbouring a higher epg counts for *A. lumbricoides* and *T. trichiura* was associated with the higher prevalence of these infections found in these rural communities. The prevalence and level of the intensity of *H. nana* had no relationship to location.

Therefore, school children from the rural communities of El Higueral, Pluma Hidalgo and Sta. Maria de Magdalena are highly likely to present more severe clinical symptoms associated with the high level of intensities of *A. lumbricoides* and *T .trichiura*

6.6.3 Association between Intestinal Parasitic Infections and Demographic Factors in School Children of the States of Sinaloa and Oaxaca

6.6.3.1 Gender

Results showed that the gender of the school children had no influence on the prevalence of the intestinal parasitic infections found in the different communities included in this study. Probably, behaviour was not sufficiently different between school males and females to unbalance the prevalence of the parasitic infections for a particular gender. Some authors have found that the prevalence of ascariasis is higher in females than in males regardless of age (Cabrera and Valeza, 1980; Shield *et al.*, 1980). Evidence for an association between prevalence and host sex is not always detected (Elkins, 1986). In some communities, in which no-'sex-related' effects had been detected in children, women were found to be more frequently infected with ascariasis than men. Probably factors such as culture and job-systems increase the risk of infection (WHO, 1967).

6.6.3.2 Age Classes

It was observed that the prevalence of protozoan infections showed a decrease with age but helminth infections remained stable and low in school children from Sinaloa. The prevalence of *A. lumbricoides* was found to be stable but low with age, and *T. trichiura* increased slightly with age. The prevalence of protozoan and helminth infections showed different trends with age in children from the State of Oaxaca. Apparently, the lack of suitable treatment, environmental and behavioural factors are more associated with the stable prevalence of *G. lamblia*, *E. coli*, *E. nana*, *H. nana*, *I butschlii*, and *E. histolytica* with age. It was also observed that levels of *A. lumbricoides* remained low with age, and *T. trichiura* decreased with age. Probably, current operations of the national administration of albendazole are apparently maintaining the low levels of infection and re-infection with *A. lumbricoides* in school children, but is showing a lower efficacy against *T. trichiura* and the re-infection event is probably responsible of the increase of *T. trichiura* with age.

Some factors such as changes in behaviour and significant immune-responses with age, access to the appropriate treatment, nutritional status, and events of re-infection are probably playing an important role in determine the presence of these infections with age in the school children from both States

Finally, it was observed that younger school children harbour higher eggs counts than older children from both States. A similar pattern has been reported by Asaolu *et al.*, (1992) who found an increase of eggs counts for ascariasis and trichuriasis with age, reaching a peak in the 10-14-year old children. Thein Hlaing *et al.*, (1984) and Arfaa and Ghadirian (1977) revealed two different patterns in the intensity of ascariasis in Burma and Iran respectively. The worm burden increased from 5 to 9 and declined in older age groups in Burma. In the Iranian study worm burdens reached a peak in the group aged 20 to 39 years old. The persistence of intestinal parasitic infections over prolonged periods of time, significant changes in behaviour and immune-responses, may have influenced these results.

6.6.3.3 Number of Family Members.

The number of family members was found not to be associated with the levels of intensity of *A. lumbricoides*, *H. nana,* and *T. trichiura* in school children from Sinaloa and Oaxaca. Several authors have found associations between large family numbers with high intensities of soil-transmitted helminths. Thein Hlaing *et al.* (1984) showed that the prevalence, number of infected individuals, and number of children harbouring more worms increased with larger family numbers. Asaolu *et al.* (1992) observed that the mean epg of *A. lumbricoides*, *T. trichiura*, and hookworm increased with increasing household size. The absence of association observed between household size and the levels of intensity of these infections in the school children in this study may result from the twice yearly national administration of albendazole in school children which is reducing the number of infective eggs in the environment and subsequently any intra-family transmission

CHAPTER 7

NUTRITIONAL STATUS, DAILY NUTRIENT INTAKES, AND INTESTINAL PARASITIC INFECTIONS IN SCHOOL CHILDREN FROM THE STATES OF OAXACA AND SINALOA.

7.1 Relationship between Nutritional Status and Intestinal Parasitic Infections in School Children from the States of Sinaloa and Oaxaca.

The weight and height of 260 school children from the States of Sinaloa and Oaxaca were measured in order to investigate the influence of the nutritional status determined by the Z scores of the ratios H/A, W/A, and W/H in the prevalence of the intestinal parasitic infections. The general results for the nutritional status of these children were as follows (Table 7.1). A high percentage of the school children in this study showed mild malnutrition in agreement to the three ratios (Z H/A, Z W/A, Z W/H).

Table 7.1 The Nutritional Status using the Z Score for the H/A, W/A, and W/H ratios in 260 School Children from States of Sinaloa and Oaxaca.

	Indicators of the Nutritional Status		
Nutritional Status	**Z H/A**	**Z W/A**	**Z W/H**
Severe Malnutrition (<-3)	**11 (4.2%)**	**1 (0.4%)**	**2 (0.8%)**
Moderate Malnutrition (-3, -2)	**48 (18.5%)**	**24 (9.2%)**	**6 (2.3%)**
Mild Malnutrition (-2,-1)	**61 (23.5%)**	**72 (27.7%)**	**22 (8.6%)**
Normal (-1,+1)	**126 (61%)**	**145 (55.8%)**	**186 (72.6%)**
Obesity degree 1 (>+1,+2)	**10 (3.8%)**	**11 (4.2%)**	**31 (12.1%)**
Obesity degree 2 (>+2,+3)	**4 (1.5%)**	**7 (2.7%)**	**9 (3.5%)**
	n=260	**n=260**	**n=256**

(controlling for sex, age, height, and weight) showed a significant higher Z W/H for uninfected than that for infected children (X^2=3.7941, $p<0.05$). A significant difference was also observed for Z W/H between uninfected and infected children with protozoan (X^2=3.502, $p<0.05$), helminth infections (X^2=2.3855, $p<0.05$), multiple infections (X^2=8.532, $p<0.05$), pathogenic parasitic infections (X^2=1.630, $p<0.05$), *E. coli* (X^2=4.007, $p<0.05$), *E. nana* (X^2=8.366, $p<0.05$), *H. nana* (X^2=0.6759, $p<0.05$), and *T. trichiura* (X^2=2.2918, $p<0.05$). Pearson's correlation showed that the nutritional status determined by the indicator W/H was negatively associated with the prevalence of parasitic infections (RC=-0.1385, p=0.05), multiple infections (RC=-0.1385, $p<0.05$), and pathogenic parasitic infections (RC= -0.1525, $p<0.05$).No statistical association was observed using Z scores for the ratios H/A and W/A and the prevalence of the different intestinal parasitic infections in school children of the States of Sinaloa and Oaxaca. Comparative analysis between both States (chi-square) showed a higher prevalence of malnutrition according to the ratio Z H/A (X^2= 6.4896, $p<0.05$) in Oaxaca (72.3%) than that found in Sinaloa (30.8%). In addition, a higher prevalence of malnutrition for the ratio Z W/A (X^2= 7.95, $p<0.05$) was found in Oaxaca (61.3%) than that in Sinaloa (15.4%). There was no significant difference in the prevalence of malnutrition according to the ratio Z W/H (X^2= 0.049, $p>0.05$) between Sinaloa (8%) and Oaxaca (11.7%).The comparison of the prevalence of intestinal infections between children with different degree of malnutrition, showed a higher prevalence of protozoan infections (X^2= 0.5019) and *G. lamblia* (X^2= 0.2380) in children with mild malnutrition (27.7%) than those found in normal nourished children in the States of Sinaloa and Oaxaca (55.8%) according to the ratio Z W/A ($p<0.05$). There was no statistical difference in the prevalence of the rest of each species of parasites between these children ($p>0.05$). No significant difference was found in the prevalence of the different intestinal parasitic infections between children with mild (27.7%) and moderate (9.2%) malnutrition according to the ratio Z W/A ($p>0.05$). A significant lower prevalence of *G. lamblia* (9.2%) and *I. butschlii* (21.8%) was observed in normal nourished school children than that observed (16% and 32% respectively) in children with mild malnutrition ($p<0.05$) according to the ratio Z H/A. No significant difference was found in the prevalence of parasitic infections between children normally nourished (61%) and moderate malnutrition (18.5%), according to the ratio Z H/A ($p>0.05$). There was a low prevalence of severe malnutrition for the ZH/A and statistical analysis were not considered using this degree of malnutrition. A significant lower prevalence of helminth infections, protozoan infections, and trichuriasis was observed in normal nourished school children (51.5%, 6.2%, and 14.9% respectively) than that in children with mild malnutrition (81.2%, 43.1% and 38% respectively) ($p<0.05$), according to the ratio Z W/A. There was a low prevalence of moderate and severe malnutrition for the Z W/A and statistical analysis were not considered using these degrees of malnutrition.

7.1.1 Association between the Intestinal Parasitic Infections and Nutritional Status of Rural School Children from the State of Sinaloa.

The weight and height of 68 and 52 school children were measured from Las Puentes and El Higueral respectively in the State of Sinaloa. In agreement with the ratio H/A, 12.5% and 3.3% of the children (n= 120) presented mild and moderate malnutrition respectively, for the ratio W/A 9.1% and 2.5% presented mild and moderate malnutrition, and for the ratio W/H 5.8% and 0.8%, presented mild and moderate malnutrition respectively. The nutritional status of these school children for each community is shown in the Table 7.2.

Table 7.2 The Nutritional Status According to the ZH/A, ZW/A, and ZW/H Indicators in 120 School Children from the State of Sinaloa.

	Indicators of the Nutritional Status					
	Las Puentes			El Higueral		
Nutritional Status	**ZH/A**	**ZW/A**	**ZW/H**	**ZH/A**	**ZW/A**	**ZW/H**
Severe Malnutrition (<-3)	0	0	0	0	0	0
Moderate Malnutrition (-3, -2)	2 (2.9%)	2 (2.9%)	1 (1.4%)	2 (3.8%)	1 (2%)	0
Mild Malnutrition (-2,-1)	6 (9%)	6 (9%)	3 (4.4%)	9 (17.3%)	5 (9.6%)	4 (8%)
Normal (-1,+1)	50 (73.5%)	47 (69%)	50 (73.5%)	37 (71.1%)	42 (81%)	39 (78%)
Obesity degree 1 (>+1,+2)	7 (10.3%)	9 (13.2%)	11 (16.1%)	4 (7.6%)	2 (4%)	5 (10%)
Obesity degree 2 (>+2,+3)	3 (4.4%)	4 (6%)	3 (4.4%)	0	4 (8%)	2 (4%)
	n=68	n=68	n=68	n=52	n=52	n=50

GLM (adjusting for sex, age, height, and weight) showed no significant difference in the Z-scores of the nutritional indicators H/A, W/A, and W/H between infected and uninfected school children for each community. Pearson's correlation showed no statistical association between the Z scores for the nutritional indicators H/A, W/A, and W/H, and the prevalence in Sinaloa.

7.1.1.1 Association between the Intestinal Parasitic Infections and Mild, Moderate, and Severe Nutritional Status in Rural School Children of the State of Sinaloa.

A significant difference was found in the prevalence of parasitic infections (X^2= 9.4, p= 0.002), helminth infections (X^2= 4.8, p= 0.03), and multiple infections (X^2= 5.6, p= 0.018) between normal nourished children (n=87) and mild malnourished children (n=15) according to the indicator Z H/A. There was no statistical difference in the prevalence for each parasite species between these children (P>0.05) (Table 7.3). A statistical difference was found in the prevalence of intestinal parasitic infections (X^2= 3.9, p= 0.047), helminth infections (X^2= 4.0, p= 0.045), *G. lamblia* (X^2=6.2, p=0.012) between normal nourished children (n=89), and mild malnourished children (n=11) according to the ratio ZW/A. There was no statistical difference in the prevalences for the rest of species of parasites between these children (P>0.05) (Table 7.3).Pearson's correlation showed no relationship between the different parasitic infections in children with mild malnutrition according to the ratios Z H/A and Z W/A (p> 0.05). There were few children with moderate and severe malnutrition according to the 3 nutritional indicators Z W/A, Z H/A, and Z W/H, and few children with mild malnutrition according to the Z W/H indicator in children from Sinaloa (Table 7.2), statistical comparison using this information was not considered.

Table 7.3 The Prevalence of the Different Intestinal Parasitic Infections between Normal and Mild Nourished School Children from the State of Sinaloa according to the Z H/A and W/A Indicators.

	ZH/A		ZW/A	
	Normal (n=87)	Mild (n=15)	Normal (n=89)	Mild (n=11)
	Prevalence (%)			
Parasitic Infection	72.9*	90*	75*	87.5+
Helminth Infections	54*	70*	50*	86*
Protozoan Infection	70.2	80	70	75
Multiple Infection	64.8*	80*	65	75
E. coli	62.1	70	65	50
E. nana	43.2	50	42.5	35
G. lamblia	2.7	10	2.5*	12.5*
T. trichiura	24.3*	70*	22.5	35
A. lumbricoides	5.4	3.2	2.5	0
I. butschlii	8	10	20	25

*Statistical difference between Normal and Mild Malnourished School Children ($p<0.05$).(Chi-square).

7.1.2 Association between the Intestinal Parasitic Infections and Nutritional Status in School Children of the State of Oaxaca.

The weight and height were measured of 39, 30,25,20, and 26 school children from Lomas, La Era, La Lobera, Pluma Hidalgo, and Sta. Ma. Magdalena respectively in the State of Oaxaca. In agreement with the ratio H/A, 32.8%, 29.3%, and 7.8% of the total children (n= 140) presented mild, moderate, and severe malnutrition respectively, for the ratio W/A 42.1%, 15%, and 0.7% presented mild, moderate, and severe malnutrition, and finally for the ratio W/H 15.7%, 5.5%, and 1.4%, presented mild, moderate, and severe malnutrition. The appendix 11 shows the nutritional status of the school children for each community in Oaxaca

GLM (controlling for sex, age, height, and weight) showed a significantly higher Z score for the ratio W/H in uninfected than that for infected children ($X^2=6.3815$, $p<0.05$) in Oaxaca. A significantly higher Z score was found for the same ratio in uninfected than that in infected children with protozoan infections ($X^2=3.3485$, $p<0.05$), helminth infections ($X^2=3.7912$, $p<0.05$), multiple infections ($X^2=7.3619$, $p<0.05$), pathogenic parasites

(X^2=4.0595, p<0.05), *E. coli* (X^2=3.004, p<0.05), *E. nana* (X^2=8.006, p<0.05), and *H. nana* (X^2=2.617, p<0.05). The same analysis for each community showed a significant higher Z score for the indicator W/H in uninfected than that for infected children with helminths (X^2=1.2031, p<0.05) in Pluma Hidalgo. No statistical difference in the Z scores for the nutritional indicators (H/A, W/A, and W/H) between uninfected and infected children was observed for the rest of the communities (Lomas de San Jacinto, La Era, La Lobera, and Sta. Ma. de Magdalena)

Pearson's correlation showed a significant negative relationship between the Z W/H with *T. trichiura* (RC=-0.22, p<0.05). No association was found between the Z H/A and W/A, and the prevalence of intestinal parasitic infections for each community of the State of Oaxaca.

7.1.2.1 Association between the Intestinal Parasitic Infections and Mild, Moderate, and Severe Nutritional Status in Rural School Children from State of Oaxaca.

There was no statistical difference in the prevalence of intestinal parasitic infections (X^2= 0.88, p=0.34), helminth infections (X^2= 0.0, p=1.0), protozoan infections (X^2= -2.09, p=0.14), and multiple infections (X^2= 0.72, p=0.4) between normal nourished children (n=39) and mild malnourished children (n=46) according to the nutritional indicator Z H/A. There was no statistical difference in the prevalence of the rest of the parasites between these children (p>0.05) (Table 7.4).

There was a significant higher prevalence of parasitic infections (X^2= 9.1, p=0.002), protozoan infections (X^2= 14.2, p=0.0001), *G. lamblia* (X^2= 4.5, p=0.034), *A. lumbricoides* (X^2= 8.4, p=0.003), and *I. butschlii* (X^2= 4.9, p=0.025) in mild malnourished children (n=59), than those found in normal nourished children (n=56) according to the indicator Z W/A. There was no statistical difference in the prevalence of the rest of each species of parasites between these children (p>0.05) (Table 7.4).

There was a significant higher prevalence of helminth infections (X^2= 22.9, p=0.0000) in mild malnourished children (n=22), than in normal nourished children (n=90) according to the nutritional Z W/H indicator. There was no statistical difference in the prevalence of the rest of each species of parasites between these children (p>0.05). Pearson's correlation showed no relationship between the different intestinal parasitic infections in children with mild malnutrition according to the ratios Z H/A, Z W/A, and Z W/H ($p > 0.05$).

A statistical difference was found in the prevalence of protozoan infections ($X^2 = 4.6$, $p=0.03$) between normal nourished children (n=39) and moderate malnourished children (n=44) according to the nutritional indicator Z H/A. There was no statistical difference in the prevalence of the rest of each species of parasites between these children. There was a significant higher prevalence of *A. lumbricoides* ($X^2 = 8.4$, $p=0.003$) in moderate malnourished children (n=21), than those in normal nourished children (n=56) according to the nutritional indicator Z W/A. There was no statistical difference in the prevalence of the rest of each species of parasites between these children ($p>0.05$) (Table 7.4).

Because there were few school children with moderate malnutrition, according to the ratio Z W/H (n=1), and children with severe malnutrition, according to the 3 ratios (n=11 with Z H/A, n=1 with Z W/A, and n=2 with Z W/H), in Oaxaca, statistical comparisons using these degree of malnutrition were not considered.

There was a significant higher prevalence of parasitic infections ($X^2 = 9.1$, $p=0.002$), protozoan infections ($X^2 = 14.2$, $p=0.0001$), *G. lamblia* ($X^2 = 4.5$, $p=0.034$), *A. lumbricoides* ($X^2 = 8.4$, $p=0.003$), and *I. butschlii* ($X^2 = 4.9$, $p=0.025$) in mild malnourished children (n=59), than those found in normal nourished children (n=56) according to the indicator Z W/A. There was no statistical difference in the prevalence of the other species of parasites between these children ($p>0.05$) (Table 7.4).

There was a significant higher prevalence of helminth infections ($X^2 = 22.9$, $p=0.0000$) in mild malnourished children (n=22), than in normal nourished children (n=90) according to the nutritional Z W/H indicator. There was no statistical difference in the prevalence of the other species of parasites between these children ($p>0.05$). Pearson's correlation showed no relationship between the different intestinal parasitic infections in children with mild malnutrition according to the ratios Z H/A, Z W/A, and Z W/H ($p > 0.05$).

Table 7.4 The Prevalence of Intestinal Parasitic Infections between Normal, Mild, and Moderate Nourished School Children from the State of Oaxaca according to the Ratios Z H/A, W/A and W/H.

	ZH/A			ZW/A			ZW/H	
	Normal (n=39)	Mild (n=46)	Moderate (n=44)	Normal (n=56)	Mild (n=59)	Moderate (n=21)	Normal (n=90)	Mild (n=22)
	Prevalence (%)							
Parasitic Infection	68.4	75.5	73.8	63.4*	83.8*	61.1	74.4	83.3
Helminth Infections	50	51.1	52.4	46.2	56.4	44.4	5*	83.3*
Protozoan Infection	55	66	54.7	48*	75.8*	33.3	62.7	58.3
Multiple Infection	50	57.7	50	44.2	59.6	50	53.2	75
E. coli	39	48.8	31	42.3	46.7	31.1	42.5	33.3
E. nana	44.7	37.8	26	36.5	41.9	31.1	38.3	25
E. histolytica	5.2	8.9	4.7	3.8	9.6	5.5	7.4	8.3
H. nana	21	26.6	21.4	17.3	25.8	16.6	23.4	33.3
G. lamblia	28.9	35.5	30.9	25*	40.3*	22.2	31.9	33.3
T. trichiura	5.26	11.1	14.3	5.8	12.9	11.1	8.5	33.3
A. lumbricoides	7.9	11.1	9.5	3.8*	16.1*	16.6*	9.6	25
I. butschlii	7.9	24.4	0	11.5*	24.2*	16.6	22.3	8.3

*** Statistical Difference in the Prevalence of Parasitic Infections between Normal and Mild Malnourished or Normal and Moderate Malnourished School Children ($p<0.05$) (Chi-square).**

Logistic regression (RC= -2.30, $p<0.05$) and Pearson's correlation analysis ($r= -0.22$), $p<0.05$) showed a significant negative relationship between protozoan infections and moderate malnutrition according to the ratio Z W/A. No relationship was found between the prevalence of the different intestinal parasitic infections and moderate malnutrition according to the ratio Z H/A ($p>0.05$)

7.2 Association between the Intestinal Parasitic Infections, Anthropometric Indicators, and Nutrient Intake in School Children of the States of Sinaloa and Oaxaca.

In addition to the coproparasitological screening and anthropometric measurements, 24-hour recalls were applied to 190 school children of the States of Sinaloa (n=52) and Oaxaca (n=138). The average daily intake of nutrients is reported in daily units and their percentages of the recommended allowances in the Fig. 7.1, approved by the Food and Nutrition Board and the Report Review Committee of the National Research (1989). (descriptive information in the appendix 12).

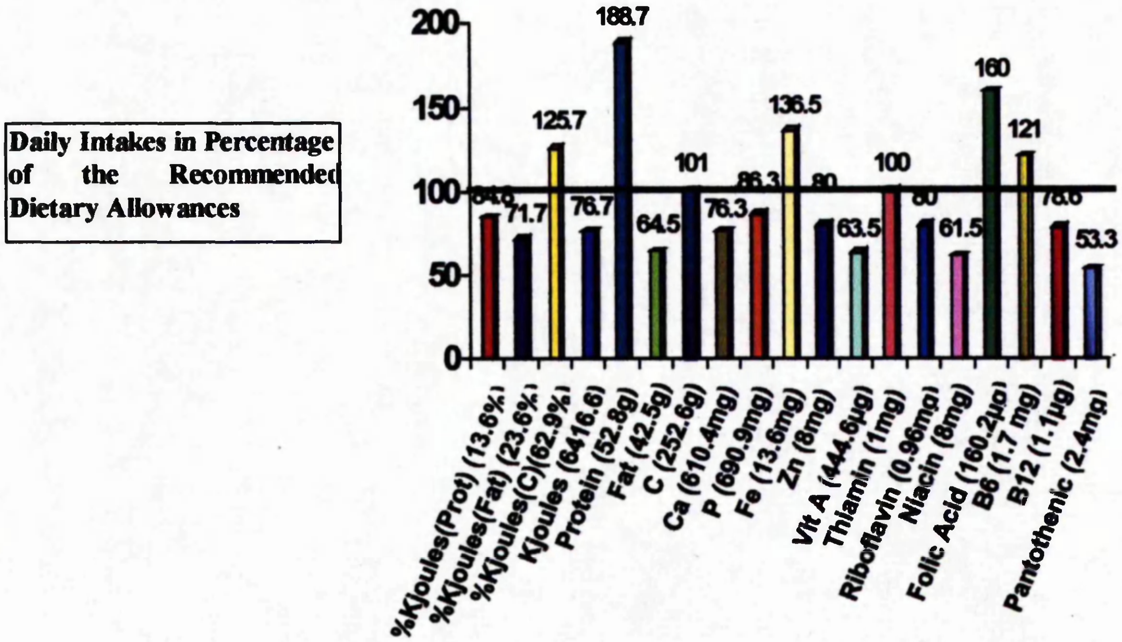


Fig. 7.1 The Average Daily Intake of Nutrients Estimated from the 24-hr Recall Method for 190 School Children from the States of Sinaloa and Oaxaca.

The percentage of school children from the States of Sinaloa and Oaxaca who did not reach the recommended dietary allowances for different daily intake of nutrients and the mean percentage of the recommended dietary allowances recorded for each nutrient are shown in the (Table 7.5)

Table 7.5 School Children from the States of Sinaloa and Oaxaca who did not Reach the Recommended Dietary Allowances and the Mean Daily Nutrient Intakes Expressed in Percentage from the Recommended Allowances.

Nutrient	Percentage of School Children who did not Meet the Recommended Dietary Allowances (total=190)	Mean Percentage of the Recommended Daily Requirements
Energy	82.8 (n=157)	68.6
Protein	11.9 (23)	78.6
Carbohydrates	47.9 (91)	74.7
Fat	82.8 (157)	51.9
Calcium	75.5 (144)	59.5
Phosphorus	68.2 (130)	64.6
Iron	28.1 (54)	75.7
Zinc	76 (145)	61
Vitamin A	75 (143)	28.6
Thiamin	51 (97)	72.3
Riboflavin	75.6 (144)	61.9
Niacin	87 (165)	53.5
Folate	37.5 (71)	65.3
Vitamin B6	39 (74)	68.5
Vitamin B12	73.9 (141)	28.7
Pantothenic Acid	92.7 (176)	46.2

The percentages of school children from Oaxaca who did not meet the recommended intake of daily requirements for energy, protein, fat, Ca, Zn, thiamin, riboflavin, niacin, vitamin E, vitamin B6, vitamin B12, folic acid, and carbohydrates were found to be significantly higher than those from Sinaloa (Chi-square) (Table 7.6). No significant difference was found in the percentages of children between Sinaloa and Oaxaca for iron, vitamin A, and pantothenic acid ($p>0.05$) (Table 7.6).

Table 7.6 Comparison of the Prevalence of School Children who did not Reach the Recommended Daily Requirements between the States of Sinaloa and Oaxaca.

	Percentage of School Children Who did not Meet the Recommended Dietary Allowances		
Nutrient	Sinaloa (total=52)	Oaxaca (total=138)	X^2 and p
Energy	71.1 (n=37)	89.8 (n=124)	9.03, 0.002
Protein	3.8 (2)	15.3 (21)	7.4, 0.006
Fat	67.3 (35)	90.6 (125)	14.3, 0.0001
Calcium	61.5 (32)	82.5 (114)	9.8, 0.001
Zinc	57.7 (30)	84.7 (105)	16.2, 0.0000
Thiamin	34.6 (18)	58 (80)	10.6, 0.001
Riboflavin	57.7 (30)	86.1 (119)	19.2, 0.0000
Niacin	75 (39)	93.4 (116)	10.8, 0.001
Vitamin E	34.6 (18)	51 (70)	5.2, 0.022
Vitamin B6	25 (13)	45.2 (62)	7.9, 0.004
Vitamin B12	55.8 (29)	82.5 (114)	15.6, 0.0000
Folate	13.4 (7)	4.4 (6)	4.1, 0.0425
Carbohydrates	61.5 (32)	83 (115)	10.9, 0.0009
Iron	28.8 (15)	29 (40)	0.000, 1.0
Vitamin A	75 (39)	75.7 (105)	0.000, 1.0
Pantothenic Acid	94.2 (49)	94.9 (131)	0.000, 1.0

Statistical significance $p<0.05$

7.2.1 Association between Daily Nutrient Intakes and Nutritional Status Investigated by the ZH/A, W/A, and W/H Indicators in School Children from the States of Sinaloa and Oaxaca.

The statistical relationship between the daily intake of nutrients, anthropometric indicators, and prevalence of intestinal parasitic infections was studied. T-test (independent) analysis showed a significant lower daily intakes of energy (Kjoules and Kcal) ($t=3.5$, $df= 188$, $p<0.05$), protein ($t=2.7$, $df= 188$, $p<0.05$), carbohydrates ($t=0.08$, $df=188$, $p<0.05$, fat ($t=4.2$, $df=188$, $p<0.05$) calcium (Ca) ($t=2.2$, $df=188$, $p<0.05$), folic acid ($t=2.3$, $df=188$, $p<0.05$), vitamin B6 ($t=2.0$, $df=188$, $p<0.05$), and iron (Fe) ($t=2.1$, $df=188$, $p<0.05$) in malnourished children than those in nourished children according to Z-score of the ratio H/A.

There was also a significantly smaller daily intake of energy (Kjoules/Kcal.) ($t=3.2346$, $df= 188$, $p<0.05$), protein ($t=2.6$, $df= 188$, $p<0.05$), fat ($t=4.5$, $df=188$, $p<0.05$), calcium (Ca) ($t=2.0$, $df=188$, $p<0.05$), folic acid ($t=2.9$, $df=188$, $p<0.05$), vitamin B6 (t=3.0, df=188, p<0.05), iron (Fe) (t=2.2, df=188, p<0.05), and zinc (t=3.0, df=188, p<0.05) in malnourished children than those in nourished children according to the ratio W/A. No significant difference was found in the daily intakes between malnourished and nourished children according to the ratio W/H.

Logistic regression analysis showed no relationship between daily nutrient intakes (energy, protein, carbohydrates, and fat) and malnourished and nourished children according to the Z-score for the ratio H/A (RC=-0.001, RC= -0.007, RC= 0.006, and RC=-0.01 respectively) (p>0.05), for the ratio W/A (RC=-0.0002, RC= -0.001, RC= 0.004, and RC=0.02 respectively (p>0.05), and for the ratio W/H (RC= -0.007, RC= -0.005, RC= 0.02, and RC=0.04 respectively (p>0.05)

7.2.2 Association between Daily Nutrient Intakes and the Prevalence of Intestinal Parasitic Infections in School Children from the States of Sinaloa and Oaxaca.

T-test analysis showed no significant difference in the daily energy, protein, carbohydrates, and fat intakes between uninfected against infected school children with parasitic infections (t=0.47, df=188, p>0.05, t=1.1, df=188, p>0.05, t=-0.18, df=188, p>0.05, and t=0.05, df=188, p>0.05 respectively), multiple infections (t= -0.56, df=188, p>0.05, t=0.01, df=190, p>0.05, t=-0.74, df=188, p>0.05, and t=-1.0, df=188, p>0.05 respectively), helminth infections (t= -0.20, df=188, p>0.05, t=0.86, df=188, p>0.05, t=-1.29, df=188, p>0.05, and t= -0.27 df=188, p>0.05 respectively), and protozoan infections (t= -0.58, df=188, p>0.05, t= -0.26, df=188, p>0.05, t= -0.74, df=188, p>0.05, and t=-0.87, df=188, p>0.05 respectively).

Logistic regression showed no relationship between daily nutrient intakes (energy, protein, carbohydrates, and fat) with prevalence of parasitic infections (RC=-0.004, RC= 0.0004, RC= 0.01, and RC=0.04, respectively) (p>0.05), multiple infections (RC= - 0.001, RC= -0.005, RC= 0.003, and RC=0.031, respectively (p>0.05), helminth infections (RC=-0.003, RC= -0.018, RC= 0.01, and RC=0.03 respectively (p>0.05), and protozoan infections RC=-0.002, RC= -0.003, RC= 0.007, and RC=0.03 respectively (p>0.05).

Correlation analysis (Pearson) showed no relationship between the nutrient intakes (energy, protein, carbohydrates, and fat) and parasitic infections (r= -0.03, r=-0.08, r= 0.1, and r=-0.00 respectively) ($p>0.05$), multiple infections (r= 0.04, RC=-0.0009, r= 0.05, and r=-0.31 respectively) ($p>0.05$), helminth infections (r= 0.02, r= -0.06, r= 0.09, and r= 0.02 respectively) ($p>0.05$) and protozoan infections (r= 0.04, r= 0.01, r= 0.05, and r= 0.06 respectively) ($p>0.05$). However, there was a significant positive relationship between family monthly income and intake of energy (0.22), protein (0.21), carbohydrates (0.20), and fat (0.30) ($p<0.05$).

7.2.2.1 Relationship between the Intestinal Parasitic Infections, Anthropometric Indicators, and Nutrient Intakes in School Children from the State of Sinaloa.

In addition to the coproparasitological screening and anthropometric measurements, 24-hour recalls were applied to 52 school children from the Community of El Higueral in Sinaloa. The average daily intake of nutrients are reported in daily units and their correspondent percentages of the recommended daily requirements in the Fig.7.2. (Descriptive information in appendix 12).

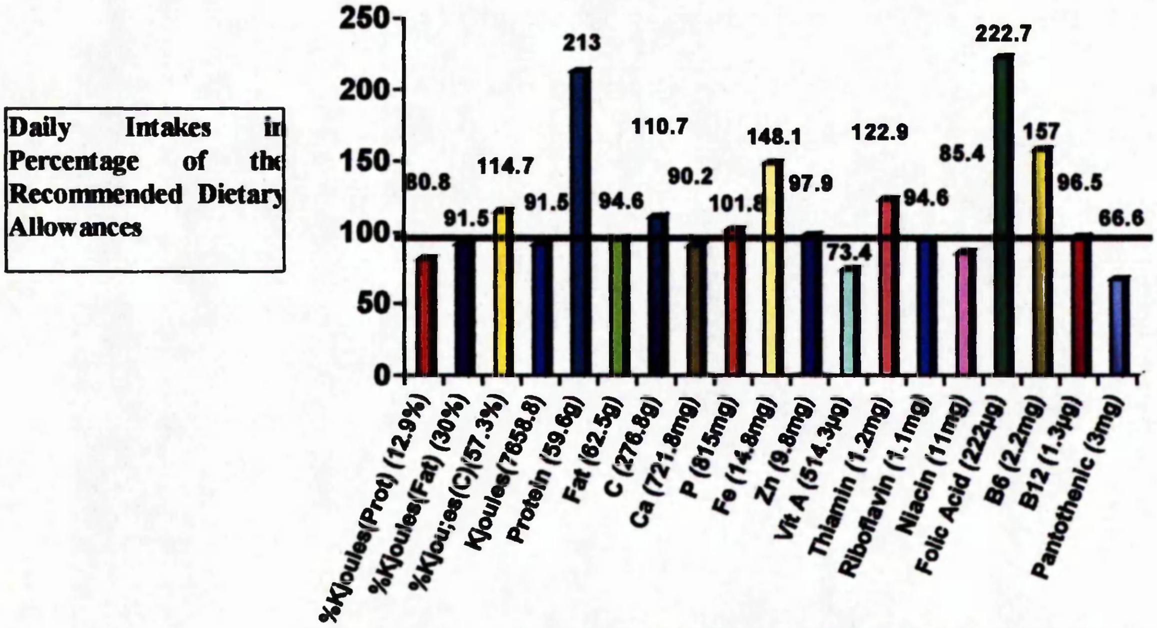


Fig. 7.2 The Average Daily Intake of Nutrients Estimated by 24-Hour Recall Method in 52 School Children in the State of Sinaloa.

The percentage of school children from Sinaloa who did not reach the recommended dietary allowances for different nutrient daily intakes and the percentage of the recommended dietary allowances recorded for each nutrient are shown in the Table 7.7

Table 7.7 School Children from the State of Sinaloa who did not Reach the Recommended Dietary Allowances and the Mean Daily Nutrient Intakes Expressed in Percentage of the Recommended Allowances.

Nutrient	Percentage of School Children Who did not Meet the Recommended Dietary Allowances (total=52)	Mean Percentage of the Recommended Daily Requirement
Energy	69.2 (n=36)	75.9
Protein	3.8 (2)	84.1
Carbohydrates	40.4 (21)	76.5
Fat	67.3 (35)	70.2
Calcium	61.5 (32)	62.1
Phosphorus	59.6 (31)	68.4
Iron	28.8 (15)	78
Zinc	57.7 (30)	68
Vitamin A	75 (39)	39.5
Thiamin	34.6 (18)	75.5
Riboflavin	57.7 (30)	71.3
Niacin	75 (39)	70.4
Folate	13.5 (7)	68.7
Vitamin B6	25 (13)	75.2
Vitamin B12	55.7 (29)	38.7
Pantothenic Acid	94.2 (49)	58.4

7.2.2.1.1 Association between Daily Nutrient Intakes and Nutritional Status Investigated by the ZH/A, ZW/A, and ZW/H Indicators in School Children from the State of Sinaloa.

T-test (independent) analysis showed no significant difference in the daily intakes of energy (Kjoules and Kcal.) ($t= 0.59$, $df= 50$, $p>0.05$), protein ($t=0.40$, $df= 50$, $p>0.05$), carbohydrates ($t=0.84$, $df=50$, $p>0.05$, fat ($t= 0.26$, $df=50$, $p>0.05$) between malnourished and nourished children from Sinaloa according to the ratio H/A. The same was observed between daily intakes of calcium (Ca), phosphorus (P), zinc (Zn), vitamin A, riboflavin, niacin, folic acid, vitamin E, vitamin B6, vitamin B12, and pantothenic acid respectively.

Logistic regression showed no relationship between daily nutrient intakes (energy, protein, carbohydrates, and fat) and malnourished and nourished children according to the Z-score for the ratio H/A (RC= 0.001, RC= -0.005, RC= -0.009, and RC=-0.01 respectively) ($p>0.05$). Because there were few malnourished school children from the rural community El Higueral according to the ZW/A and ZW/H, statistical associations between daily nutrient intakes and nutritional status considering these indicators were not considered.

7.2.2.1.2 Association between Daily Nutrient Intakes and the Prevalence of Intestinal Parasitic Infections in School Children from the State of Sinaloa.

T-test analysis showed no significant difference in the daily intakes for energy, protein, carbohydrates, and fat between uninfected and infected school children from Sinaloa with parasitic infections (t= -0.97, df=50, $p>0.05$, t=-0.40, df=50, $p>0.05$, t=-1.3, df=50, $p>0.05$, and t= -0.76, df=50, $p>0.05$ respectively), multiple infections (t= -1.4, df=50, $p>0.05$, t= -0.95, df=50, $p>0.05$, t=- 2.01, df=50, $p>0.05$, and t= -0.88, df=50, $p>0.05$ respectively), helminth infections (t= -1.7, df=50, $p>0.05$, t= -1.20, df=50, $p>0.05$, t= -2.09, df=50, $p>0.05$, and t= -1.19 df=50, $p>0.05$ respectively), and protozoa infections (t= 0.83, df=50, $p>0.05$, t= -0.45, df= 50, $p>0.05$, t= -1.4, df=50, $p>0.05$, and t= -0.4, df=50, $p>0.05$ respectively).

Logistic regression showed no relationship between daily nutrient intakes (energy, protein, carbohydrates, and fat) and prevalence of parasitic infections (RC=-0.009, RC= 0.07, RC= 0.35, and RC=0.99, respectively) ($p>0.05$), multiple infections (RC –0.06, RC= -0.02, RC= 0.25, and RC=0.61, respectively ($p>0.05$), helminth infections (RC=-0.05, RC= -0.19, RC= 0.22, and RC=0.5 respectively ($p>0.05$), and protozoan infections RC=-0.05, RC= 0.14, RC= 0.19, and RC=0.48 respectively ($p>0.05$).

Correlation analysis (Pearson) showed no relationship between the nutrient intakes (energy, protein, carbohydrates, and fat) and parasitic infections (r= 0.13, r= 0.05, r= 0.18, and r= 0.10 respectively) ($p>0.05$), multiple infections (r= 0.20, r= 0.13, r= 0.27, and r=-0.12 respectively) ($p>0.05$), helminth infections (r= 0.23, r= 0.16, r= 0.28, and r= 0.16 respectively) ($p>0.05$) and protozoan infections (r= 0.11, r= 0.06, r= 0.19, and r= 0.05 respectively) ($p>0.05$). In addition, there was no relationship between family monthly income and intake of energy (-0.19), protein (-0.21), carbohydrates (-0.22), and fat (-0.11) ($p>0.05$).

7.2.2.2 Relationship between the Intestinal Parasitic Infections, Anthropometric Indicators, and Nutrients Intake in School Children from the State of Oaxaca.

In addition to the coproparasitological screening and anthropometric measurements, 24-hour recalls were applied to 138 school children in Oaxaca. The average daily nutrient intakes are reported in daily intakes units and their correspondent percentages of daily requirements in the Fig. 7.3 (Descriptive information in appendix 12)

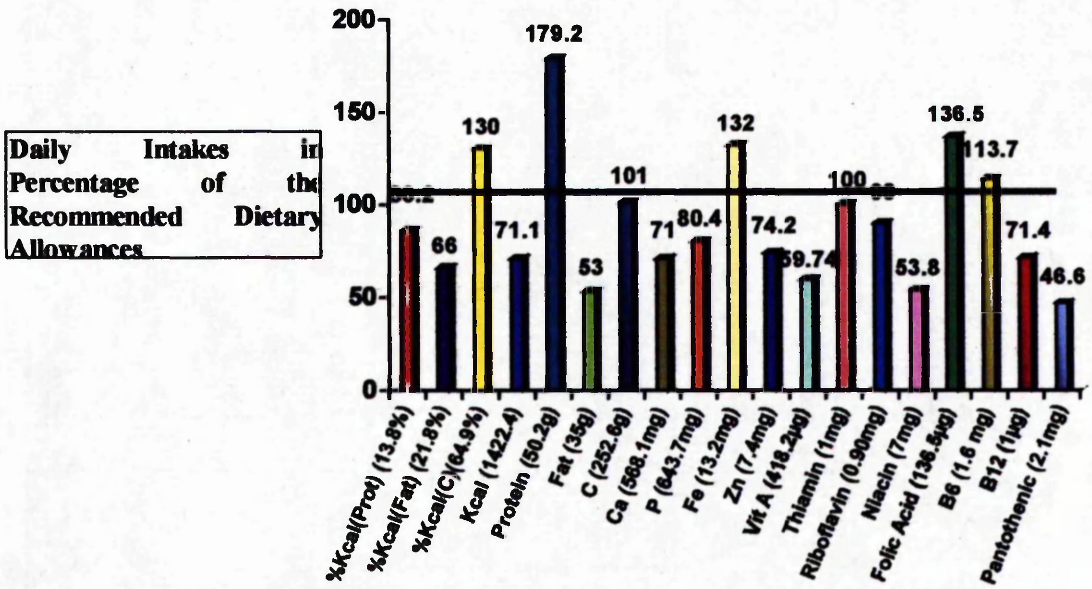


Fig. 7.3 The Average Daily Intake of Nutrients Estimated by 24-Hour Recall Method in 138 School Children from the State of Oaxaca.

The percentage of school children from the State of Oaxaca who did not reach the recommended dietary allowances for different nutrient daily intakes and the mean percentage from the recommended dietary allowances recorded for each nutrient are shown in the Table 7.9 (appendix 13).

Table 7.9 School Children from the State of Oaxaca who did not Reach the Recommended Dietary Allowances and the Mean Daily Nutrient Intakes Expressed in Percentage from the Recommended Allowances.

Nutrient	Percentage of School Children (n=138)	Mean Percentage from the Recommended Daily Requirement
Energy	87.9 (n=121)	66.5
Protein	15 (21)	78.1
Carbohydrates	50.7 (70)	74.1
Fat	88.6 (122)	46.8
Calcium	80.7 (111)	58.8
Phosphorus	71.4 (99)	63.4
Iron	27.8 (38)	74.9
Zinc	82.8 (114)	59.2
Vitamin A	75 (104)	24.6
Riboflavin	83.6 (115)	59.5
Niacin	91.4 (126)	48.3
Folate	46.4 (64)	65
Vitamin B6	44.3 (61)	67.1
Vitamin B12	80.7 (111)	26.1
Pantothenic Acid	92.1 (127)	41.6

7.2.2.2.1 Association between Daily Nutrient Intakes and Nutritional Status Investigated by the ZH/A, ZW/A, and ZW/H Indicators in School Children from the State of Oaxaca.

T-test analysis showed significant smaller daily intakes of energy (Kjoules and Kcal.) ($t= 1.5$, $df= 136$, $p<0.05$), protein ($t=-2.0$, $df= 136$, $p<0.05$), and fat ($t= 1.85$, $df=136$, $p<0.05$) in malnourished children than those in nourished children from Oaxaca according to the ratio H/A. No significant difference ($p>0.05$) was observed between the daily intakes for the rest of the nutrients (carbohydrates, calcium, phosphorus, iron, zinc, vitamin A, thiamin, riboflavin, niacin, folic acid, vitamin E, vitamin B6, vitamin B12, and pantothenic acid between malnourished and nourished children for the ratio H/A.

In addition, there were significant smaller daily intakes of energy (Kjoules and Kcal.) (t= 1.5, df= 136, p<0.05), protein (t= 1.8, df= 136, p<0.05), and fat (t= 2.1, df=136, p<0.05) in malnourished children than those in nourished children for the ratio W/A. No significant difference (p>0.05) was observed in the daily intakes of the rest of the nutrients (carbohydrates, calcium, phosphorus, iron, zinc, vitamin A, thiamin, riboflavin, niacin, folic acid, vitamin E, vitamin B6, vitamin B12, and pantothenic acid between malnourished and nourished children for the ratio W/A.

No significant difference was found in the daily intakes of energy (t=0.56, df=136, p>0.05), protein (t=-0.15, df=136, p>0.05), carbohydrates (t= -0.3, df=136, p>0.05) and fat (t=0.78, df=136, p>0.05) between nourished and malnourished children according to the ratio the ratio W/H. The same was observed between the daily intakes of the rest of nutrients.

Logistic regression showed no relationship between daily nutrient intakes (energy, protein, carbohydrates, and fat) and malnourished and nourished children according to the Z-score for the ratio H/A (RC=-0.0005, RC= -0.008, RC= 0.004, and RC=-0.008 respectively) (p>0.05), for the ratio W/A (RC=-0.001, RC= -0.003, RC= 0.006, and RC=0.007 respectively (p>0.05), and for the ratio W/H (RC= -0.008, RC= -0.043, RC= 0.03, and RC=0.054 respectively (p>0.05)

7.2.2.2.2 Association between Daily Nutrient Intakes and the Prevalence of Intestinal Parasitic Infections in School Children from the State of Oaxaca.

T-test (independent) analysis showed no significant difference for the intakes of daily energy, protein, carbohydrates, and fat between uninfected and infected school children in Oaxaca with parasitic infections (t= 1.8, df= 136, p>0.05, t=1.6 df=136, p>0.05, t=-0.82, df=136, p>0.05, and t= 1.2, df=136, p>0.05 respectively), multiple infections (t= 1.3, df=136, p>0.05, t=0.80, df=136, p>0.05, t=-0.82, df=136, p>0.05, and t= 0.57, df= 136, p>0.05 respectively), helminth infections (t= 1.5, df=136, p>0.05, t= 1.78, df=136, p>0.05, t= 0.06, df=136, p>0.05, and t= 1.40 df= 136, p>0.05 respectively), and protozoan infections (t= 0.58, df=136, p>0.05, t= 0.19, df=136, p>0.05, t= 0.33, df=136, p>0.05, and t= 0.09, df=136, p>0.05 respectively).

Logistic regression showed no relationship between daily nutrient intakes (energy, protein, carbohydrates, and fat) and the prevalence of parasitic infections (RC=-0.004, RC= 0.01, RC= 0.01, and RC=0.03, respectively) ($p>0.05$), multiple infections (RC –0.004, RC= 0.01, RC= 0.010, and RC=0.03, respectively) ($p>0.05$), helminth infections (RC=-0.004, RC= 0.007, RC= 1.57, and RC=0.03 respectively) ($p>0.05$), and protozoan infections RC=-0.003, RC= -0.01, RC= 0.009, and RC=0.03 respectively ($p>0.05$)

Pearson correlation showed no relationship between the nutrient intakes (energy, protein, carbohydrates, and fat) and parasitic infections (r= -0.15, r=-0.13, r= -0.07, and r=-0.10 respectively) ($p>0.05$), multiple infections (r= -0.10, r=-0.06, r= -0.071, and r=-0.05 respectively) ($p>0.05$), helminth infections (r= -0.12 r= -0.151, r= -0.005, and r= -0.12 respectively) ($p>0.05$) and *protozoan infections* (r= -0.05, r= -0.01, r= -0.03, and r= -0.008 respectively) ($p>0.05$). However, there was a significant positive relationship between family monthly income and intake of energy (r= 0.27), protein (0.27), carbohydrates (0.16), and fat (0.35) ($p<0.05$).

7.2.2.2.3 Brief Information about the Associations between the Prevalence of Intestinal Parasitic Infections, Anthropometric Measurements, and Daily Nutrient Intakes in School Children for each Community in the State of Oaxaca.

The percentages of school children who did not meet the recommended daily nutrient intakes in Oaxaca are shown in the appendix 13. Statistical analysis were carried out for each community, using t-test analysis to detect differences for the daily intake of nutrients between malnourished and nourished children according to the ratios H/A, W/A, and W/H, logistic regression and Pearson's correlation analysis to look for associations between daily nutrient intakes and intestinal parasitic infections. T-test analysis showed a statistical smaller difference in the daily intakes of energy (Kjoules/Kcal) (t= 2.08, df= 28, $p<0.05$), and protein (t= 2.2, df= 28, $p<0.05$) in malnourished children than those in nourished children according the ratio Z H/A in the community La Era. Pearson's correlation showed a significant positive association between family monthly income and intake of energy (RC= 0.44), protein (0.45), and carbohydrates (0.41) ($p>0.05$) in Sta Maria de Magdalena. It was not found other relevant results in the rest of the communities.

7.3 Conclusions and Recommendations

The prevalence of malnutrition investigated for the ratios Z H/A and Z W/A was found to be higher in school children from Oaxaca than in Sinaloa. No difference was found between the prevalences of malnutrition investigated by Z W/H in the school children from both States. As was found in the Fourth Mexican Survey of Nutrition in pre-school children from rural communities in 1998 (Avila-Curiel *et al.*, 1998), mild malnutrition was found to be the most common level of malnutrition in the school children in this study. All degrees of malnutrition (mild, moderate, and severe) were found to be more prevalent in school children from Oaxaca than those in Sinaloa. Although, normal nourished children showed a higher prevalence of parasitic infections than malnourished children, no association was found between the nutritional status and parasitic infections in Sinaloa. In addition, no difference was found in the Z scores for the three ratios (H/A, W/A, and W/H) between uninfected and infected children in Sinaloa. It has been recognised that malnutrition may increase the susceptibility to these infections, or infections may deteriorate the nutritional status of the host. There is evidence that intestinal parasitic infections may interfere with growth development (Solomons and Keusch, 1981) but this has not been always confirmed (Gupta *et al.*, 1977; Burke, 1978; Willet *et al.*, 1989; Stephenson *et al.*, 1980), because is though that the nutritional impact of infection depends on the degree and rate of infection and the nutritional status of the host (Solomons and Keush, 1981). The absence of association between parasitic infection and nutritional status in Sinaloa could be due to a number of factors. First, even when mild malnutrition for the ratio ZH/A was relatively high (17%) (Table 7.2), the negative effects by parasitic infections were not the major factor associated with malnutrition in Sinaloa. Second, the low prevalence of moderate and severe malnutrition reduced the chances of finding a statistical association between nutritional status and parasitic infections. Third, it must be regarded that the prevalence of helminth infections is lower in Sinaloa that in Oaxaca. Probably helminth species produce a greater nutritional impact than protozoan species. Fourth, Dewey (1983) failed to find an association between nutritional status and parasitic infections in pre-school children (aged 2-4 years) in the Mexican State of Tabasco, despite a high percentage of children with ascariasis and trichuriasis were judged to have moderate or severe intensity of infection. It is probable that the high prevalence of parasitic infections in the pre-school children from Tabasco and school children from Sinaloa is also hiding any statistical association between parasitic infections and nutritional status. Finally, it must be understood that the children in this study have probably been infected with one or more parasites at some time in their lives. The artificial distinction between infected and uninfected individuals hides any negative significant effect that could be really observed between an infected and uninfected individual with time (Stephenson *et al.*, 1980).

In Oaxaca, uninfected children showed better nutritional status than infected children. It is difficult to explain this when all children are equally exposed to parasitic infections at some time in their lives. This may be explained by the kind of parasite producing infection, predisposition, frequency of re-infection, and level of intensity of infection. Children from Oaxaca showed a higher prevalence of helminth infections than those from Sinaloa. It was also found that children with malnutrition investigated for the three ratios (ZW/H, W/A, and H/A) showed a higher prevalence of parasitic infections. Only, moderate malnutrition for Z W/A was found to be associated with intestinal parasitic infections. It seems to be that parasitic infections were not severe enough to be associated with mild malnutrition, but probably their persistence led them to be one of the major factors increasing the severity of malnutrition in these children.

A high percentage of school children in this study did not meet the recommended daily requirements for some of the assessed nutrient intakes estimated by the 24-hour recall methodology. The percentage of school children subjected to low nutrient requirements was higher in Oaxaca than in Sinaloa. Wyatt and Triana (2000) reported low daily incomes for energy (56.6%), fat (25%), calcium (34%) and zinc (51%) in the basis of the RDA, in 124 oaxaquenan pre-school children of low-income families. This finding was also accompanied by mild malnutrition for the ratio ZH/A in 51.6% of the children. In our study low daily intakes were also found for the same nutrients [energy (71.1%), fat (53%), calcium (71%), and zinc (74.2%)] in Oaxaca and Sinaloa, and 32.8% of the children showed mild malnutrition for the ratio ZH/A.

No significant association was found between smaller daily intakes and nutritional status investigated for the three ratios (Z H/A, Z W/A, and ZW/H) in Sinaloa. In Oaxaca, smaller daily intakes were associated with malnutrition investigated for the ratios Z H/A and Z W/A (no association with Z W/H). No association was found between daily intakes and parasitic infections in both States.

It is evident that the children have been chronically subjected to sub-optimal daily requirements of nutrients and probably this is the major cause for the different degrees of malnutrition observed. The higher percentage of malnutrition in Oaxaca compared with that in Sinaloa, has suggested that school children from Oaxaca have been subjected for longer to stress processes (infections or diseases) or prolonged imbalance in the energy intakes than those in Sinaloa. Therefore, school children in this study had to adapt to lack of nutrients (homeostasis) resulting in a new balance between weight and height with time. It means, that height has not shown a significant increase with time and, current weight is appropriate to the current height (homeorresis) (Flores, 1995). It may also explain the low prevalence of malnutrition described for the ratio Z W/H observed in this study. The ratio W/H was not appropriate indicator to investigate for malnutrition in this study.

Otherwise, an impaired immune system and a prolonged lack of nutrients, would favour the continue re-infection (Solomons and Keusch, 1981). It probably explains the stable prevalence of intestinal parasitic infections with age in Oaxaca. On the contrary, the more competitive immune system could be involved in decreasing parasitic infections given the less impaired nutritional status in Sinaloa.

CHAPTER 8

SOCIO-ECONOMIC STATUS AND INTESTINAL PARASITIC INFECTIONS IN SCHOOL CHILDREN FROM THE STATES OF SINALOA AND OAXACA.

8.1 Economic Activity and Education of the School Children's Parents in the States of Sinaloa and Oaxaca.

A socio-economic study was conducted on parents (271 mothers and 250 fathers) of 271 school children from the States of Sinaloa and Oaxaca (the father was absent in 21 families). The socio-economic characteristics for these school-children are shown in the Table 8.1.

167 (61.6%) mothers were unemployed (housewife) and the rest 104 (38.3%) had different jobs with household servant, merchant, day labourer, and seamstress were the most common. 34 (13.6%) fathers were unemployed and the rest 216 (86.4%) were retired and the others involved in masonry, merchant, day labourer, and fishing. 164 (60.5%) mothers and 193 (77.2%) fathers had not completed their primary school education

There was no statistical difference in the proportions of employed mothers between Sinaloa (34.61%) and Oaxaca (40.1%)(X^2= 0.53, p=0.46). The same was observed between employed fathers (90.3% and 88.3% for Sinaloa and Oaxaca respectively) (X^2=0.05, p=0.82).

No statistical difference was observed in the proportions of mothers with completed primary school between Sinaloa (34.6%) and Oaxaca (43%) (X^2= 0.53, p=0.46). However, the proportion of fathers with completed primary school was significantly higher in Oaxaca than that in Sinaloa (11.5% and 29.9% for Sinaloa and Oaxaca respectively) (X^2=9.03, p=0.002). The average monthly income per family was estimated in 2.5 times the minimal wage (around 9 and 10 American dollars) in the families from both States (Sinaloa and Oaxaca) and the socio-economic status index (total sum of the scores for education and employment of the parents, family members, family monthly income, size and quality of the material used in the construction of the house) was better in Sinaloa than in Oaxaca (Table 8.1).

Table 8.1 Socio-economic Characteristics of 271 Families from which the School-Children of the States of Sinaloa and Oaxaca were recruited for this Study.

Socio-economic characteristics	Proportion, Percentage, and Average
Mother	271
Unemployed	167 (61.6%)
Employed	104 (38.5%)
No completed-primary	164 (60.5%)
Average of Education in Years	4.6 (±3)
Father	250
Unemployed	34 (13.4%)
Employed	216 (79.7)
No completed primary	193 (77.2%)
Average of >Education in Years	3.9 (±3.4)
Absent	21 (7.8%)
Family monthly income	2.5 minimal wage (around $9.00 USD per day)
Household characteristics:	
Number of Rooms per Family (average)	3 (84%)
Material used for Walls	
Block/cement	101 (37.3%)
Adobe	42 (15.5%)
Carrizo/mud	11.5 (4.2%)
Board laminate	20 (7.4%)
Board laminate/wood	12 (4.4%)
Carrizo/mud	19 (7%)
Material used for Roof	
Concrete	105 (38.7%)
Metal laminate/wood	139 (51.3%)
Metal laminate/palm	9 (3.3%)
Board laminate/wood	130 (48%)
Asbestos/wood	5 (1.9%)
Material used for Floor	
Bare-earth	116 (42.8%)
Cement	155 (57.2%)
Dispose of Faecal Materials	
Defecation in Open Area	60 (22.2%)
Pit	176 (65%)
Latrine	34 (12.7%)
Socio-economic Status Index	28.9 (SD=9.5)
Socio-economic Status Index in Sinaloa	34.0 (SD=10.3)*
Socio-economic Status Index in Oaxaca	27.0 (SD=8.5)*

Significantly higher in Sinaloa than in Oaxaca, t=-4.8, p=0.0000

The materials used for flooring, roofing, walls and number of rooms were considered to qualify the housing conditions in which each child was living during the parasitological study.

The frequency of use of the materials in the construction of houses in the States of Sinaloa and Oaxaca was as follows: combination block with cement (37.3%) was the most frequent material used for walls. (Table 8.1).

The combinations metal laminate with wood (51.3%), concrete (48%), and board laminate with wood (38.7%) were the most frequent materials used for roof.

Bare-earth was found as a floor in 42.8% of the houses and cement in 57.2%. Most of the families (65%) had a pit for disposing of faecal material. A system of clean water supply was not available in the communities included in this study.

The statistical analysis (t-tests) showed that the housing conditions were better for uninfected school children than those with *G. lamblia* (t=2.66, df= 269, p<0.05), and *A. lumbricoides* (t=2.14, df= 269, p<0.05). There was no significant difference between uninfected and infected children for intestinal parasitic infections (t=0.40, df=269, p>0.05), multiple infections (t=1.06, df=269, p>0.05), protozoan infections (t=-0.40, df=269, p:0.05) and helminth infections (t=0.92, df=269, p>0.05).

Regression analysis showed the housing conditions were an influencing factor for the prevalence of *G. lamblia* (RC= -0.09, p<0.05) and *A. lumbricoides* (RC=-0.15, p<0.05), but no relationship was observed between housing quality and parasitic infections (RC= -0.01, p>0.05), multiple infections (RC= -0.02, p>0.05), protozoan infections (RC= -0.014, p>0.05), and helminth infections (RC= -0.02, p>0.05).

Pearson's correlation showed a positive and significant relationship for family monthly income with housing conditions (RC=0.15, p<0.05), floor material (RC=0.24, p<0.05), and roof material (RC=0.17, p<0.05). No relationship between income and number of rooms (RC=0.05, p>0.05), and wall material (RC= 0.07, p>0.05) was observed.

The statistical analysis (Chi-square) showed a higher significant difference in the prevalence of different intestinal parasitic infections in school children with unemployed mothers than those in children with employed mothers excepting for *E. histolytica*, *E. nana*, *G. lamblia*, *A. lumbricoides*, and *I. butschlii* (Table 8.2) (p>0.05).

Table 8.2 Statistical Comparison in the Prevalence of the Different Intestinal Parasitic Infections between School Children with Unemployed and Employed Mothers in the States of Sinaloa and Oaxaca.

Infection	Unemployed Mother (n=167)	Employed Mother (n=107)	X^2 and p Significance p<0.05
	Prevalence of the Infection		
Intestinal Parasitic Infection	**81**	**61.6**	**8.76, 0.003**
Pathogenic Parasites	**68.9**	**49.3**	**6.67, 0.009**
Helminth Infection	**59.5**	**39.7**	**7.22, 0.007**
Protozoan Infection	**74.4**	**46.6**	**15.2, 0.000**
Multiple Infection	**68.9**	**36.9**	**19.2, 0.0000**
E. coli	**54.3**	**31.5**	**9.9, 0.001**
E. nana	**47.4**	**23.3**	**11.6, 0.0006**
E. histolytica	**7.7**	**1.4**	**3.2, 0.07**
H. nana	**27.6**	**16.4**	**2.96, 0.08**
G. lamblia	**27.6**	**17.8**	**2.4, 0.12**
T. trichiura	**20.7**	**8.21**	**5.02, 0.024**
A. lumbricoides	**7.8**	**2.7**	**1.8, 0.17**
I. butschlii	**16.4**	**12.4**	**0.37, 0.54**

The statistical analysis (Chi-square) showed no significant difference in the prevalence of intestinal parasitic infections between children with mothers and fathers with completed and uncompleted primary school (parasitic infections X^2= 0.41, p=0.52 and X^2= 1.73, p=0.18, for mothers and fathers respectively).

Pearson's correlation showed a positive and significant relationship between the employed mothers and family monthly income (FMI) (RC=0.17, p<0.05), and a negative and significant relationship between FMI with multiple infection (RC=-0.19, p<0.05), *E. nana* (RC= -0.12, p<0.05), *H. nana* (RC= -0.11, p<0.05), *E. histolytica* (RC= -0.13, p<0.05), *A. lumbricoides* (RC= -0.16, p<0.05).

No relationship was found between FMI with employed fathers or fathers with completed primary school.

8.1.1 Economic Activity and Education of the School Children's Parents in the State of Sinaloa.

Socio-economic information from parents (120 mothers and 120 fathers) of 120 school children from the state of Sinaloa was collected. 78 (65.3%) mothers and 11 fathers (9.6%) were unemployed and the remaining 42 mothers (36.6%) and 109 fathers (90.8%) had different economic activities. 78 (65%) mothers and 106 (88.4%) fathers did not have their primary school completed (Table 8.3)

Table 8.3 Economic Activity and Education of the School Children's Parents in the State of Sinaloa.

Economic and Educational Status of Parents	Frequency and Percentage
Mothers with Primary School Completed Average of Education in Years	42 (35%) 5 (±3.7)
Fathers with Primary School Completed Average of Education in Years	14 (12%) 4.0 (±3.5)
Employed Mothers:	42 (35%)
Day Labour or Fishing	78 (65%)
Household Servant	42 (35%)
Employed Fathers:	109 (91%)
Day Labour	71 (59%)
Fishing	23 (19%)
Masonry	11 (9%)
Merchant	6 (5%)
Driver	6 (5%)
Household Servant	2 (2%)
Retired	1 (1%)

Chi-square showed significant difference in the prevalence of multiple infections and protozoan infections in children with unemployed and those with employed mothers (X^2=13.4, p=0.0002 and X^2=13.1 p=0.0002 respectively). No difference was found in the prevalence of intestinal parasitic infections (X^2=1.3, p=0.24) and helminth infections (X^2=2.02, p=0.15). In addition no significant difference was observed in the prevalence of intestinal parasitic infections between children with employed and unemployed fathers (X^2= 0.26, p=0.60).

The statistical analysis (Chi-square) showed no significant difference in the prevalence of intestinal parasitic infections between children with mothers and fathers with completed and uncompleted primary school (X^2= 1.3, p=0.24 and X^2= 3.0, p=0.08, for mothers and fathers respectively).

The regression analysis showed no relationship between the prevalence of the different intestinal parasitic infections and the economic activity of the parents. The Pearson's correlation showed no relationship between the FMI and employed mothers or fathers, or mothers/fathers with completed primary school. However, it was found a negative significant relationship between FMI and parasitic infection (RC= -0.40, $p<0.05$), multiple infection (RC= -0.25, $p<0.05$), protozoan (RC= -0.35, $p<0.05$), and *A. lumbricoides* (RC= -0.21, $p<0.05$).

The statistical analysis between the prevalence of intestinal parasitic infections and the economic activity of the parents for each community in Sinaloa showed no relevant meaningful.

8.1.2 Economic Activity and Education of the School Children's Parents in Oaxaca.

The parents (151 mothers and 130 fathers) of 151 school children from the state of Oaxaca were subjected to socio-economic study. 89 (58.9%) mothers and 13 (10%) fathers were unemployed and the rest 62 mothers (41%) and 117 fathers (90%) had different economic activities. 85 (56.29%) mothers and 90 (69.2%) fathers did not have their primary school completed (Table 8.4).

Table 8.4 Economic Activity and Education of the School Children's Parents in the State of Oaxaca.

	Frequency and Percentage
Mothers with Primary School Completed Average of Education in Years	65 (43%) 4.5 (±2.8)
Fathers with Primary School Completed Average of Education in Years	47 (31%) 3.8 (± 3.2)
Employed Mothers:	62 (41%)
Day Labour or Fishing	33 (22%)
Household Servant	95 (63%)
Merchant	23 (15%)
Employed Fathers:	117 (90%)
Day Labour	65 (50%)
Masonry	52 (40%)
Driver, Household Servant, Police, Merchant, Retired	13 (10%)

The statistical analysis (Chi-square) showed a high significant difference in the prevalence for the different intestinal parasitic infections, pathogenic parasites, helminth infections, protozoan infections, multiple infections, *E. coli*, *E. nana*, *E. histolytica*, *H. nana*, and *T. trichiura* in children with unemployed than those with employed mothers (Table 8.5).

Table 8.5 Statistical Comparison of the Prevalence of the Different Intestinal Parasitic Infections between School Children with Unemployed and Employed Mothers in the State of Oaxaca

Infection	Unemployed Mother (n=89)	Employed Mother (n=62)	X^2 and p Significance p<0.05
	Prevalence of the Infection		
Intestinal Parasitic Infection	81.7	58.2	11.4, 0.0007
Pathogenic Parasites	71.9	49	9.2, 0.0024
Helminth Infection	58.5	36.4	8.85, 0.003
Protozoan Infection	69.5	43.6	12.7, 0.0004
Multiple Infection	65.8	32.7	20.5, 0.0000
E. coli	48.8	25.4	10.4, 0.001
E. nana	48.8	16.4	22.0, 0.0000
E. histolytica	11	1.8	7.18, 0.007
H. nana	28	14.5	5.1, 0.024
G. lamblia	36.6	23.6	3.5, 0.06
T. trichiura	13.4	3.6	5.5, 0.018
A. lumbricoides	8.5	3.6	1.5, 0.215
I. butschlii	20.7	12.7	1.82, 0.177

Logistic regression analysis showed the employed mothers was associated with low prevalence of intestinal parasitic infections (RC=-1.16, $p<0.05$), multiple infections (RC= -1.37, $p<0.05$), helminth infections (RC= -0.90, $p<0.05$), protozoan infections (RC= -1.0801, $p<0.05$), *E. coli* (RC=-1.02, $p<0.05$), *E. nana* (RC= -1.58, $p<0.001$), *E. histolytica* (RC= -1.89, $p<0.05$), and *T. trichiura* (RC= -1.41, $p<0.05$).

However, no significant difference was observed in the prevalence of intestinal parasitic infections in children with employed and unemployed fathers (X^2= 2.3, p=0.13). The regression analysis showed no relationship between the prevalence of intestinal parasitic infections and economic activity of the father.

Also, the statistical analysis (Chi-square) showed no significant difference in the prevalence of intestinal parasitic infections between mothers and between fathers with completed and uncompleted primary school (X^2= 2.0, p=15, and X^2= 6.0, p= 0.014, for mothers and fathers respectively) Logistic regression showed no relationship between the prevalence of intestinal parasitic infections in school children and education of the parents.

The Pearson's correlation showed a negative and significant relationship between employed mothers and prevalence of parasitic infections (r= -0.25, $p<0.05$), multiple infection (r= -0.32, $p<0.05$), protozoan infection (r= -0.25, $p<0.05$), helminth infections (r= -0.21, $p>0.05$) and *T. trichuria* (r= -0.16, $p<0.05$). Also, it was found a positive and significant relationship between the employed mothers and FMI (r=0.25, $p<0.05$).

The statistical analysis for each community in Oaxaca showed no significant relationship between economic activity and level of education of the parents with the prevalence of the different intestinal parasitic infections in the school children.

8.2 Housing Conditions and Prevalence of Intestinal Parasitic Infections in School Children of the States of Sinaloa and Oaxaca.

8.2.1 Housing Conditions and Prevalence of Intestinal Parasitic Infections in Sinaloa.

Information about the housing conditions of 120 school children in the state of Sinaloa was collected. The frequency of the materials used for construction of houses in Sinaloa is shown in the Table 8.6.

Table 8.6 Frequency of the Different Materials used in the Construction of Households of the Families of the Recruited School Children in the State of Sinaloa.

Material used for Walls	Frequency and Percentage
Block/cement	76 (63.5%)
Adobe	19 (15.4%)
Carrizo/mud	14 (11.5%)
Board laminate	7 (5.8%)
Adobe	5 (4%)
Material used for Roof	
Concrete	90 (75%)
Metal laminate/wood	14 (11.5%)
Metal laminate/palm	9 (7.7%)
Board laminate/wood	5 (3.8%)
Asbestos/wood	2 (1.9%)
Material used for Floor	
Bare-earth	44 (36.5%)
Cement	76 (63.5%)
Number of Rooms per Family (average)	4 (90%)
Disposal of Faecal Material	
Defecation in open areas	9 (7.7%)
Pit	104 (86.5%)
Latrine	7 (5.7%)

Bare-earth was observed as a floor in 36.5% of the houses and cement in 63.5%. 86.5% of the houses had a pit for disposing faecal material (Table 8.6)

No difference in housing conditions was found between uninfected and infected children with different intestinal parasitic infections, but logistic regression analysis showed the housing conditions was an influencing factor for the prevalence of parasitic infections (RC= -0.18, p<0.05), multiple infections (RC= -0.18, p<0.05), protozoan infections (RC= -0.17, p<0.05) *E. coli* (RC=-0.21, p<0.05), and *T. trichiura* (RC= -0.10, p<0.05). No relationship was found between housing conditions with helminth infections (RC= -0.06, p>0.05), *H. nana* (RC= -0.02, p>0.05), *G. lamblia* (RC= -0.02, p>0.05), *A. lumbricoides* (RC= -0.17, p<0.05) and *I. butschlii* (RC= -0.17, p>0.05).

Pearson's correlation showed no relationship between family monthly income with housing conditions (r= -0.12, p>0.05), floor material (=0.13, p>0.05), roof material (r= -0.09, p<0.05), wall material (r= -0.20, p>0.05), and number of rooms (r=-0.05, p>0.05).

Chi-square showed no difference was found in the prevalence of parasitic infections between children with latrine and /or pit in their houses with those defecating in open areas (p>0.05). It is probably because a low proportion of families was using latrine or a large proportion using pit making difficult to find some statistical difference.

8.2.2 Housing Conditions and Prevalence of Intestinal Parasitic Infections in School Children of the State of Oaxaca.

Information about housing conditions of 151 school children in Oaxaca was collected.

The frequency of the materials used for construction of houses in Oaxaca (Lomas de San Jacinto, La Era, La Lobera, Pluma Hidalgo, and Sta. Maria de Magdalena) is shown in the Table 8.7

Table 8.7 Frequency of the Different Materials used in the Construction of Households of the Families of the Recruited School Children in the State of Oaxaca.

Material used for Walls	Frequency and Percentage
Metal laminate/wood	60 (40%)
Block/cement	25 (16.8%)
Adobe	24 (16.1%)
Brick	21 (13.9%)
Board laminate/wood	13 (8.8%)
Carrizo/mud	5 (3.6%)
Material used for Roof	
Metal laminate/wood	126 (83.2%)
Concrete	14 (9.5%)
Tile	5 (3.6%)
Board laminate/wood	4 (2.9%)
Board laminate/mud	1 (0.7%)
Material used for Floor	
Bare-earth	72 (47.5%)
Cement	79 (52.5%)
Number of Rooms per Family (average)	3 (94%)
Disposal of Faecal Material	
Defecation in open areas	42 (27.8%)
Pit	86 (57%)
Latrine	23 (15%)

Bare-earth and cement were observed as a floor in 47.5% and 52.5% respectively. 57% of the houses had a pit for disposing faecal material (Table 8.7). The statistical analysis (t-tests) showed better housing conditions for uninfected than those for infected children with *T. trichiura* (t=2.1, df= 149, p<0.05) and *A. lumbricoides* (t=2.1, df= 149,

$p<0.05$). No difference in housing conditions was observed between uninfected and infected children with intestinal parasitic infections ($t=-0.52$, $df= 149$, $p>0.05$), multiple infections ($t=0.91$, $df= 118$, $p>0.05$), protozoan infections ($t=-0.95$, $df=149$, $p>0.05$), helminth infections ($t= 0.92$, $df=118$, $p>0.05$), *E. coli* ($t=0.90$, $df= 149$, $p>0.05$), *E. nana* ($t= 0.21$, $p>0.05$), *E. histolytica* ($t=1.23$, $p>0.05$), *H. nana* ($t=0.85$, $p>0.05$) *G. lamblia* ($t=1.04$, $p>0.05$), and *I. butschlii* ($t= -0.63$, $p>0.05$).

Logistic regression showed no relationship between housing conditions and the prevalence of parasitic infections (RC= 0.03, $p>0.05$), multiple infections (RC= -0.02, $p>0.05$) protozoan infections (RC= -0.067, $p>0.05$) *E. coli* (RC= -0.04, $p>0.05$), *E. nana* (RC= 0.04, $p>0.05$), *E. histolytica* (RC= -0.1, $p>0.05$), *H. nana* (RC=0.02, $p>0.05$) *G. lamblia* (RC= -0.05, $p>0.05$), *T. trichiura* (RC= -0.21, $p>0.05$) and *A. lumbricoides* (RC= -0.20, $p>0.05$), and *I. butschlii* (RC= -0.03, $p>0.05$).

Pearson's correlation showed no relationship between family monthly income and housing conditions ($r= 0.08$, $p>0.05$), roof material ($r= 0.07$, $p>0.05$), wall material ($r= 0.05$, $p>0.05$), and number of rooms ($r= -0.04$, $p>0.05$). However a positive significant relationship between income and floor material ($r=0.24$, $p<0.05$) was observed.

Chi-square showed a significant lower prevalence of parasitic infections, helminth infections, and protozoan infections in children with latrine and /or pit in their houses compared with those defecating in open areas ($p>0.05$).

8.3 Discussion.

The proportion of employed mothers and employed fathers of the school children from Sinaloa and Oaxaca was found not to be different. The level of education between the mothers and between the fathers of the school children from both states was found not to be different ($t= -0.92$, $p=0.35$ and $t= -0.42$, $p=0.67$ respectively)

No association was observed between the economic activity or level of education of the parents and the prevalence of intestinal parasitic infections in school children from Sinaloa. The economic activity of the parents was not associated with the family monthly income in Sinaloa. It was observed that 70% and 50% of the school children's families were receiving economic support from the government of Sinaloa and relatives respectively. It may explain the lack of an association between the economic contribution and level of education of the parents with the family monthly income in Sinaloa. In addition, the economic support from sources different to those supplied by parents are probably contributing significantly to the family monthly income which was found to be a factor of influence in the lower prevalence of different intestinal parasitic infections in Sinaloa.

In Oaxaca the role of the mother was remarkable. The economic activity of the mother contributed significantly to the family monthly income and the prevalence of the different intestinal parasitic infections in Oaxaca. Most of them (55%) were performing two or more unskilled jobs at the same time. Only 27% and 10% of the children's families in Oaxaca were receiving economic support from the government of Oaxaca and relatives respectively. It must also be regarded that 13.9% of the fathers in these families were absent. The level of education of the parents showed no association with both the family monthly income and the prevalence of intestinal parasitic infections in Oaxaca. Perform of unskilled jobs may explains the lack association between father education and family monthly income. The income from fathers was found not to be associated with both the family monthly income and the prevalence of the different intestinal parasitic infections in Oaxaca. Ismail *et al.*, (1989) found that the low educational background of mothers was a significant risk factor in the transmission of *E. vermicularis* in Sri Lanka. Kan *et al.*, (1992) indicated that parental educational level and occupation and maternal employment affected the prevalence of soil-transmitted helminthiases among pre-school children in Malaysia. Holland *et al.*, (1988) also found that ascariasis occurred more frequently in Panamanian children of mothers with the least formal education.

The family monthly income was found to be associated with the lower prevalence of intestinal parasitic infections and better housing conditions in the school children from both States. Better housing conditions were found for uninfected than for infected children with *T. trichiura* and *A. lumbricoides* in Oaxaca. The income was associated with better daily nutrient intakes in Oaxaca,

In Kuala Lumpur, Malaysia, Kan (1991) found an association between the poor purchasing power, poor nutritional status, and poor living conditions with high prevalence of infectious diseases. Unfortunately, the family monthly income is a factor not easily modifiable because it is strongly associated with political decisions. It is known that the population wishes to find fair paid jobs. However, badly paid jobs are more common when jobs are available. Results from this study have shown that economic contributions from the government authorities are able to produce important benefits for the general population if they are used properly.

The quality of the housing conditions was determined by the quality of the material used for floor, roof, and walls, and number of rooms. No difference in housing conditions was observed for uninfected children than infected in Sinaloa. The family monthly income was positively associated with the quality of floor and roof material. The use of latrine and/ or pit was associated with a lower prevalence of parasitic infections in Oaxaca. Ghani and Oothuman (1991) found that the prevalences of *Ascaris*, *Trichuris*, and hookworm were higher in areas without piped water, proper housing and toilets facilities regardless of race and place in rural and urban areas of Malaysia. In addition, Lai and Yang (1991) found that toilets in the houses were an important factor in the lower risk of worm infection in Selangor, Malaysia.

In Oaxaca, the family monthly income was found to be associated with the quality of the floor material. There was a negative association between floor material and prevalence of *A. lumbricoides* and *T. trichiura*. The floor material was a factor contributing to the surviving and transmission of *A. lumbricoides* and *T. trichiura* in the school children from Oaxaca.

Holland *et al.*, (1988) revealed that the prevalence of single and multiple helminth infections was significantly higher in children living in housing made of wood or bamboo than in those living in housing made of concrete blocks.

The current programme of educational activities promoted by primary health workers of the national deworming campaign has probably reduced the impact of the low level of education of the parents on the prevalence of these infections. A high percentage of families of the school children in this study drunk boiled or commercial purified water (65% and 78% in Sinaloa and Oaxaca respectively) and washed their hands (75% and 85% in Sinaloa and Oaxaca respectively). However, an association was not found between the use of safe water or clean hands and reduced prevalence of parasitic infections, probably as a result of lack continuity to follow these rules. Yu *et al.*, (1992), revealed that human behaviours such as non-washing hands were important factors to maintain the high endemicity of soil-transmitted helminths in China.

In Malaysia, Ismail *et al* (1989), showed that the personal and health factors were unrelated to re-infection with intestinal nematodes. In contrast, environmental and socio-economic factors such as cleanliness of the living area, soil-pollution, type of drinking water and family monthly income were found to have a significant impact on transmission of parasitic infections

Health education makes people aware and responsible for their own health, to create the demand for, and to involve the community for the use and maintenance of latrine building, clean piped water, and continuity of personal behaviour. Some chemotherapeutic interventions are often hampered by re-infections because of imposed culturally non-acceptable and defective latrines and septic tanks, and the discharge of untreated sewage into rivers, streams and ponds, and poor encourage of people on health education (Albonico *et al.*, 1999). Some studies in different regions in the world have associated a reduced prevalence and intensity of infection with an improvement in sanitation and education (Tanner *et al.*, 1987; Sorensen *et al.*, 1994). However, other studies have failed to find such associations (Muller *et al.*, 1989). The effect of improved sanitation on parasitic infections is expected to take some time to achieve measurable impact (Huttly, 1990). The combination of the national deworming programme, with an appropriate health education, and an accepted and viable clean water and faecal material disposal systems may result in an immediate reduction in the prevalence of parasitic infections not only in Sinaloa and Oaxaca, but also in the country.

CHAPTER 9

SYMPTOMATOLOGY AND INTESTINAL PARASITIC INFECTIONS IN SCHOOL CHILDREN OF THE STATES OF SINALOA AND OAXACA.

9.1 Symptomatology and Prevalence of Intestinal Parasitic Infections in School Children of the States of Sinaloa and Oaxaca.

A total of 192 school children from Sinaloa (n=52) and Oaxaca (n=140) were subjected to physical examination at the time of faecal sample collection. The frequency of clinical symptoms (headache and abdominal pain) and signs (respiratory infections, allergy, vomit, fever, and diarrhoea) in these school children are shown in the Fig. 9.1.

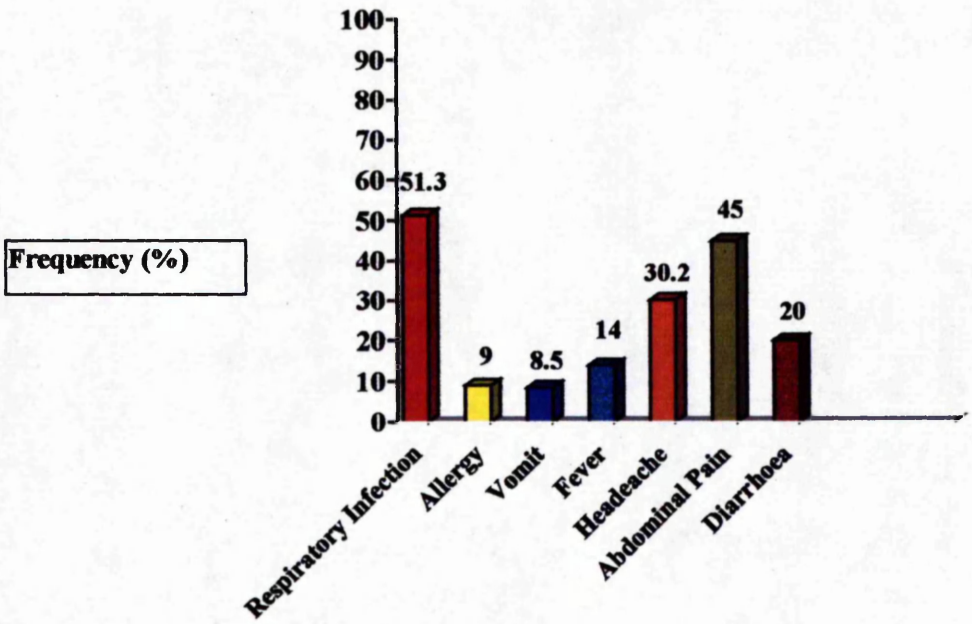


Fig 9.1 Frequency of Clinical Symptoms in 192 School Children from the States of Sinaloa and Oaxaca

The frequency of clinical symptom and signs in 52 school children in Sinaloa is shown in the Fig 9.2.

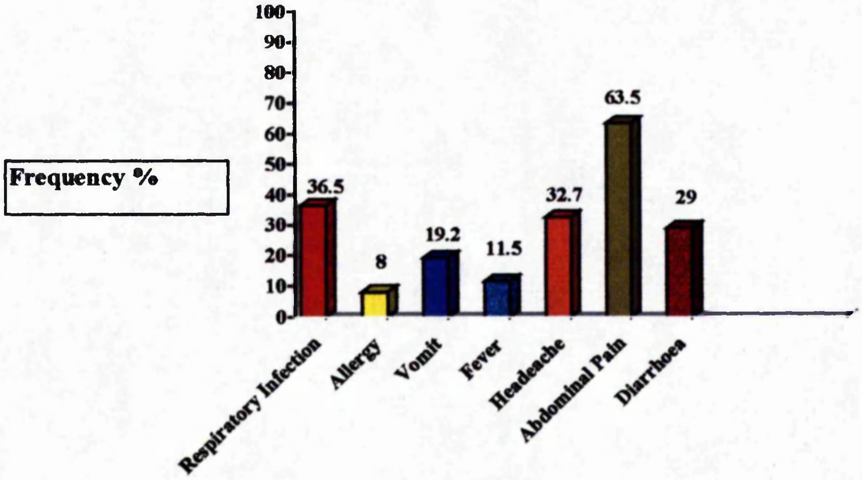


Fig 9.2 Frequency of Clinical Symptoms in 52 School Children from the State of Sinaloa.

The frequency of clinical symptoms of 140 school children in Oaxaca is shown in the Fig 9.3.

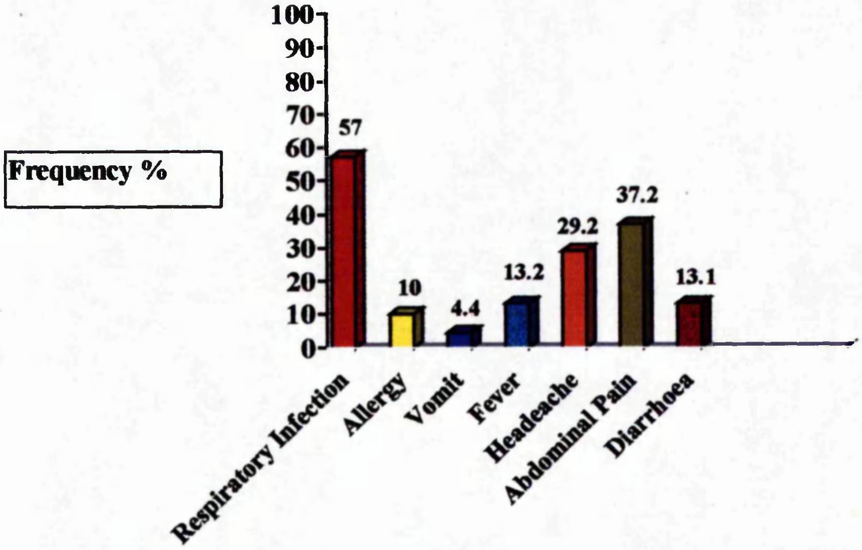


Fig 9.3 Frequency of Clinical Symptoms in 140 School Children from the State of Oaxaca.

The statistical comparison (Chi-square) of the frequency of clinical symptoms between school children from Sinaloa and Oaxaca showed a significant higher frequency of respiratory infections in Oaxaca (56.9%) than those in Sinaloa (36.5%). A significant higher frequency of vomiting, abdominal pain, and diarrhoea was observed in Sinaloa than those in Oaxaca. There was no significant difference in the frequency of allergies, headache, and fever between the school children from both States (Table 9.1).

Table 9.1 Frequency of Clinical Symptoms and Signs in 192 School Children from Sinaloa and Oaxaca

Signs and Symptoms	Sinaloa n=52	Oaxaca n=140	X^2 and p
Respiratory Infections	**36.5% (n=19)**	**56.9% (n=80)**	**7.26, 0.007**
Vomiting	**19.2% (10)**	**4.3% (6)**	**9.62, 0.002**
Abdominal Pain	**63.4% (33)**	**37.2% (52)**	**12.5, 0.0004**
Diarrhoea	**28.8% (15)**	**13.1% (18)**	**6.01, 0.014**
Allergy	**8% (4)**	**10% (14)**	**0.06, 0.80**
Headache	**32.7% (17)**	**29.2% (41)**	**0.09, 0.75**
Fever	**11.5% (6)**	**13.2% (19)**	**0.04, 0.82**

The relationship between the general prevalence of parasitic infections and clinical symptoms was investigated. Chi-square showed a significant lower frequency for some symptoms and signs between uninfected and infected children (Table 9.2).

Table 9.2 Comparison of the Frequency of Clinical Symptoms between Uninfected and Infected in 192 School Children from the States of Sinaloa and Oaxaca.

Clinical Symptom	Frequency in Percentage		
	Uninfected	Infected	X^2, p
	Intestinal Parasitic Infections		
Respiratory Infection	38% (73)	56% (108)	5.8, 0.016
	Protozoan Infections		
	41% (79)	56.6% (109)	3.9, 0.047
	Multiple Infections		
	42.7% (82)	57% (109)	3.9, 0.047
Abdominal Pain	Intestinal Parasitic Infections		
	32.2% (62)	50.9% (98)	5.9, 0.01
	Pathogenic Infections		
	32.9% (63)	51.7% (100)	6.6, 0.009
	Helminth Infections		
	34.7% (67)	53.2% (102)	6.5, 0.01
	Protozoan Infections		
	28.3% (54)	54% (104)	12.9, 0.0003
	Multiple Infections		
	31.3% (60)	54.4%(104)	9.9, 0.001
	E. coli		
	36.2% (70)	54% (104)	5.8, 0.015
	E. nana		
	37.3% (72)	56% (108)	6.5, 0.010
Diarrhoea	*T. trichiura*		
	14.4% (28)	31.7% (61)	7.3, 0.006
	A. lumbricoides		
	15% (29)	37.5% (72)	11.4, 0.0007
Vomiting	T. trichiura		
	5.9% (11)	23.3% (45)	10.3, 0.001
Fever	10% (19)	28% (54)	9.3, 0.002

Logistic regression showed that pathogenic intestinal parasitic infections (RC= 3.8, $p<0.05$), and helminth infections (RC= 0.05, $p<0.05$), were associated with the frequency of allergies, *T. trichiura* with the frequency of vomiting (RC= 2.7, $p<0.05$), and fever (RC=1.63, $p<0.05$), parasitic infections (RC= 2.1, $p<0.05$) and protozoan infections (RC= 2.6, $p<0.05$) with the frequency of abdominal pain. Pearson's correlation showed a significant and positive association between abdominal pain and parasitic infections (r=

0.22, $p<0.05$), helminth infections ($r=0.18$, $p<0.05$), protozoan infections ($r= 0.23$, $p<0.05$), and multiple infections ($r=0.20$, $p<0.05$). Logistic regression showed no association between respiratory infections, allergies, vomiting, fever, headache, abdominal pain, and diarrhoea, with the different levels of intensity of *H. nana*, *T. trichiura*, and *A. lumbricoides* in the school children from both States. The same analysis showed the same results in the school children for each State and community.

9.1.1 Symptomatology and Prevalence of Intestinal Parasitic Infections in School Children of the State of Sinaloa.

The relationship between the prevalence of intestinal parasitic infections and frequency of clinical symptoms was analysed. Chi-square showed a significantly lower frequency of respiratory infections in uninfected than infected children with parasitic infections, protozoan infections, and multiple infections (Table 9.2). There was no statistical difference in the frequency of respiratory infections between uninfected and infected children with helminth infections ($X^2= 1.3$, $p=0.25$). There was no statistical difference in the frequency of respiratory infections between uninfected and infected children with each species of parasites.

Chi-square showed a significant lower frequency of abdominal pain in uninfected than infected children with protozoan infections, helminth infections multiple infections, and *G. lamblia* (Table 9.2). There was no statistical difference in the frequency of abdominal pain between uninfected and infected children with helminth infections ($X^2= 0.0$, $p>0.05$). Also, there was no statistical difference in the frequency of abdominal pain between uninfected and infected children with each species of parasites.

Chi-square showed no significant difference in the frequency of diarrhoea between uninfected than infected children with parasitic infections ($X^2= 1.9$, $p=0.16$), helminth infections ($X^2= 0.2$, $p=0.65$) protozoan infections ($X^2= 2.4$, $p=0.11$), and multiple infections ($X^2= 3.0$, $p=0.08$). There was no statistical difference in the frequency of diarrhoea between uninfected and infected children with each species of parasites.

Chi-square showed no significantly difference in the frequency of fever between uninfected than infected children with *H. nana* ($X^2= 3.1$, $p=0.07$), and *T. trichiura* ($X^2= 9.4$, $p=0.002$). There was no statistical difference in the frequency of fever between uninfected and infected children with the rest of each species of parasites. There was no significant difference in the frequency of allergy, vomiting, and headache between uninfected and infected children with the different intestinal parasitic infections (p>0.05)

Table 9.3 Comparison of the Frequency of Clinical Symptoms between Uninfected and Infected in 52 School Children from the State of Sinaloa.

Clinical Symptom	Frequency of the Clinical Symptom in Percentage		
	Uninfected	Infected	X^2, p
	Intestinal Parasitic Infections		
Respiratory Infection	4.6% (3)	43.3% (23)	40.1, 0.0000
	Protozoan Infections		
	3.1% (2)	46% (24)	47.7, 0.0000
	Multiple Infections		
	18.4% (10)	41.4% (22)	11.6, 0.0006
Abdominal Pain	Protozoan Infections		
	46.3% (24)	70.9% (37)	10.8, 0.0009
	Helminth Infections		
	46% (24)	55% (29)	4.7, 0.03
	Multiple Infections		
	42.5% (22)	74% (39)	19.7, 0.0000
	G. lamblia		
	3.2% (2)	70.3% (37)	94.0, 0.0000

Statistical Significance p<0.05

Logistic regression showed no relationship between the different intestinal parasitic infections and clinical symptoms and signs. Pearson's correlation showed a significant and positive correlation between abdominal pain with multiple infections (r= 0.42, p<0.05), and protozoan infections (r= 0.31, p<0.05), and fever with *E. coli* (X^2= 0.28, p<0.05), *E. nana* (X^2= 0.28, p<0.05), *H. nana* (X^2= 0.34, p<0.05), and *T. trichiura* (X^2= 0.39, p<0.05). No relationship was observed between respiratory infections, allergy, vomiting, headache, and diarrhoea with the rest of each species of parasites.

9.1.2 Symptomatology and Prevalence of Intestinal Parasitic Infections in School Children of the State of Oaxaca.

A total of 140 school children from Oaxaca (Lomas de San Jacinto, n=39, La Era, n= 30, La Lobera, n= 25, Pluma Hidalgo, n=20, Sta. Ma. Magdalena, n= 26) were subjected to physical exploration. The frequencies of signs and clinical symptoms in the school children from Oaxaca are shown in the Fig 9.3. The appendix 14 shows the frequency of symptomatology and the results of the statistical analysis between the prevalence of parasitic infections and frequency of clinical symptoms per each community.

The relationship between the prevalence of parasitic infections and frequency of clinical symptoms was analysed. Chi-square showed a significant lower frequency of abdominal pain in uninfected than that in infected children with protozoan infections (X^2= 0.79, p<0.05), and *A. lumbricoides* (X^2= 0.1.4, p<0.05) (Table 9.3). There was no statistical difference in the frequency of abdominal pain between uninfected and infected children with the rest of each species of parasites. A significant lower frequency of diarrhoea was observed in uninfected than that in infected children with helminth infections (X^2= 0.50, p<0.05), *G. lamblia* (X^2= 0.20, p<0.05) and *A. lumbricoides* (X^2= 0.59, p<0.05). There was no significant difference in the frequency of diarrhoea between uninfected and infected children with the rest of the parasites (p>0.05).

Finally, there was no statistical difference in the frequency of respiratory infections, allergies, vomiting, fever, and headache between uninfected and infected children with each species of parasites (p>0.05)

Table 9.4 Comparison of the Frequency of Clinical Symptoms between Uninfected and Infected in 140 School Children from the State of Oaxaca.

Clinical Symptom	Frequency of the signs and Clinical Symptom in Percentage		
	Uninfected	Infected	X^2, p
Diarrhoea	Helminth Infections		
	6.5% (9)	19.5% (27)	40.1, 0.0000
	Giardia lamblia		
	10% (14)	20% (28)	47.7, 0.0000
	A. lumbricoides		
	10% (14)	33% (46)	11.6, 0.0006
Abdominal Pain	Protozoan Infections		
	27% (38)	44% (62)	10.8, 0.0009
	A. lumbricoides		
	33% (46)	69% (97)	

Statistical Significance p<0.05

Logistic regression showed that helminth infections (RC= 2.6, p<0.05) were an influencing factor in the frequency of allergies, *A. lumbricoides* in the frequency of fever (RC= 3.0, p<0.05), and parasitic infections (RC=2.0, p<0.05), protozoan infections (RC= 2.1, p<0.05) and multiple infections (RC= 1.5, p<0.05) in the frequency of abdominal pain.

No relationship was observed between respiratory infection, vomiting, headache, and diarrhoea with the rest of each species of parasites.

Pearson's correlation showed a significant positive correlation between abdominal pain and protozoan infections ($r= 0.17$, $p<0.05$), and *A. lumbricoides* ($r= 0.26$, $p<0.05$), and diarrhoea with parasitic infections ($r= 0.24$, $p<0.05$), pathogenic parasitic infections ($r= 0.21$ $p<0.05$) helminth infections ($r=0.25$, $p<0.05$) and multiple infections $r= 0.1931$, $p<0.05$), and headache with *A. lumbricoides* ($r= 0.23$, $p<0.05$). No relationship was observed between respiratory infection, allergy, vomiting, and fever with the rest of each species of parasites ($p>0.05$).

9.2 Discussion and Conclusion

Respiratory infections were more frequent in Oaxaca than Sinaloa. However, vomiting, abdominal pain and diarrhoea were more frequent in Sinaloa. No difference was observed in the frequency of allergies and fever between school children from both States.

The prevalence of parasitic infections was positively associated with the frequency of respiratory infections, abdominal pain, diarrhoea, vomiting, and fever in the school children in this study. It was also observed that the prevalence of helminth infections was positively associated with allergies (skin rush). No association was found between the different levels of intensities of *H. nana*, *A. lumbricoides*, and *T. trichiura* with the signs clinical symptoms recorded in this study.

In Sinaloa, the prevalence of intestinal parasitic infections was associated with respiratory infections, abdominal pain, and fever. In Oaxaca, the prevalence of parasitic infections was associated with abdominal pain, allergies, fever and diarrhoea. Allergies and fever were closely associated with helminth infections.

It is well known that a direct relationship between the presence of infection (prevalence and level of intensity) and clinical symptoms is usually complicated by the presence of other bacteria or virus infections, nutritional status, and immune response of the host. It may explains the poor association observed between the level of intensities of *H. nana*, *A. lumbricoides*, and *T. trichiura* with symptomatology in the school children from both States.

Previous surveys which included information collected about symptoms and signs in presence of different intestinal parasitic infections, have revealed that diarrhoea, vomiting, reduced appetite, abdominal and epigastric pain, fever, malaise, nausea, allergic reactions such as asthma or skin rush, and steatorrhoea can accompany ascariasis. (Tripathy *et al.*, 1972; Lagundoye, 1972; Maxwell *et al.*, 1968). Trichuriasis usually shows a very strong, positive correlation between intensity and pattern of symptoms (Jung and Beaver, 1951; Gilman *et al.*, 1983), although, light infections may result in massive infantile trichuriasis (Kouri and Valdez, 1952) characterised by diarrhoea, abdominal pain, nausea, and vomiting including anaemia (Bundy, 1986). Heavy infections by *H. nana*, as many as 2000 worms may produce enteritis. Light infections produce either no symptoms or vague abdominal disturbances with or without diarrhoea, lack of appetite, vomiting, and dizziness (Neva and Brown, 1994). *E. vermicularis* is relatively innocuous and may be accompanied by poor appetite, weight loss, hyperactivity, enuresis, insomnia, irritability, grinding of the teeth, abdominal pain, and nausea (Haswell-Elkins *et al.*, 1987). Abdominal discomfort, severe diarrhoea accompanied by steatorrhoea, have been experienced from people suffering of giardiasis. (Nash *et al.*, 1987), and abdominal discomfort, weakness, neurasthenia, malaise, constipation, diarrhoea, have been observed in amoebiasis (Shulman *et al.*, 1999).

CHAPTER 10

TRICHURIASIS AND ITS ASSOCIATION WITH NUTRITIONAL AND IRON STATUS, SOCIO-ECONOMIC CONDITIONS, AND MORBIDITY IN SCHOOL CHILDREN FROM A RURAL COMMUNITY OF THE STATE OF SINALOA, MEXICO.

10.1 Introduction.

Trichuriasis is regarded as one of the major intestinal parasitic infections which extends through the countries of the tropics and sub-tropics. In 1979 the WHO reported that trichuriasis affected 500-700 million people per year and was causing morbidity in 100 thousand people per year, mainly in some areas of Africa, Asia, and Latin America (Walsh and Warren, 1979). Current trends have revealed that trichuriasis is affecting 1,049 million people, causing morbidity in 220 million people, and a mortality rate of 10,000. Trichuriasis is still a serious public health problem in the world (Crompton, 1999).

The above estimates acquire more significance when there is evidence of an association between trichuriasis and malnutrition. Studies by Gilman *et al.*, (1983), Cooper and Bundy (1986), Cooper *et al.*, (1990), Robertson *et al.*, (1992), Callender *et al.*, (1993), and Simeon *et al.*, (1994) have suggested that trichuriasis is capable to deteriorate the childhood growth through pathological conditions such as diarrhoea or dysentery, abdominal pain, nausea, vomiting, and rectal prolapse (Gilman, *et al.*, 1983; Bundy, 1986).

In addition, trichuriasis has long been associated with poor iron status and anaemia (Otto, 1935; Getz, 1945; Brown, 1954; Wong and Tan, 1961; Layrisse *et al.*, 1967; Robertson *et al.*, 1992; Rambdath *et al.*, 1994). Iron deficiency is the most common form of malnutrition in the world, affecting over one billion people and one third of them have developed anaemia (West, 1996). WHO reported that the prevalence of iron deficiency anaemia in children from 5 to 12 years old in Africa was 49%, in Latin America 26%, in Asia 72%, and in developing countries overall around 46% (Scrimshaw, 1993).

Mexico has shown significant progress in public health as a result of the accelerated economic development in the last forty years. However, intestinal parasitic infections are still a persistent problem of public health in the Mexican population, particularly in pre-school and school children living in rural areas. A review of published and scientific data over nearly 40 years in Mexico (Quihui, 1995) revealed that trichuriasis remains as one of the most important helminthiasis in the country, particularly in school children with a mean nation-wide prevalence of 13%. Because this figure was estimated on the basis of limited information, it is assumed that the true figure could be higher.

Malnutrition is still affecting a large proportion of the Mexican child population. The Fourth National Survey of Nutrition (Avila-Curiel *et al.*, 1998), showed that malnutrition was affecting 42.7% of pre-school children according to the indicator of Height/Age (H/A), 59.9% according to the indicator Weight/Age (W/A), and 18.9% according to the indicator Weight/Height (W/H).

However, there are few recent data about iron deficiency and anaemia in Mexico. Kaufer and Casanueva (1990) reported a high percentage of anaemia in the population from 12 to 40 years old from urban areas of Distrito Federal. Unpublished data by Diaz *et al.*, (1995), revealed a prevalence of 25% of trichuriasis in children aged 0-14 years from rural and sub-urban communities of the State of Sinaloa and around 17% of these children were found to be at risk of anaemia (marginal normal levels of haemoglobin) due to poor iron status.

There are multiple factors associated with iron-deficiency and anaemia such as poor dietary quality, nutrient bioavailability, acute or chronic diseases, infection and re-infection rates, and socio-economic status. Trichuriasis has been recognised as a risk factor for iron deficiency-anaemia and its severity will depend on the nutritional status and immune response of the host, and previous infections.

The primary objective of this study was to investigate a possible association between trichuriasis and nutritional iron status in school children from a rural population of the State of Sinaloa, Mexico. It is expected that results from this study may lead to an increase in the operations carried out by the current Mexican deworming campaign in the most affected and vulnerable populations of the whole country. In addition, it will help to use more effectively and efficiently the limited human and financial resources for the improvement of health, life quality, and productivity of individuals in a short period of time.

10.2 Brief Description of Logistic and Methodological Activities Involved in this Study.

This study was conducted in the rural community of El Higueral located 70 Km. Southwest of the city of Culiacan, Sinaloa, Mexico. Its population has a low socio-economic status according to the General Census of Population and Household (1990). From a total of 101 school children aged 72-120 months, screened for coproparasitological analysis, 52 (22 females and 30 males) were recruited for this study. The parents signed a consent form to accept their childrens' participation in this study (appendix 4). The children were classified into a control group (n=24, with 10 females and 14 males), and an experimental group (n= 28 with 12 females and 16 females). Each stool sample collected was analysed by the Kato Katz technique to identify and to estimate egg counts of *T. trichiura*, and Faust and Ritchie techniques (Markell and Voge, 1976) to identify other helminth and protozoan species.

Children were recruited when they were aged 6 to 10 years old, infected only with *T. trichiura* (experimental group), absence of other intestinal parasitic infections (control group), normal concentrations of urinary indoxyl sulfate (indican) indicating no intestinal bacterial overgrowth, no malaria (*P. vivax* has been reported in that area), no evidence of chronic diseases or disability, no iron supplement, medication, or recent albendazole administration, because all of them may contribute to an imbalance in the biochemical indicators of iron status (see Chapter 4).

Questionnaires were designed to obtain particular information about the child's family, health records of the child and his/her family, child's habits, education and economic activity of the parents, monthly family income, expenses in food per week, economic support by the local government or relatives, and household conditions. Nominal and ordinal scales were used to evaluate the socio-economic variables (appendix 5).

Blood samples were collected before and after albendazole and placebo treatments in fasting conditions, at baseline and 5 weeks after treatment for measurements of haemoglobin, haematocrit, red blood cell counts, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), serum iron, total iron binding capacity (TIBC), transferrin saturation, and ferritin. Following standard procedures, weight and height measurements were carried out. In the presence of the child, the mother or the primary caregiver was asked to report on what her child had eaten during the last 24 h, using a semi-quantitative dietary questionnaire. The presence or absence of clinical symptomatology such as signs (respiratory infections, allergy, vomiting, fever, and diarrhoea) and symptoms (headache and abdominal pain) was defined by their frequency after a week of daily home visits before and after albendazole and placebo treatments using a qualitative questionnaire.

Albendazole was administered orally in a single dose (400 mg) (RIMSA Laboratories, Mexico). A sugar solution (1 g of sugar in 5 ml. of water with no albendazole) carefully bottled and sealed was also administered orally as a placebo. The bottle for placebo was identical in appearance (size, shape, and colour) to that for the albendazole. The administration of treatments was carried out in presence of the field-workers.

The physician who collected the syptomatological information, and the technicians who helped to perform the biochemical, haematological and anthropometric measurements had no access to the parasitological data to avoid biased information

10.3 Results.

10.3.1 Levels of Intensity for Trichuriasis in 24 Rural School Children (Experimental Group) from the State of Sinaloa.

The epg counts was carried out in the experimental group (n=24) and results are shown in the Fig. 10.1:

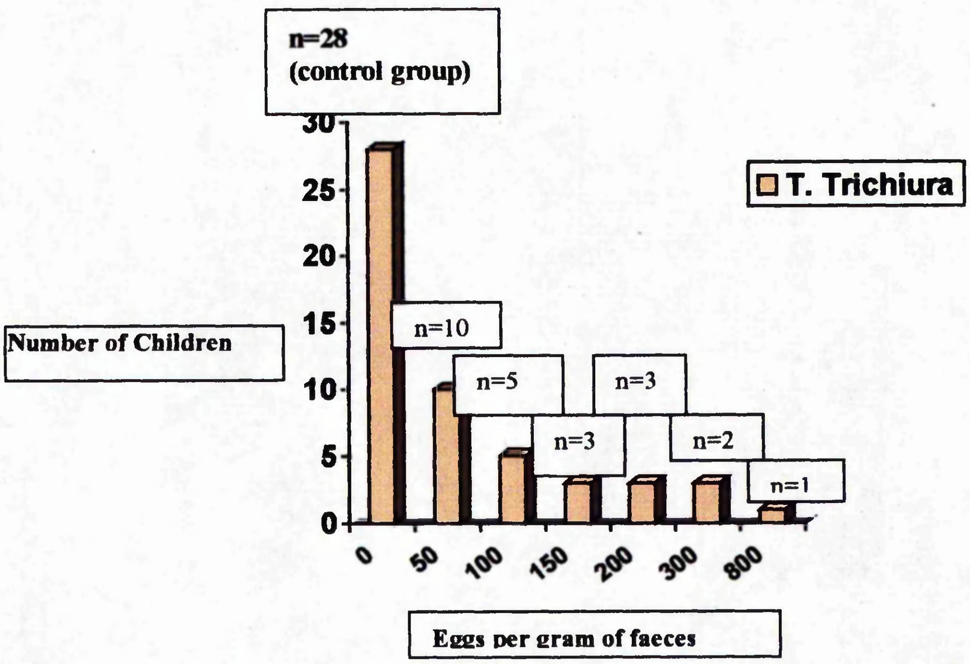


Fig 10.1 Distribution of the Number of Eggs per Gram of Faeces for *T. trichiura* in 24 Rural School Children from the State of Sinaloa.

The mean epg was 150 (SD=154, SE=33, Range 50-800, Confidence limits 95% 82-218).

10.3.2 Association between Trichuriasis and Children's Characteristics at Baseline.

10.3.2.1 Socio-economic Conditions.

Logistic regression analysis showed that the parents' education was an influencing factor (RC=- 0.23, p=0.03) for the presence of trichuriasis in the recruited children. The same analysis showed no association between trichuriasis and the rest of the socio-economic variables included in this study (economic activity and education of the parents, family monthly income, economic support, number of family members, and household conditions) (p>0.05). In addition, Spearman's correlation analysis showed a negative and significant association between the different levels of intensity of *T. trichiura* and the parents' education (r=-0.35, p=0.04). The same analysis showed no association between trichuriasis and the rest of the socio-economic variables (p>0.05).

10.3.2.2 Nutritional Status.

Mild and moderate malnutrition according to Z scores expressed by H/A, W/H, and W/H were observed as follows Table 10.1.

Table 10.1 Distribution of the Nutritional Status Investigated by the Z H/A, W/A, and W/H indicators between Uninfected and Infected School Age Children from a Rural Community of the State of Sinaloa.

	Uninfected (n=24)			**Infected (n=28)**		
Z-Score	**Normal**	**Mild**	**Moderate**	**Normal**	**Mild**	**Moderate**
H/A	**21 (87%)**	**3 (13%)**	**0**	**20 (72%)**	**6 (21%)**	**2 (7%)**
W/A	**23 (96%)**	**1 (4%)**	**0**	**23 (82%)**	**5 (18%)**	**0**
W/H	**23 (96%)**	**1 (4%)**	**0**	**25 (89%)**	**3 (11%)**	**0**

Malnutrition was observed in 20.8% of uninfected children (n=24) and 57.2% of *Trichuris*-infected children (n=28). Although the prevalence of mild and moderate malnutrition were found to be higher but no significant (X^2= 1.7, p=0.18) in *Trichuris*-infected children than uninfected children, logistic regression showed no association between trichuriasis and the nutritional status of these school children (RC=-0.001 and p= 0.66, RC= -0.005 and p= 0.51, and RC= -0.003 and p= 0.75 for malnutrition expressed by the Z H/A, W/A, and W/H indicators respectively). Spearman's correlation analysis showed no association between trichuriasis and nutritional status stated by the 3 indicators (r= 0.21 and p= 0.12, r=0.16 and p= 0.25, and r=0.10 and p= 0.48 for H/A, W/H, and W/H respectively).

10.3.2.3 Daily Nutrient Intakes.

The mean daily intakes of energy, calcium, niacin, vitamin B12, riboflavin, and pantothenic acid (Table 10.2 and Table 10.3) were lower than those recommended dietary allowances in both uninfected and *Trichuris*-infected school children.

Table 10.2 Uninfected School Children from the State of Sinaloa who did not Reach the Recommended Dietary Allowances and the Mean Daily Nutrient Intakes Expressed in Percentage of the Recommended Allowances.

Nutrient	Uninfected School Children (n=24)	Percentage Consumed of the Recommended Daily Requirement
Energy	20 (83.3%)	83.3
Fat	12 (50%)	72
Carbohydrates	12 (50%)	76.7
Calcium	16 (66.6%)	61.3
Phosphorus	14 (58.3%)	67.8
Iron	8 (33.3%)	80.5
Zinc	13 (54.1%)	67.9
Vitamin A	13 (54.1%)	44
Thiamin	12 (50%)	75.5
Riboflavin	17 (70.8%)	75
Niacin	18 (75%)	68
Folate	3 (12%)	78.8
Vitamin B6	6 (24%)	76.3
Vitamin B12	13 (54.1%)	48.7
Pantothenic Acid	23 (95.8%)	56.8

Table 10.3 *Trichuris*-Infected School Children from the State of Sinaloa who did not Reach the Recommended Dietary Allowances and the Mean Daily Nutrient Intakes Expressed in Percentage of the Recommended Allowances.

Nutrient	Infected School Children (n=28)	Percentage Consumed of the Recommended Daily Requirement
Energy	16 (57.1%)	73.3
Protein	2 (7.1%)	84.1
Fat	11 (39.3%)	68.2
Carbohydrates	9 (32.1%)	76.2
Calcium	16 (57.1%)	62.9
Phosphorus	11 (39.2%)	69.2
Iron	7 (25%)	75
Zinc	17 (60.7%)	68
Vitamin A	20 (71.4%)	36.4
Thiamin	6 (21.4%)	75.3
Riboflavin	13 (46.4%)	66.5
Niacin	21 (75%)	72
Folate	4 (14.3%)	61.2
Vitamin B6	7 (25%)	74.3
Vitamin B12	19 (67.8%)	33.4
Pantothenic Acid	26 (92.8%)	59.8

Logistic regression showed a significant and negative association between *T. trichiura* and lower daily intakes of protein (RC= -6.72, p=0.03), and vitamin B12 (RC=-3.14, p=0.02) in the *Trichuris*-infected school children. No association between *T. trichiura* against the rest of the daily nutrient intakes was observed in these children (p>0.05).

In addition, there was a negative and significant association between mild and moderate malnutrition in the 52 school children and lower intakes of energy (RC= -5.67, p=0.04) and protein (RC= -4.56, p=0.03). No association was observed between malnutrition and the rest of the daily nutrient intakes (p>0.05).

10.3.2.4 Symptomatology.

Logistic regression showed a positive and significant association between *T. trichiura* and respiratory infections (RC= 1.23, p=0.04) abdominal pain (RC= 1.45, p=0.04) and diarrhoea (RC= 1.33, p=0.03). No association was observed between *T. trichiura* and the prevalence of allergy, vomiting, fever, and headache (Table 10.4). Again, Spearman's correlation analysis showed a positive and significant association between *T. trichiura* and the prevalence of respiratory infections (r=0.3081, p=0.05), abdominal pain (r= 0.45, p=0.04), and diarrhoea (r=0.46, p=0.04). No association was observed between trichuriasis and the rest of the observed clinical symptoms (p>0.05).

Table 10.4 Trichuriasis and its Association with Different Clinical Symptoms Recorded from 28 School Children from El Higueral of the State of Sinaloa.

Symptom	Regression Coefficient	Standard Error	p
Respiratory Infections	**1.2321**	**0.6021**	**0.0407***
Allergy	**0.2513**	**1.0414**	**0.8093**
Vomiting	**-0.2144**	**0.7160**	**0.7646**
Fever	**1.0445**	**0.9163**	**0.2543**
Headache	**0.8827**	**0.6043**	**0.1440**
Abdominal Pain	**1.4567**	**0.5674**	**0.0435***
Diarrhoea	**1.3365**	**0.6574**	**0.0357***

Statistically significant (p<0.05)

10.3.2.5 Iron Status.

Higher levels of intensity of *T. trichiura* were accompanied with lower mean concentrations of MCH, MCHC, and haemoglobin, (F= 1.28 and p= 0.28, F= 0.86 and p= 0.53, and F= 1.43 and p= 0.23 respectively) and a higher mean concentration of TIBC in the experimental group F= 2.3 and p= 0.050) (ANOVA analysis). These associations were not significantly (p>0.05).

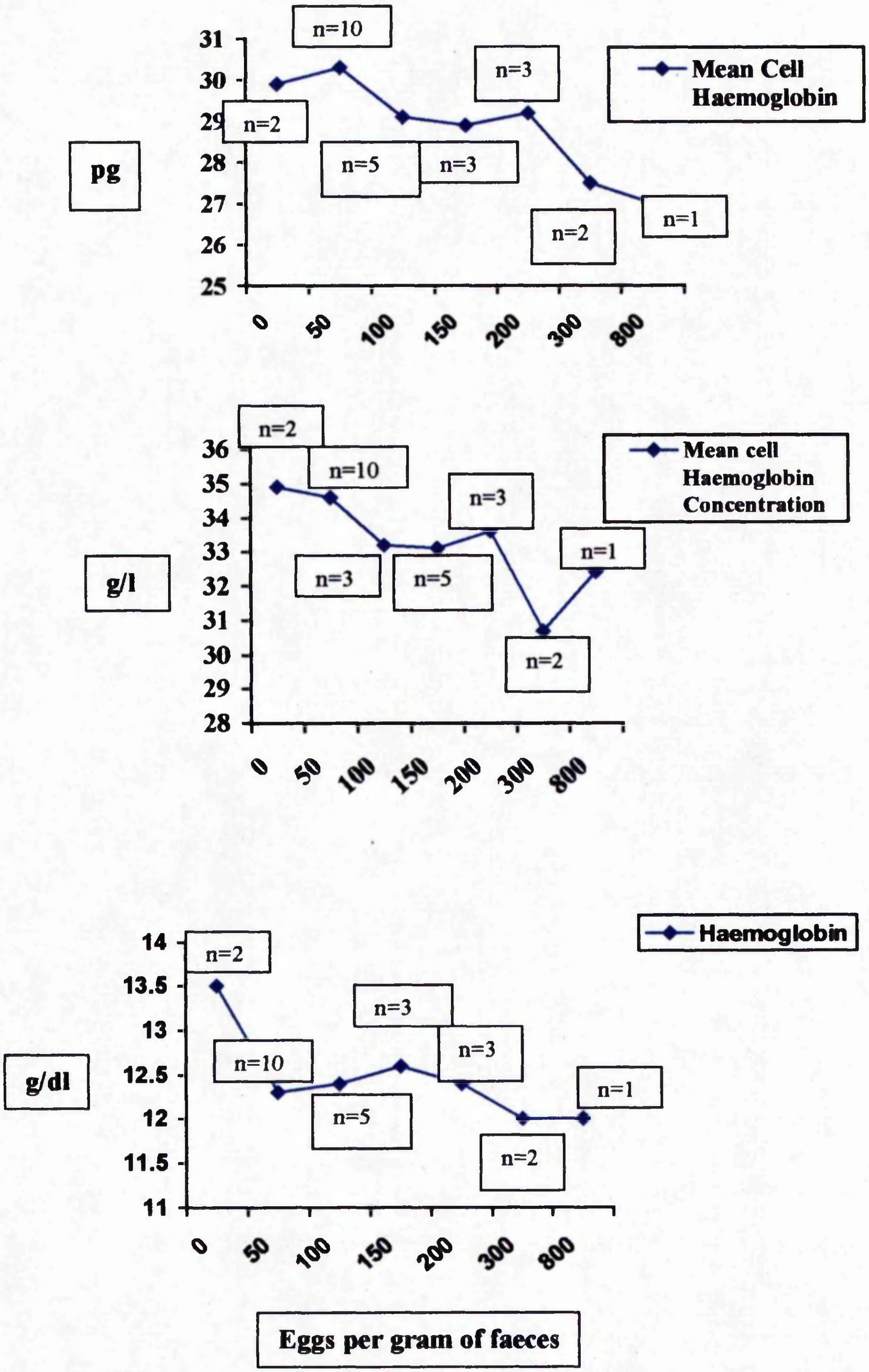


Fig 10.2 Relationship between the Levels of Intensity for *T. trichiura* and Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration and Haemoglobin, in 24 Rural School Children from the State of Sinaloa.

Spearman's correlation analysis showed a negative and significant association between the level of intensity of *T. trichiura* and the mean concentrations of haematocrit ($r= -0.38$, $p=0.04$), and MCH ($r=-0.48$, $p=0.008$). No correlation was observed between the level of intensity of *T. trichiura* and the rest of the mean concentrations of haematological and iron biochemical indicators ($p>0.05$). Because values below the cut-off for these indicators were not observed neither in the control or experimental group, logistic regression to analyse the association between trichuriasis and low iron status was no carried out.

Considering the value <12 µg/L of ferritin as a level of risk for iron deficiency, odds ratio by using the chi-square test showed that *T. trichiura* was not a risk factor for the presence of marginal normal levels of serum ferritin concentration in 10 *Trichuris*-infected school children ($X^2= 0.27$, $p= 0.15$) but low daily nutrient intakes of vitamin B12 ($X^2= 0.57$, $p= 0.04$) and riboflavin ($X^2= 0.68$, $p=0.03$) were found to be risk factors for the presence of marginal normal levels of serum ferritin concentration.

10.3.2.6 Comparison of Characteristics between the Control and Experimental Groups at Baseline.

At baseline (pre-albendazole and placebo treatments), mean ages for the experimental (n=28) and control groups (n=24) were 96.2 ± 18.8 and 95.9 ± 14.3 months respectively ($t= -0.0586$, $p=0.9535$, $df=50$). Comparison (t-test and GLM ANOVA), of the different variables between experimental and control groups are shown in Table 10.5. Indican concentrations (mg. indican/ mg. creatinine) showed no evidence of intestinal bacterial overgrowth in the recruited school children before (0.07 ± 0.04 and 0.06 ± 0.04 in the control and experimental groups respectively) ($t= 0.18$, $p>0.05$) and after treatment (0.10 ± 0.04 and 0.08 ± 0.04 in the control and experimental groups respectively) ($t= 1.4$, $p>0.05$). Also, there was no evidence of intestinal bacterial overgrowth in the same group after treatment (0.10 ± 0.05 and 0.07 ± 0.04, $t= 2.78$, $p> 0.05$ in the control group, and 0.08 ± 0.05 and 0.07 ± 0.04, $t=0.88$, $p>0.05$ in the experimental group). Malaria was not detected in these children.

Although malnutrition was observed in 20.8% of uninfected children (n=24) and 57.2% of *Trichuris*-infected children (n=28), no statistical difference was found in the mean average of the Z scores for the 3 indicators (Z H/A, Z W/A, and Z W/H), between the groups (t tests) ($p > 0.05$). No statistical difference was found in the mean daily nutrient intakes between the groups ($p > 0.05$). In both groups (control and experimental) the mean average for daily energy, calcium, niacin, zinc, vitamin B12, riboflavin, and pantothenic acid intakes were found to be below the recommended dietary allowances

There was no statistical difference in the mean average of the haematological values and biochemical indicators of iron between the groups ($p > 0.05$). It was observed that 5 uninfected school children and 11 *Trichuris*-infected school children showed haemoglobin values and serum ferritin concentrations close to the cut off values <12 g/L and < 12 μg/L respectively. However, no children showed evidence of iron deficiency or anaemia. Although the percentages for families with a family monthly income less than a minimal wage ($ 4 USD per day), bare-earth floor, and fathers with primary school completed were higher in the control group than in the experimental group, no statistical difference was found in the socio-economic levels between the groups (Table 10.5).

Electricity supply in the household was not taken into account during the socio-economic data collection, because most of the households were irregular settlements and did not have formal arrangements to request for electricity service.

Chi-square showed a higher significant difference in the frequency of respiratory infections, fever, headache, and abdominal pain ($p < 0.05$), in the experimental group than in the control group (Table 10.5, Fig.10.1).

Table 10.5 Comparative Statistical Analysis of the Children's Characteristics at Baseline (Pre-Albendazole and Placebo Treatments)

Nutritional Status			
Variable	**Control (n=24)**	**Experimental (n=28)**	**t values and p**
Z H/A	-0.0320 ± 1.0	-0.4510± 0.9491	t=1.54, p=0.1284
Z W/A	0.06891± 0.9361	-0.1028± 1.1004	t=0.4922, p=0.6247
Z W/H	0.1017 ± 0.7769	0.3011 ± 0.9446	t=-0.8059, p=0.4242
Mean Daily Intakes			
Energy (Kjoules)	7271.61 ± 2204.4	8380.70 ± 3030.64	t= -1.4864, p=0.1434
Energy (Kcal)	1730.63 ± 508.85	1980.511 ± 750.12	t= -1.3812, p=0.1733
Protein (g)	55.768 ± 20.702	66.826 ± 33.218	t= -1.4116, p= 0.1642
Fat (g)	53.257 ± 22.399	69.214 ± 43.909	t= - 1.6084, p= 0.1140
Carbohydrates (g)	274.143 ± 92.064	302.791 ± 115.110	t= -0.9795, p= 0.3320
Sodium (mg)	1376.85 ± 715.75	1667.32 ± 908.55	t= -0.6722, p= 0.5045
Potassium (mg)	1479.85 ± 733.65	2037.17 ± 1219.31	t=1.4044, p= 0.1663
Calcium (mg)	738.82 ± 421.60	775.79 ± 369.76	t= -0.3369, p= 0.7375
Phosphorus (mg)	665.09 ± 320.47	905.92 ± 630.046	t= -2.3955, p= 0.0560
Iron (mg)	12.51 ± 4.817	15.015 ± 11.532	t= -2.1774, p= 0.0641
Zinc (mg)	8.209 ± 4.009	11.873 ± 8.533	t= -1.9270, p= 0.0596
Vitamin A (µg)	715.425 ±541.60	1857.32 ± 704.25	t= -0.9991, p=0.3225
Thiamin (mg)	1.3055 ± 0.4266	1.4846 ± 0.7346	t= - 2.8117, p= 0.0701
Riboflavin (mg)	1.0418 ± 0.3961	1.0135 ± 0.6379	t= -1.1421, p= 0.2588
Niacin (mg)	11.036 ± 5.731	12.047 ± 6.455	t= -0.5920, p= 0.5559
Vitamin C (mg)	82.12 ± 78.64	158.86 ± 415.5	t= -0.8900, p= 0.3777
Vitamin E (mg)	9.0367 ± 8.317	15.718 ± 17.427	t= -1.7164, p= 0.0922
Vitamin B6 (mg)	1.9381 ± 1.3568	3.0320 ± 3.129	t= -1.5874, p= 0.1187
Vitamin B12 (µg)	1.3547 ± 1.5509	0.8610 ± 1.1129	t= 1.6018, p = 0.1155
Folate (µg)	195.718 ± 218.7	327.25 ± 476.45	t= - 1.2436, p= 0.2194
Pantothenic Acid (mg)	2.6550 ± 2.01	3.5368 ± 4.1774	t= - 0.9437, p= 0.3498
Iron Status			
Haemoglobin (g/l)	128.9 ± 6.90	127.8 ± 9.62	t= 0.4910, p= 0.6255
Haematocrit (%)	38.4 ± 2.084	38.25 ± 2.10	t= 0.2576, p= 0.7978
Red Blood Cells (Million/L)	$4.42 \times 10^{12} \pm 0.21 \times 10^{12}$	$4.36 \times 10^{12} \pm 0.28 \times 10^{12}$	t= 0.8642, p= 0.3916
MCV (fl)	86.77 ± 2.19	87.73 ± 2.81	t= -1.3529, p= 0.1821
MCH (pg)	29.09 ± 0.728	29.25 ± 1.09	t= - 0.6128, p= 0.5427
MCHC (g/l)	336.35 ± 13.41	335.52 ± 20.6	t= 0.2182, p= 0.8281
Serum Iron (µg/dl)	101.83 ± 49.65	90.42 ± 38.55	t= 0.9316, p= 0.3560
TIBC (µg/dl)	386.75 ± 83.68	360.928 ± 114.95	t= 0.9121, p= 0.3660
Transferrin Saturation (%)	37.23 ± 22.58	38.26 ± 15.81	t= -0.1941, p= 0.8468
Ferritin ((µg/l)	37.12 ± 22.09	33.68 ± 16.88	t= 0.4946, p= 0.6230

Socio-economic Conditions			
Variable	**Control (n=24)**	**Experimental (n=28)**	**X^2 value and p**
Employed Mother (%)	70.8 (17)	71.4 (20)	X^2= 0.0, p= 1.0
Mother with Completed Primary School (%)	12.5 (3)	14.3 (4)	X^2= 0.044, p= 0.83
Employed Father (%)	91.6 (22)	98 (27)	X^2= 3.4, p= 0.062
Father with Completed Primary School	14.3 (4)	8.3 (2)	X^2= 1.3, p= 0.25
Families with <1 Minimal Wage ($ 4 USD per day) (%)	33.3 (8)	21.4 (6)	X^2= 3.06, p= 0.08
Families with < 3 Rooms (%)	50 (12)	60.7 (17)	X^2= 1.63, p= 0.20
Families with Bare Earth Floor (%)	33.3 (8)	21.4 (6)	X^2= 2.06, p= 0.08
Families with Clean Piped Water (%)	0	0	
Pit for Faeces Disposal	91.6 (22)	89.2 (25)	X^2= 0.05, p= 0.81
Latrines	7.4 (2)	10.8 (3)	
Families with more than 4 children	16.6 (4)	25 (7)	X^2= 1.9, p= 0.16
Drinking Water (purified or boiled)	66.6 (16)	60.7 (17)	X^2= 0.53, p= 0.46
Socio-economic Status Index	56.3 (14) (SD=16.9)	49.5 (14) (SD=16.5)	t= 1.8, p= 0.079, df=50

Symptomatology			
Respiratory Infections (%)	25 (6)	46 (11)	X^2= 8.7, p= 0.003
Allergy (%)	8.3 (20)	7.1 (2)	X^2= 0.00, p= 1.0
Vomiting (%)	20.8 (5)	26.8 (8)	X^2= 0.5, p= 0.4
Fever (%)	4.2 (1)	17.8 (5)	X^2= 7.6, p= 0.005
Headache (%)	20.8 (5)	42.8 (12)	X^2= 38.6, p= 0.00
Abdominal Pain (%)	54.1 (13)	71.4 (20)	X^2= 5.4, p= 0.019
Diarrhoea (%)	29.2 (7)	28.6 (8)	X^2= 0.000, p= 1.0

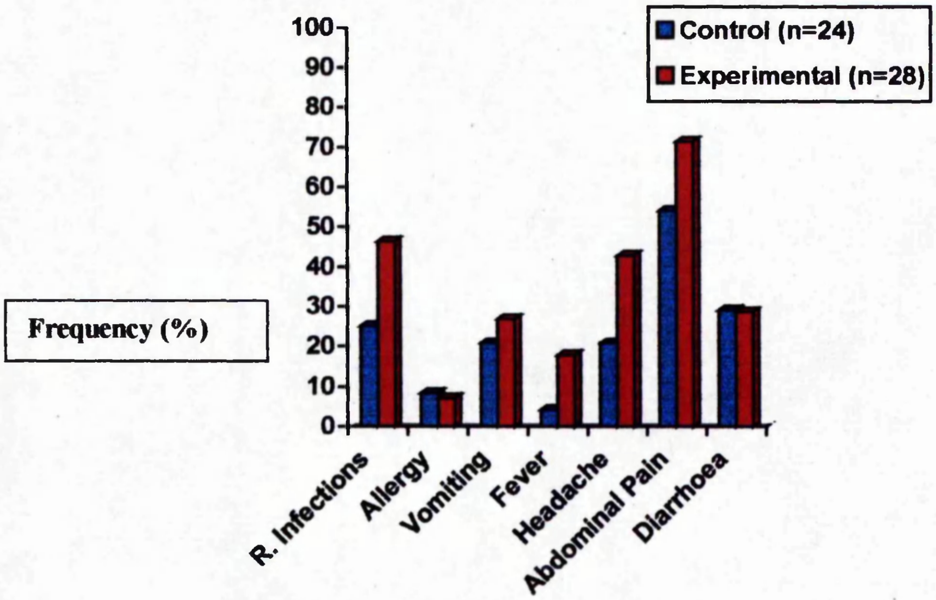


Fig. 10.3 Frequency of Clinical Symptoms observed in 52 School Age Children from the Rural Community of El Higueral in the State of Sinaloa before Albendazole and Placebo Treatments.

10.3.1.7 Administration of Albendazole and Placebo to the Experimental and Control Groups.

Egg counts in the *Trichuris*-infected children ranged from 50 to 800 (epg). The mean epg was 150 (SD=154, SE=33, Confidence limits 95%, 82-218). The experimental group was treated with albendazole, and the control groups received placebo. Stool samples were collected again on days 14th and 28th after treatment in the control group to ensure they remained uninfected. Stool samples were also collected in the experimental group on day 28 to determine the effectiveness for the albendazole treatment (Table 10.6). It was found that only 8 (28.6%) from 28 *Trichuris*-infected school children showed no egg counts after treatment. A second and third albendazole treatments were given again (on days 29 and 57 after the first treatment) for two consecutive days in those children with egg counts to ensure complete deworming (Table 10.6).

Table 10.6 Effectiveness of the Albendazole Treatment in 28 *Trichuris*-Infected School Children from the Rural Community El Higueral in the State of Sinaloa.

Day	Before Treatment	After Treatment		
	Arithmetic Mean (epg)	Arithmetic Mean (epg)	Cure Rate (%)	Eggs Counts Reduction (%)
1th Treatment	117			
28th		45	28.6	61.5
2nd Treatment	45			
28th		5	92	88.8
3rd Treatment	5			
28th		0	100	100

10.3.1.8 Comparison of Nutritional Status, Daily Nutrient Intakes, Iron Status, and Symptoms between Groups after Albendazole and Placebo Treatments.

Although a significant increase for the ratio Z H/A and significant decrease for the ratio Z W/H in the experimental group after treatment was observed, there was no significant difference in the Z Scores for the 3 ratios (H/A, W/A, W/H) between control and experimental groups (Table 10.7). Although an increase was observed in some mean daily nutrient intakes (potassium, phosphorus, vitamin B12, vitamin A) or decrease in others (folate, vitamin B6) in both groups, there was no significant difference in the mean daily nutrient intakes between the groups after treatment. An increase in the concentration of serum ferritin was observed in the experimental group. No significant difference was observed in the haematological values and biochemical indicators between the groups after treatment.

There was an increase in the frequency of respiratory infections in the experimental group and allergy in the control group. A decrease was observed in the frequency of vomiting and diarrhoea in both groups. The changes in the frequency of respiratory infections, allergy, vomiting, fever, and fever were significant between the groups after treatment (Table 10.7, Fig. 10.2).

Table 10.7 Comparative Statistical Analysis of the Nutritional Status, Daily Nutrient Intakes, Iron Status and Symptomatology between the Groups 28 days after Albendazole and Placebo Treatments.

Nutritional Status			
Variable	**Control (n=24)**	**Experimental (n=28)**	**t values and p**
Z H/A	-0.0566 ± 0.9207	-0.3721± 0.9565	t=1.2061, p=0.2334
Z W/A	0.0429 ± 0.9801	-0.1764± 1.0880	t=0.7583, p=0.4518
Z W/H	0.0886 ± 0.8327	0.0548 ± 1.0288	t=-0.1265, p=0.8998
Mean Daily Intakes			
Energy (Kjoules)	6780.75± 2082.4	8134.5 ± 3308.5	t=-1.7309, p= 0.0896
Energy (Kcal)	1622.62 ± 496.49	1944.21 ± 790.75	t= -1.7213, p= 0.0913
Protein (g)	53.90 ± 18.691	60.715 ± 31.120	t= -0.9361, p= 0.3537
Fat (g)	61.160 ± 31.654	64.771 ± 34.852	t= - 0.3885, p= 0.6992
Carbohydrates (g)	244.115 ± 75.763	268.241 ± 122.358	t= -2.5731, p= 0.0623
Sodium (mg)	1341.78 ± 637.82	1480.79 ± 879.92	t= -0.6423, p= 0.5235
Potassium (mg)	1301. 536 ± 640.51	1574.94 ± 1025.06	t= -1.1303, p= 0.2637
Calcium (mg)	592.415 ± 331.46	764.32 ± 467.78	t= -1.5046, p= 0.1387
Phosphorus (mg)	695.18 ± 347.77	855.41 ± 519.49	t= -1.2836, p= 0.2051
Iron (mg)	12.75 ± 5.0692	15.3522 ± 9.728	t= -1.1779, p= 0.2444
Zinc (mg)	10.244 ± 4.5838	8.6605 ± 5.0916	t= -1.1706, p= 0.2473
Vitamin A (µg)	1422.48 ± 2723.93	720.83 ± 761.05	t= 1.1909, p=0.2520
Thiamin (mg)	1.0907 ± 0.5353	1.2833 ± 0.7349	t= 1.0640, p= 0.2924
Riboflavin (mg)	1.095 ± 0.5585	1.1705 ± 0.5622	t= -0.4847, p= 0.6300
Niacin (mg)	10.087 ± 5.0720	11.068 ± 6.3833	t= -0.6066, p= 0.5468
Vitamin C (mg)	96.779 ± 107.245	82.985 ± 103.389	t= -0.4714, p= 0.6393
Vitamin E (mg)	10.5077 ± 10.6	9.188 ± 6.683	t= 0.5448, p= 0.5882
Vitamin B6 (mg)	1.8218 ± 0.8531	1.8267 ± 1.0619	t= -0.0182, p= 0.9855
Vitamin B12 (µg)	2.1457 ± 2.6040	1.0682 ± 1.2114	t= 1.9584, p = 0.0557
Folate (µg)	180.96 ± 107.99	177.11 ± 120.69	t= 0.1201, p= 0.9049
Pantothenic Acid (mg)	2.8739 ± 1.5233	2.6868 ± 1.5229	t= 0.4415, p= 0.6607

Iron Status			
Haemoglobin (g/l)	130.22 ± 7.19	129.08 ± 6.51	t= 0.5971, p= 0.5531
Haematocrit (%)	38.62 ± 2.51	37.60 ± 1.64	t= 1.7512, p= 0.0860
Red Blood Cells (Million/L)	4.45 X 10^{12} ± 0.25 X 10^{12}	4.42 X 10^{12} ± 0.17 X 10^{12}	t= 0.5479, p= 0.5861
MCV (fl)	86.70 ± 2.12	85.42 ± 2.16	t= 2.1353, p= 0.0507
MCH (pg)	29.24 ± 0.575	29.31 ± 0.966	t= - 0.3062, p= 0.7622
MCHC (g/l)	337.65 ± 13.96	343.74 ± 18.8	t= -1.3035, p= 0.1983
Serum Iron (µg/dl)	94.916 ± 41.89	94.25 ± 33.35	t= 0.0639, p= 0.9493
TIBC (µg/dl)	363.04 ± 66.75	370.35 ± 99.07	t= -0.3068, p= 0.7603
Transferrin Saturation (%)	37.11 ± 18.38	36.49 ± 17.02	t= 0.1246, p= 0.9013
Ferritin ((µg/l)	38.76 ± 29.40	49.32 ± 31.17	t= -1.2505, p= 0.2169

Symptomatology			
Respiratory Infections (%)	20.1	64.3	X^2= 37.9, p= 0.00
Allergy (%)	16.5	5	X^2= 5.3, p= 0.02
Vomiting (%)	3.3	17.8	X^2= 9.4, p= 0.002
Fever (%)	6.5	20.4	X^2= 7.5, p= 0.006
Headache (%)	22	44	X^2= 9.9, p= 0.001
Abdominal Pain (%)	50	42.3	X^2= 0.98, p= 0.32
Diarrhoea (%)	20.1	15	X^2= 0.55, p= 0.45

Statistically significant between the groups (p<0.05).

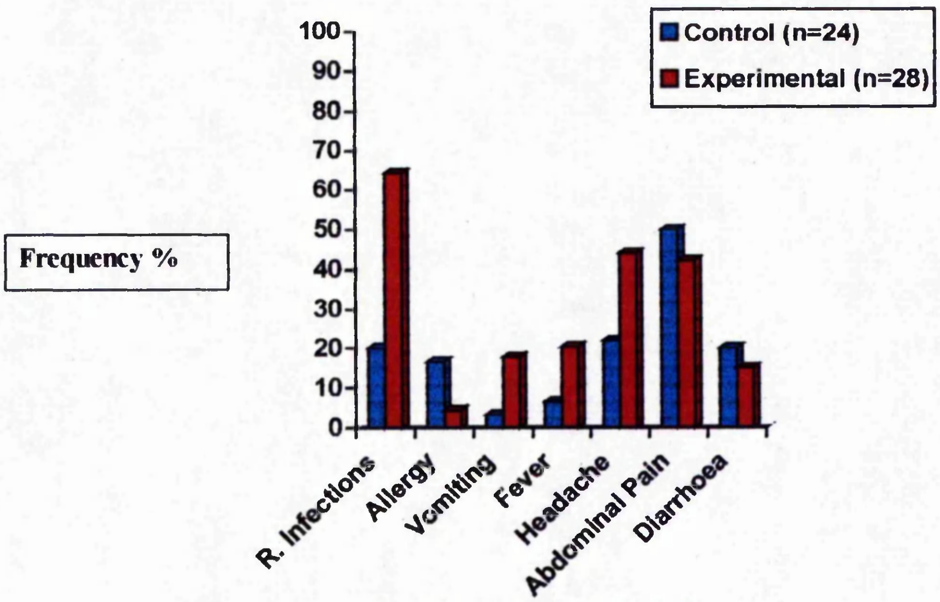


Fig. 10.4 Frequency of Clinical Symptoms observed in 52 School Age Children from El Higueral in the State of Sinaloa after Albendazole and Placebo Treatments

10.3.1.9 Comparison of Nutritional Status, Daily Nutrient Intakes, Iron Status, and Symptoms in the Experimental Group after Treatment.

A comparative analysis (paired t-test) showed a significant increase for the ratio H/A and a significant decrease for the ratio W/H after treatment (Table 10.8). No significant difference was observed for the ratio Z W/A before and after treatment (Table 10.8). A slight decrease was observed in some daily nutrient intakes in these school children. However, comparative analysis showed no significant difference in the daily nutrient intakes before and after treatment. It was also found that the recommended daily dietary allowances for energy, calcium, niacin, vitamin B12, and pantothenic acid were not reached for these children before and after treatment.

Slight increases were observed in haemoglobin, MCH, and TIBC, and significant increases in MCHC and serum ferritin (adjusting by sex, nutritional status, daily nutrient intakes, and clinical symptoms) in the experimental group after treatment. Analysis focusing on these changes (Spearman's correlation) showed a positive and significant association between haemoglobin and haematocrit (r=0.55, p= 0.002), haemoglobin with MCH (r= 0.63, p= 0.0002), and haemoglobin with MCHC (r= 0.64, p= 0.0001). It was also found that increases in the concentrations of haemoglobin, MCH, and MCHC were negative and significantly associated with daily nutrient intakes of vitamin B12 (r= -0.38, p=0.05, r=-0.44, p=0.01, and r= 0.35, p= 0.04 respectively) and riboflavin (r= -0.37, p=0.045, r= -0.33, p=0.05, and r= -0.40, p= 0.03 respectively).

The same analysis showed a negative and significant association between TIBC with transferrin saturation (r= -0.7850, p= 0.008). Although a significant increase of serum ferritin concentration after albendazole treatment was found in these children, no association was found between ferritin with biochemical indicators of iron (p>0.05). In addition, no association was observed between the rest of haematological and biochemical indicators of iron (p>0.05). Serum ferritin concentration showed no association with daily intakes of energy (r= 0.02, p= 0.90), protein (r= -0.12, p = 0.54) fat (r= -0.11, p= 0.55) and carbohydrates (r= -0.02, p= 0.90). The same was observed between ferritin concentration with the rest of the daily nutrient intakes included in this study (p>0.05). Ferritin also showed no association with respiratory infections (r= 0.21, p= 0.26), allergies (r=-0.08, p= 0.66), vomiting (r= 0.05, p= 0.79), fever (r= 0.01, p= 0.93), headache (r= -0.3, p= 0.12), abdominal pain (r= -0.20, p= 0.29), and diarrhoea (r= -0.12, p= 0.51).

Although it was observed an increase in the frequency of respiratory infections and a decrease in the frequency of abdominal pain, and diarrhoea after treatment (Table 10.8, Fig. 10.3), there was no significant difference in the frequency of the different clinical symptoms in the experimental group before and after albendazole treatment (p>0.05).

Table 10.8 Comparative Statistical Analysis of the Nutritional Status, Daily Nutrient Intakes, Iron Status and Symptomatology in the Experimental Group before and after Albendazole Treatment.

Nutritional Status			
Variable	**Pre- Treatment**	**Post-Treatment**	**t values and p**
Z H/A	-0.4510± 0.9491	-0.3721± 0.9565	t=2.1513, p=0.0405*
Z W/A	-0.1028± 1.1004	-0.1764± 1.0880	t= -2.9812, p=0.0514
Z W/H	0.3011 ± 0.9446	0.0548 ± 1.0288	t=-3.5246, p=0.0015*
Mean Daily Intakes			
Energy (Kjoules)	8380.70 ± 3030.64	8134.5 ± 3308.5	t=-0.5129, p= 0.6121
Energy (Kcal)	1980.511 ± 750.12	1944.21 ± 790.75	t= -0.3279, p= 0.7455
Protein (g)	66.826 ± 33.218	60.715 ± 31.120	t= -1.0435, p= 0.3059
Fat (g)	69.214 ± 43.909	64.771 ± 34.852	t= - 0.6469, p= 0.5231
Carbohydrates (g)	302.791 ± 115.110	258.241 ± 122.358	t= -0.1829, p= 0.1562
Sodium (mg)	1667.32 ± 908.55	1480.79 ± 879.92	t= -0.4743, p= 0.6391
Potassium (mg)	2037.17 ± 1219.31	1574.94 ± 1025.06	t= -1.1725, p= 0.2512
Calcium (mg)	775.79 ± 369.76	764.32 ± 467.78	t= -0.1573, p= 0.8761
Phosphorus (mg)	905.92 ± 630.046	855.41 ± 519.49	t= -1.3805, p= 0.1787
Iron (mg)	15.015 ± 11.532	15.3522 ± 9.728	t= -1.1848, p= 0.2464
Zinc (mg)	11.873 ± 8.533	8.6605 ± 5.0916	t= -1.9945, p= 0.0562
Vitamin A (µg)	1857.32 ± 704.25	720.83 ± 761.05	t= 1.1419, p=0.2635
Thiamin (mg)	1.4846 ± 0.7346	1.2833 ± 0.7349	t= 1.2098, p= 0.2368
Riboflavin (mg)	1.0135 ± 0.6379	1.1705 ± 0.5622	t= -0.2775, p= 0.7834
Niacin (mg)	12.047 ± 6.455	11.068 ± 6.3833	t= -0.6168, p= 0.5425
Vitamin C (mg)	158.86 ± 415.5	82.985 ± 103.389	t= -0.9819, p= 0.3348
Vitamin E (mg)	15.718 ± 17.427	9.188 ± 6.683	t= -1.7776, p= 0.0867
Vitamin B6 (mg)	3.0320 ± 3.129	1.8267 ± 1.0619	t= -1.9060, p= 0.0673
Vitamin B12 (µg)	0.8610 ± 1.1129	1.0682 ± 1.2114	t= 0.6834, p = 0.5001
Folate (µg)	327.25 ± 476.45	177.11 ± 120.69	t= 1.5965, p= 0.1220
Pantothenic Acid (mg)	3.5368 ± 4.1774	2.6868 ± 1.5229	t= -0.9564, p= 0.3473

Iron Status			
Haemoglobin (g/l)	127.8 ± 9.62	129.08 ± 6.51	t= 0.7507, p= 0.4593
Haematocrit (%)	38.25 ± 2.10	37.60 ± 1.64	t= -1.4916, p= 0.1474
Red Blood Cells (Million/L)	4.36 X 10^{12} ± 0.28 X 10^{12}	4.42 X 10^{12} ± 0.17 X 10^{12}	t= 1.1814, p= 0.2473
MCV (fl)	87.73 ± 2.81	87.42 ± 2.16	t= -0.5789, p= 0.8065

MCH (pg)	29.25 ± 1.09	29.91 ± 0.966	t= 0.2266, p= 0.6224
MCHC (g/l)	335.52 ± 20.6	343.74 ± 18.8	t= 1.9415, p= 0.0427*
Serum Iron (µg/dl)	90.42 ± 38.55	94.25 ± 33.35	t= 0.4719, p= 0.6408
TIBC (µg/dl)	360.928 ± 114.95	370.35 ± 99.07	t= 0.3677, p= 0.7159
Transferrin Saturation (%)	38.26 ± 15.81	36.49 ± 17.02	t= 0.4719, p= 0.6408
Ferritin ((µg/l)	33.68 ± 16.88	49.32 ± 31.17	t= 3.3188, p= 0.0025*

Symptomatology			
Respiratory Infections (%)	46	64.3	X^2= 5.8, p= 0.01
Allergy (%)	7.1	5	X^2= 0.08, p= 0.076
Vomiting (%)	26.8	17.8	X^2= 1.9, p= 0.16
Fever (%)	17.8	20.4	X^2= 0.13, p= 0.71
Headache (%)	42.8	44	X^2= 0.020, p= 0.88
Abdominal Pain (%)	71.4	42.3	X^2= 15.9, p= 0.000
Diarrhoea (%)	28.6	15	X^2= 4.3, p= 0.004

10.3.1.10 Comparison of Nutritional Status, Daily Nutrient Intakes, Iron Status, and Symptoms in the Control Group before and after Treatment.

There was no significant difference between the Z scores for H/A, W/A, and W/H indicators before and after placebo treatments in the control group (Table 10.9). A slight decrease in some daily nutrient intakes in these school children was observed. However, comparative analysis showed no significant difference in the daily nutrient intakes before and after placebo treatment. It was also found that recommended daily dietary allowances for energy, calcium, phosphorus, riboflavin, niacin, and pantothenic acid were not reached for these children before and after placebo treatment.

Slight increases in haemoglobin and serum ferritin were observed in the control group after treatment. These changes were accompanied by a significant and positive association (Spearman's correlation) between haemoglobin with haematocrit (r= 0.68, p= 0.0002), and haemoglobin with red blood cells (r= 0.90, p= 0.000). Spearman's correlation analysis showed no association between ferritin with haematological and biochemical indicators of iron (p>0.05). No additional associations were found between the haematological and biochemical indicators of iron before and after placebo treatment (p>0.05).

Although an evident increase in the frequency of allergies, and a significant decrease in the frequency of vomiting (Table 10.9, Fig. 10.3). No significant difference was observed in the frequency of the rest of the different clinical symptoms in the control group before and after placebo treatment ($p>0.05$).

The control group showed an evident increase in the frequency of allergies and a decrease of vomiting and diarrhoea after placebo treatment. The frequency of respiratory infections, abdominal pain, fever, and headache remained stable in this group. Again, all changes observed were not significant

Table 10.9 Comparative Statistical Analysis of the Nutritional Status, Daily Nutrient Intakes, Iron Status and Symptomatology in the Control Group before and after Placebo Treatment.

Nutritional Status			
Variable	**Pre- Treatment**	**Post-Treatment**	**t values and p**
Z H/A	-0.0320 ± 1.0	-0.0566 ± 0.9207	t=-0.5759, p=0.5702
Z W/A	0.06891± 0.9361	0.0429 ± 0.9801	t= -0.5414, p=0.5934
Z W/H	0.1017 ± 0.7769	0.0886 ± 0.8327	t= -0.1708, p=0.8659
Mean Daily Intakes			
Energy (Kjoules)	7271.61 ± 2204.4	6780.75± 2082.4	t=-0.9329, p= 0.3605
Energy (Kcal)	1730.63 ± 508.85	1622.62 ± 496.49	t= -0.8741, p= 0.3911
Protein (g)	55.768 ± 20.702	53.90 ± 18.691	t= -0.3114, p= 0.7582
Fat (g)	53.257 ± 22.399	61.160 ± 31.654	t= - 1.0485, p= 0.3053
Carbohydrates (g)	274.143 ± 92.064	244.115 ± 75.763	t= -2.5487, p= 0.0579
Sodium (mg)	1376.85 ± 715.75	1341.78 ± 637.82	t= -0.1954, p= 0.8467
Potassium (mg)	1479.85 ± 733.65	1301. 536 ± 640.51	t= -0.9246, p= 0.3647
Calcium (mg)	738.82 ± 421.60	592.415 ± 331.46	t= -1.5294, p= 0.1398
Phosphorus (mg)	665.09 ± 320.47	695.18 ± 347.77	t= 0.3613, p= 0.7211
Iron (mg)	12.51 ± 4.817	12.75 ± 5.0692	t= 0.1879, p= 0.8525
Zinc (mg)	8.209 ± 4.009	10.244 ± 4.5838	t= 1.6908, p= 0.1043
Vitamin A (µg)	715.425 ±541.60	1422.48 ± 2723.93	t= 1.3509, p=0.1898
Thiamin (mg)	1.3055 ± 0.4266	1.0907 ± 0.5353	t= 0.7221, p= 0.4775
Riboflavin (mg)	1.0418 ± 0.3961	1.095 ± 0.5585	t= 0.3740, p= 0.7118
Niacin (mg)	11.036 ± 5.731	10.087 ± 5.0720	t= -0.5759 p= 0.5703
Vitamin C (mg)	82.12 ± 78.64	96.779 ± 107.245	t= 0.5436, p= 0.5919
Vitamin E (mg)	9.0367 ± 8.317	10.5077 ± 10.6	t= 0.5021, p= 0.6203
Vitamin B6 (mg)	1.9381 ± 1.3568	1.8218 ± 0.8531	t= -0.3292, p= 0.7449
Vitamin B12 (µg)	1.3547 ± 1.5509	2.1457 ± 2.6040	t= 1.0202, p = 0.3182
Folate (µg)	195.718 ± 218.7	180.96 ± 107.99	t= -0.2727, p= 0.7875
Pantothenic Acid (mg)	2.6550 ± 2.01	2.8739 ± 1.5233	t= 0.4512, p= 0.6560

Iron Status			
Haemoglobin (g/l)	128.9 ± 6.90	130.22 ± 7.19	t= 1.0990, p= 0.2831
Haematocrit (%)	38.4 ± 2.084	38.62 ± 2.51	t= 0.5586, p= 0.5818
Red Blood Cells (Million/L)	4.42 X 10^{12} ± 0.21 X 10^{12}	4.45 X 10^{12} ± 0.25 X 10^{12}	t= 0.9416, p= 0.3561
MCV (fl)	86.77 ± 2.19	86.70 ± 2.12	t= -0.1196, p= 0.9058
MCH (pg)	29.09 ± 0.728	29.24 ± 0.575	t= 0.7135, p= 0.4827
MCHC (g/l)	336.35 ± 13.41	337.65 ± 13.96	t= 0.3169, p= 0.7542
Serum Iron (µg/dl)	101.83 ± 49.65	94.916 ± 41.89	t= -0.5677, p= 0.5757
TIBC (µg/dl)	386.75 ± 83.68	363.04 ± 66.75	t= -1.5444, p= 0.1361
Transferrin Saturation (%)	37.23 ± 22.58	37.11 ± 18.38	t= 0.2082, p= 0.8368
Ferritin ((µg/l)	37.12 ± 22.09	38.76 ± 29.40	t= 0.4359, p= 0.6669

Symptomatology			
Respiratory Infections (%)	25	20.1	X^2= 0.25, p= 0.61
Allergy (%)	8.3	16.5	X^2= 2.3, p= 0.13
Vomiting (%)	20.8	3.3	X^2= 12.6, p= 0.0003
Fever (%)	4.2	6.5	X^2= 0.10, p= 0.74
Headache (%)	20.8	22	X^2= 0.030, p= 0.86
Abdominal Pain (%)	54.1	50	X^2= 0.18, p= 0.67
Diarrhoea (%)	29.2	20.1	X^2= 1.7, p= 0.19

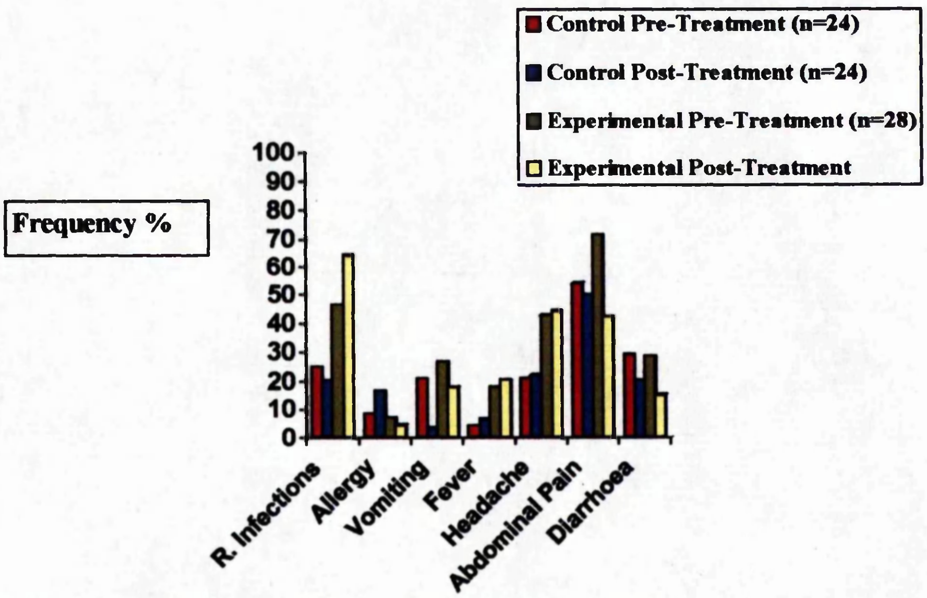


Fig. 10.5 Frequency of Clinical Symptoms observed in 52 School Age Children from the Rural Community of El Higueral in the State of Sinaloa Pre and Post Periods of Treatments.

10.4 Discussion and Conclusions

10.4.1 Trichuriasis as a Silent and Insidious Infection and Albendazole Treatment.

Trichuriasis has been characterised as a silent and insidious disease and is rarely present as an acute infection (Cooper and Bundy, 1988). This study was conducted in the rural community of El Higueral of the State of Sinaloa, where *T. trichiura* has been present as a chronic infection according to records from the health centre close to this community (a total of 150 cases of non-acute trichuriasis were confirmed from 1993 to 1996). It is difficult to treat *T. trichiura* as was evidenced by the low efficacy shown by albendazole treatment in the school children in this study. Only 28.6% of the 28 *Trichuris*-infected school-children showed no egg counts after albendazole treatment. Two and three albendazole administrations were required to ensure a complete deworming in the rest of the *Trichuris*-infected children.

A WHO report (1996) revealed that albendazole has shown lower efficacy against trichuriasis in comparison with that shown against ascariasis. Albendazole has shown cure rates and reduction in egg counts between 85% and 100% against ascariasis, and cure rates between 10% and 67%, and reduction in egg counts between 73% and 87% against trichuriasis (WHO, 1996).

In 1990, Ismael *et al.*, (APCO, 1993) in Sri Lanka assessed the efficacy of different anthelmintics in a single against STHs in 667 children aged 3-12 years. Schedules of treatment of albendazole 400mg, mebendazole 200mg, mebendazole 500 mg produced high cure rates (95.9%-99.7%) and egg count reductions (86.7%-97.4%) against light (<10000 epg) or heavy infections (>10000 epg) of ascariasis 14-21 days after treatment. In the case of light trichuriasis (<2000 epg) cure rates of 37.5%, 44.1%, and 37.1% were observed with albendazole 400mg, mebendazole 200mg and mebendazole 500mg respectively. In high intensities (>2000 epg) cure rates were poorer and ranged from 6.7% to 15%.

In 1990, Chen *et al.*, (APCO, 1993), observed a cure rate of 41.4% and egg reduction rate of 85% against trichuriasis after 2 months in 111 children who received albendazole 200mg (single administration). A poorer cure rate (23.8%) and egg reduction rate (59.5%) was observed in 63 children after 2 months in 63 children who received albendazole 400mg.

In 1990, Setasuban *et al.*, (APCO, 1993), observed better cure rates against trichuriasis after 21 days in school children who received albendazole 400mg for 3 consecutive days (Cure Rates= 100% and Egg Reduction Rate =100%), than those in school children who received albendazole 400mg for 2 consecutive days (CR=94.4% and ERR=99.8%) or albendazole 400 mg for 1 day (CR=77.7% and ERR=99.5%

In 1992 Anantaphruti *et al.*, (APCO, 1993) observed that albendazole 400mg once daily for 3 days was effective against light infections of trichuriasis (<1000 epg) (CR=68.2%) after 21 days in 90 individuals. Poorer cure rates were observed in 90 individuals with moderate infections (1001 epg-10000 epg) (CR=27.7%) and 90 individual with heavy infections (>10000epg) (CR= 7.1%).

In this study a single dose of albendazole 400mg showed a cure rate of 28.6% and a reduction in egg counts of 61.5% against trichuriasis. When albendazole was administered for two consecutive days, a cure rate of 92% and egg count reduction of 88.8% was observed. Results from this study suggest that trichuriasis is a consistent infection in populations with low income and presenting some degree of malnutrition. It was also found that the level of education of the parents was an influencing factor for the presence of trichuriasis in school children from the community of El Higueral. No other socio-economic factor was found to be associated with the presence of trichuriasis. In addition sub-optimal daily nutrient intakes were found to be more strongly associated with malnutrition in these children rather than with trichuriasis.

10.4.2 Trichuriasis and Socio-economic Status.

Although comparisons showed no statistical difference in the socio-economic status considered between control and experimental groups, results suggested that the presence of *T. trichiura* in the school children from the rural community of El Higueral in the State of Sinaloa was influenced by the level of education of the children's parents. Other socio-economic variables were not found to be determinant for the presence of *T. trichiura.* in El Higueral. Smith, *et al.*, (2001), found an inverse association between trichuriasis and the level of formal education of respondents in four rural Honduran communities.

A study with Panamanian children revealed that the prevalence of trichuriasis was significantly higher in children living in the poorest housing conditions (Holland *et al.*, 1988). However, socio-economic conditions have been found not always to be associated with the prevalence of *T. trichiura* (Stoltzfus *et al.*, 1997; Tshikuka *et al.*, 1996).

10.4.3 Trichuriasis, Nutritional Status, and Daily Nutrient Intakes.

An increase in the height of the experimental group was observed after albendazole treatment which resulted in a significant increase in the indicator Z H/A and a significant decrease in the Z W/H. Slight increases were observed for weight and height in the control group. In spite of these changes, no statistical difference was observed in the indicators Z H/A, W/A, and W/H between the groups before and after treatment. Mild and moderate malnutrition (20% and 50% in the control and experimental groups respectively) was observed in these school children, but no association was observed between trichuriasis and malnutrition. Recently, an epidemiological study carried out in Brazilian children revealed that stunting (low height/age) was significantly associated with *A. lumbricoides* and *T. trichiura* (Saldiva *et al.*, 1999). On the other hand, results from 24-h food intakes recalls, suggested that school children from this study are subjected to sub-optimal daily nutrient intakes. No difference was found in the mean daily nutrient intakes between groups before and after treatment. Lower daily nutrient intakes were found to be stronger associated with malnutrition than trichuriasis. Saldiva *et al.*, (1999), reported a lower indicator of height/age associated with inadequate protein intakes in *Trichuris-* school children. No association was found between low height/age and trichuriasis.

10.4.4 Trichuriasis and Morbidity.

Although the frequencies of respiratory infections, fever, headache, and abdominal pain were higher in the experimental group than those in the control group, there was no statistical difference in the frequency of the recorded clinical symptoms between the groups at baseline. Statistical analysis gave evidence of significant association between trichuriasis with respiratory infections, abdominal pain, and diarrhoea

Albendazole treated school children did not complain of side effects a week after treatment, although abdominal pain and diarrhoea has been reported as occasional side effects (Ismail, *et al.*, 1990; Chen, *et al.*, 1990). In this study, a decrease in the frequency of abdominal pain and diarrhoea was observed in the treated school children.

Smith, *et al.*, (2001), showed an association between trichuriasis and a recent history of diarrhoea and other infections in Honduran children aged 2-5 years old.

After albendazole treatment there was a significant decrease in the frequency of abdominal pain, diarrhoea and a significant increase in the frequency of respiratory infections in the experimental group. The frequency of allergies, fever, and headache remained stable in this group. The control group showed a significant increase in the frequency of allergies and a significant decrease of vomiting after placebo treatment. The frequency of respiratory infections, abdominal pain, fever, headache, and diarrhoea remained stable in this group.

It is difficult to explain the association between trichuriasis and the frequency of respiratory infections, although it could be evidence of an impaired immune response in these children resulting in a higher vulnerability to different infections. Albendazole treatment evidenced some degree of association between trichuriasis and abdominal pain, vomiting and diarrhoea, although reductions in their frequencies after albendazole treatment were no significant. Other kind of infections or factors are probably contributing for the presence of the clinical symptoms recorded in this study.

The relative decrease in clinical symptoms observed in the experimental group showed the potential benefits of albendazole treatment against trichuriasis. At present, a deworming campaign using albendazole in school children has been carried out in Mexico since 1993 and probably a decrease in the prevalence of trichuriasis will result in a significant decrease in morbidity in early childhood. However, consecutive days for dosage administration (regimen), time length between treatment, prevalence, level of intensity must be considered when albendazole is specifically administered to school children in communities where trichuriasis is persistent. *T. trichiura* must always be of public health importance because although it is seldom causes acute symptoms or mortality, it is cumulative in terms of the nutritional and health status of the host.

10.4.5 Trichuriasis and Iron Status.

No statistical difference was found in the mean of the different haematological and biochemical indicators of iron between *Trichuris*-infected and uninfected school children before (baseline) and after treatments (after 5 weeks). Studies by Carrera *et al.*, (1984) and Taren *et al.*, (1987) showed that approximately three or four weeks are required for lactose tolerance to return to normal values, probably associated with a recovering of the integrity of the intestinal mucosal. These findings were used to investigate for any probably change in the biochemical indicators of iron status in the albendazole treated school children during a period of 5 weeks (before and after albendazole treatment) in this study. No additional time was regarded because the high risk to infection or re-infection by the same or different intestinal parasites and the unhealthy environment conditions at which these school-children were living.

Higher levels of intensity *T. trichiura* were associated with lower mean concentrations of haemoglobin, MCH, MCHC, and higher TIBC in the experimental group, although these associations were not significant. Trichuriasis was found not to be associated with marginal normal levels of haemoglobin and ferritin concentrations. In a recent study, *T. trichuria* was found to be associated with worse iron status (lower haemoglobin, erythrocyte protoporphyrin, and serum ferritin) in school-children from Pemba Island, Zanzíbar, although the association with hookworms was strongest by far (Stoltzfus *et al.*, 1997). Robertson *et al.*, (1992) reported that 124 Panamanian school-children infected with *T. trichiura* had a significantly lower haemoglobin concentration than uninfected children, and children with both *Trichuris* and hookworm infections were significantly more likely to be associated with haemoglobin concentration < 11.5 g/dl than were uninfected. In addition, they reported that analysis of variance of mean haemoglobin concentration showed that intensities of trichuriasis > 5000 eggs/g were associated with a significantly lower blood haemoglobin concentration, and a reduction in the intensity of trichuriasis was associated with an increase in haemoglobin concentration in a year-period, although it was not statistically significant. However, other authors (Mahmood, 1966; Martinez and Beaver, 1968; Lotero *et al.*, 1974; Greenberg and Cline, 1979) showed no evidence of association between intensity and iron status even when the infection was severe. One explanation for this paradox could be the host nutritional status, sample size (individual variation), actual intensity (light and heavy), statistical data analysis, dietary iron requirements, and methodology to determine the biochemical indicators, and coproparasitological analysis (Stephenson and Holland, 1987).

After albendazole treatment, results showed significant increases in MCHC and serum ferritin in the experimental group, but no significant changes were observed in the rest of the biochemical indicators of iron. It was also observed that slight increases in haemoglobin, MCH, and TIBC were accompanied by a decrease in transferrin saturation.

Serum ferritin concentration has largely replaced serum iron determination as an index of iron storage (Worwood, 1996). However, ferritin concentration in this study was found not to be associated with iron and the rest of the haematological and biochemical indicators even in those school children with marginal levels of ferritin and haemoglobin concentrations. In addition, the increase in TIBC accompanied by a decrease in transferrin saturation, observed after albendazole treatment, is a common feature observed in iron deficiency (Gibson, 1990). In this case these changes were accompanied by a significant increase of ferritin concentration (even controlling for nutritional status, daily nutrient intakes and symptomatology) which showed no association with TIBC or transferrin saturation. Ferritin probably may has also been acting as a reactant of acute phase although was found not to be associated with the recorded clinical symptoms. The absence of anaemia and iron deficiency, viral and bacterial infections, and sub-optimal micronutrients intakes are probably factors influencing in the lack of association between ferritin and the rest of the biochemical indicators of iron, or ferritin with clinical symptoms. McSharry *et al.*, (1999) showed increased ferritin values suggesting an inflammatory response in putatively immune children compared with *Ascaris*-infected children with little serological evidence of inflammation despite their high parasite burdens in an area of Nigeria where infection is hyperendemic.

On the other hand, slight but no significant increases in the haemoglobin and ferritin concentrations were observed in the control group after placebo treatment. No additional changes were observed in the rest of the haematological and biochemical indicators of iron

Probably the period of time of five weeks between blood collections, before and after albendazole treatment, was not enough to find some statistical change and association among the biochemical indicators of iron in this study. Robertson *et al.*, (1992) could not observe statistical changes in the haemoglobin concentration in treated trichuris-infected children in a period of one year, although a tendency of increase in the haemoglobin values was reported at the end of this period. Although iron deficiency and anaemia were not found in the community of El Higueral, some school children showed marginal low values of haemoglobin and serum ferritin concentrations in this study. Significant changes in haemoglobin concentrations may be expected in the basis of the time period between measurements, number of subjects studied and their iron requirements, the intensity of parasitic infections, efficacy of the treatment, how rapidly the re-infection occurs, degree of depression of haemoglobin, iron content of the local diets and sources of iron (Stephenson and Holland, 1987).

10.4.6 Association between Iron Status and Daily Nutrient Intakes

In this study, the increases in haemoglobin, MCH, and MCHC were negatively and significantly associated with daily intakes of vitamin B12 and riboflavin. Evidence of association between vitamin B12 deficiency and high values of MCH and MCV has been reported by Gibson, (1990), and Allen, *et al.*, (2000). These associations may indicate that these vitamins are probably influencing factors in erythropoiesis or a proxy for low intakes of other micronutrients, although recommended mean daily intakes for iron, folate and vitamin A were reached for the control and experimental groups in this study. In addition, bioavailability, quality, maldigestion and malabsorption of nutrients may also be important factors of influence in the process of haematopoiesis (Chapter 4). It is likely that deficiencies in daily nutrient intakes are a stronger factor than trichuriasis influencing in the nutritional status and probably the haematopoiesis process in the school children from the community of El Higueral.

10.4.7 Trichuriasis as a Risk Factor for Iron Deficiency and Anaemia.

These children are probably at higher risk of iron deficiency or anaemia attributed to different factors including trichuriasis in combination with sub-optimal daily nutrient intakes observed in this study. Statistical analysis showed association, but not significant, between higher levels of intensity of *T. trichiura* with lower haemoglobin, MCH, and MCHC concentrations, and a significant association between higher MCH, MCHC and marginal normal values of ferritin with lower daily intakes of vitamin B12 and riboflavin. No evidence was found of sub-optimal daily intakes of iron, vitamin A and folate in the children from this study.

Additional parasitological and nutritional studies must involve determinations of plasma vitamin concentrations, mainly those related to erythropoiesis (at least vitamin B12, folate, vitamin A, and riboflavin), and to introduce the serum transferrin receptor determination which has been successful for evaluating iron status in environments where multiple factors make serum ferritin a relatively unhelpful index (Bayness, 1996).

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APPENDIX 1

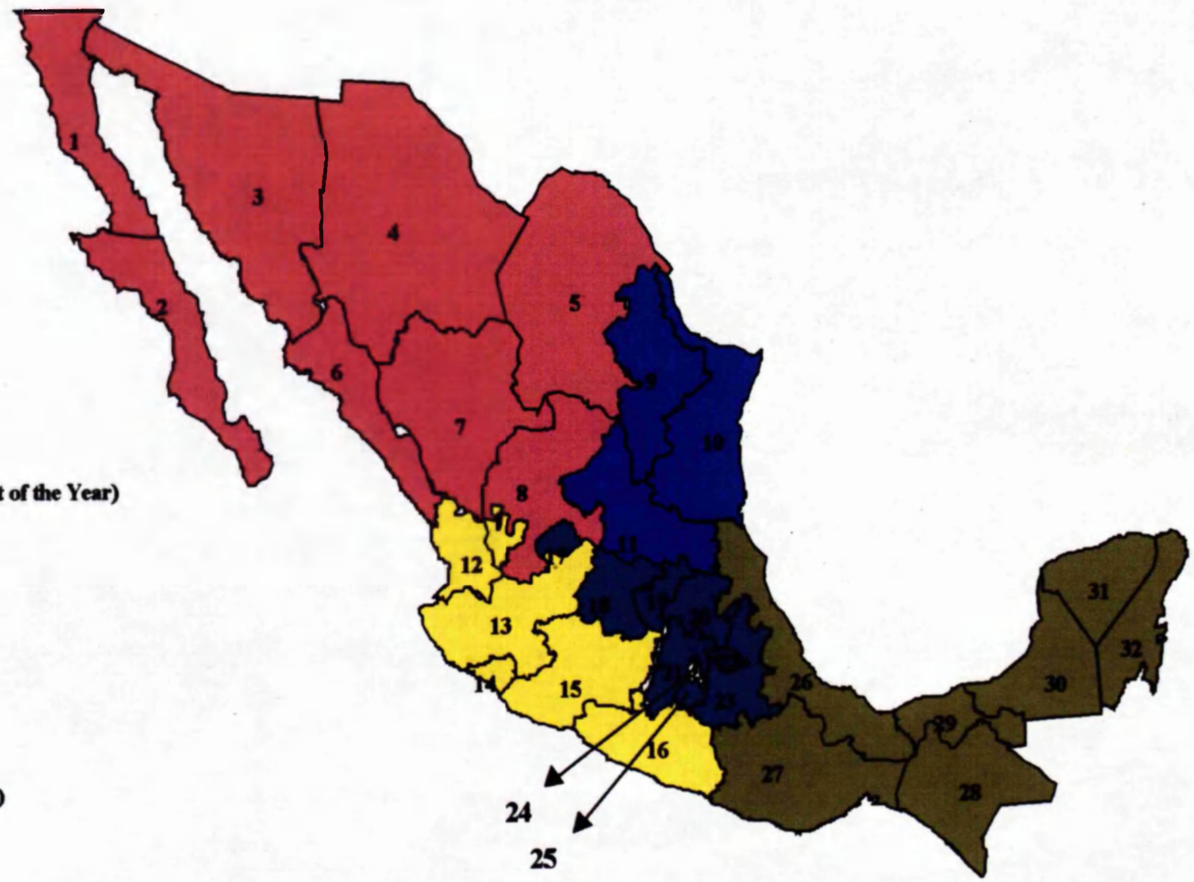
Northwest Region (Rainfall during June-September)
1. Baja California Norte (Temparate , warm and cold climate)
2. Baja California Sur (Temperate and warm climate)
3. Sonora (Warm and cold climate with desert areas)
4. Chihuahua (Temperate , warm and cold climate)
5. Coahuila(Temperate and tropical climate)
6. Sinaloa (Tropical climate)
7. Durango (Tropical climate)
8. Zacatecas (Tropical climate with desert areas)

Northeast Region (Rainfall most part of the Year)
9. Nuevo Leon (Template and warm climate)
10. Tamaulipas (Template and tropical climate)
11. San Luis Potosi (Template and tropical climate)

Southwest Region (Rainfall most part of the Year)
12. Nayarit (Tropical climate)
13. Jalisco (Tropical climate)
14. Colima (Tropical climate)
15. Guerrero (Heavyrain and tropical climate)
16. Michoacan (Heavyrain and tropical climate)

Central Region (Rainfall and template climate most part of the Year)
17. Aguascalientes
18. Guanajuato
19. Queretaro
20. Hidalgo
21. Estado de Mexico
22. Tlaxcala
23. Puebla
24. Distrito Federal
25. Morelos

Southeast Region (Heavy rainfall most part of the Year)
26. Veracruz (Tropical climate)
27. Oaxaca (Tropical climate)
28. Chiapas (Tropical climate)
29. Tabasco (Tropical climate)
30. Campeche (Tropical climate)
31. Yucatan (Template and tropical climate)
32. Quintana Roo (Template and tropical climate)



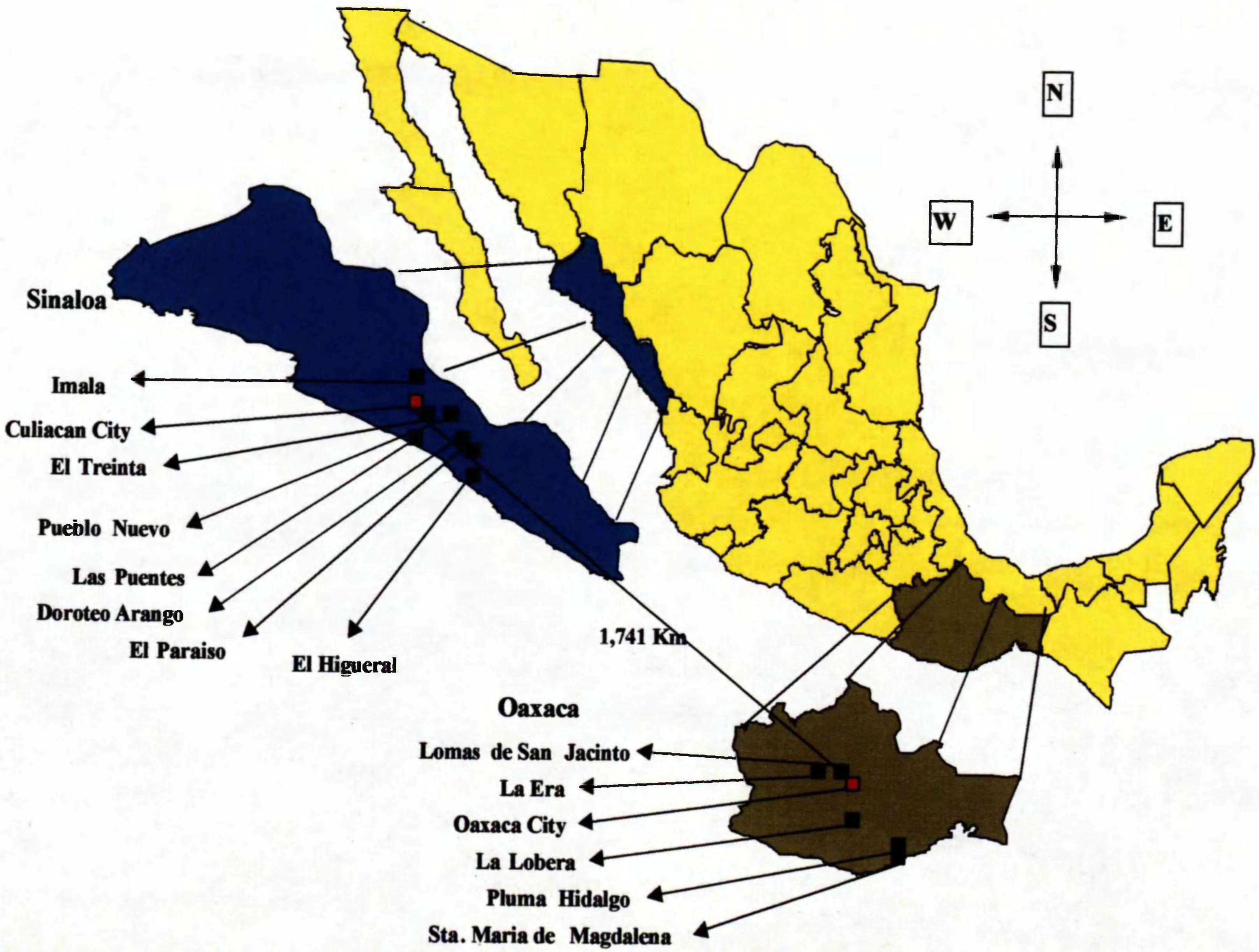
Political Division of the Mexican United States

APPENDIX 2

Socio-economic Characteristics of the Population for Each Mexican State Recorded from the XI General Census of Population and Housing (INEGI, 1990).

State	Total Population (thousands)	Illiteracy (%)	Households with bare earth floor (%)	Households without piped water (%)	Households without sewage (%)
Oaxaca	3019.6	27.5	51.4	41.9	70.1
Chiapas	3210.5	30	48.7	41.6	58.8
Guerrero	2620.6	26.8	46.9	43.1	62.4
Hidalgo	1888.4	20.6	29.3	30.0	56.5
Puebla	4126.1	19.2	28.8	28.8	51.6
Veracruz	6228.2	18.2	33.2	40.2	46.8
Morelos	3548.2	17.3	27.3	21.3	42.9
Guanajuato	3982.6	16.5	15.8	17.3	41.3
Yucatan	1363.0	15.8	17.4	28.5	53.8
Campeche	535.2	15.4	22.8	29.3	52.9
Queretaro	1051.2	15.3	15.6	16.6	43.1
San Luis Potosi	2003.2	14.9	28.5	33.3	51.3
Tabasco	1501.7	12.6	13.5	41.7	36.8
Quintana Roo	493.3	12.3	21.2	11.4	42.7
Michoacan	1195.1	11.9	20.9	11.5	30.8
Nayarit	824.6	11.3	20.8	16.5	39.9
Tlaxcala	761.3	11.1	14.2	9.6	42.1
Zacatecas	1276.3	9.9	16.1	24.7	53
Sinaloa	2204.1	9.8	22.8	20.0	45.1
Colima	428.5	9.3	19.6	7.2	17.7
Edo. Mexico	9815.8	9.0	11.9	14.9	26
Jalisco	5302.7	8.9	12.1	13.5	18.8
Aguascalientes	7199.7	7.1	6.6	4.4	13.9
Durango	1349.4	7.0	18.9	14.7	45.6
Tamaulipas	2249.6	6.9	12.9	18.6	40.2
Chihuahua	2441.9	6.1	9.0	33.5	33.5
Sonora	1823.6	5.6	17.5	9.3	34.2
Coahuila	19.72.3	5.5	8.1	8.3	31.8
Baja California Sur	317.8	5.4	13.5	11.2	34.3
Baja California Norte	1660.9	4.7	7.8	9.5	33.2
Distrito Federal	8235.7	4.0	2.1	3.7	6.2
Nuevo Leon	3098.7	4.6	6.0	7.1	19.1
Country	81,249.6	12.4	19.5	20.6	36.4

APPENDIX 3



Geographical Distribution of the Study Sites in the Mexican States of Sinaloa and Oaxaca in this Study

APPENDIX 4

INSTITUTE OF BIOMEDICAL AND LIFE SCIENCES
UNIVERSITY OF GLASGOW.
CENTRO DE INVESTIGACIÓN EN ALIMENTACIÓN Y DESARROLLO, A.C.

LETTER USED AS A FORM OF CONSENT.

Dear Mother and/or Father.

A study is being carried out to detect and identify intestinal parasitic infections which may be causing some health problems in your children.

To achieve the objectives above this study will include: measurement of weight and height, collection of stool samples, urine samples, blood samples, information about the food eaten by the child over 24 hours, medical examination, personal and socio-economic examination. All information will be confidentially treated. It will be necessary to repeat some measurements and analysis at particular time of the study. Collection of biological samples and personal data will be carried out in the primary school of the community. When necessary, some home visits may be carried out by our research personnel.

I have read and understood the reasons and objectives of the study "The Prevalence of Intestinal Parasitic Infections and their Association with Risk Factors in School Age Children from Sub-Urban and Rural Communities from two Mexican States after 7 years of an Active National Deworming Campaign" In addition, I will accept my child is included in the study if he/she wishes to participate with the freedom to leave it at any time by different reasons. I have also understood his/her participation does not threat his/her current or future health status.

Signature

Name of the Mother, Father, or Care-giver.

Address ______________________
School ______________________
Date ______________________

APPENDIX 5

"Prevalence of Intestinal Parasitic Infections and its Association with Risk Factors in School Children from Sub-Urban and Rural Communities of Two Mexican States after Seven Years of an Active Programme of Albendazole Administration".

Name of the Subject ____________________

Code Number ____________________

Institute of Biomedical and Life Sciences
University of Glasgow.
Centro de Investigación en Alimentación y Desarrollo, A.C.

Responsible ______________________ **Date** ______________________

Supervisor ______________________ **Community** ______________**Code Number** ____

Demographic Information

Name ____ ______________
Surname Names (s) Code Number

I. Information about the child.

I.1. Address ____________ Community ____________ Code Number ____________

Telephone (when possible) ____________

I.2. Birth Date______________________ ______________________
Month Date Year Months and Years

I.3. Birth Town______________________ State ______________________

I.4. Sex:____________ (1)- Male (0)- Female

I.5. How long has you been living in this community? ______________________
(Years/Months)

I.6. Is your address permanent? ______________________

I.7. Religion? ______________________

I.8. Do you know about the National Deworming Campaign? How long? ____________

I.9. The child is practising the washing-hands activity before eating or after each evacuation?
____

II. Family Information

II.1. Number of Children in the Family? (Including the child in the study)

1- ______ 2- ______ 3-______ Others numbers ______

II.2. Other relative living in your home?

1- No ______ 0- Yes ______

How many? ______________________________

Name	Sex (m/f)	Age (years)	Relationship
____________________	______	__________	____________

Institute of Biomedical and Life Sciences
University of Glasgow.
Centro de Investigación en Alimentación y Desarrollo, A.C.

Anthropometric Evaluation

Date ____________________

Code Number __________

Responsible of the Measurement ____

Child ____

Surname **Name (s)**

Birthdate ______________ **(Years and Months)**

Weight _____________ **(Kg.)**

Height _____________ **(cm.)**

Institute of Biomedical and Life Sciences
University of Glasgow.
Centro de Investigación en Alimentación y Desarrollo, A.C.

Coproparasitological Analysis

Date ____________________
Code Number __________

Responsible of the Analysis ____

Child ____
Surname **Name (s)**

Age ______________
Number of Stool Samples _________________

Stool Collection Dates _________________

Result:
1st. ________________________________
2nd ________________________________
3rd ________________________________

Intensity of the Helminth Infection (epg)
1st ________________________________
2nd ________________________________
3rd ________________________________

Date of Administration of the Parasitic Treatment ___________________________

Intensity of the Helminth Infection (epg) after Treatment.
1st ________________________________
2nd ________________________________
3rd ________________________________

Institute of Biomedical and Life Sciences
University of Glasgow.
Centro de Investigación en Alimentación y Desarrollo, A.C.

Biochemical Analysis

Date ______________
Code Number ______

Responsible of the Analysis ________________________________

Child ________________________________ ____________
Surname **Name (s)**

1. Urinary Indoxil Potassium Sulfate (indican) (mg indican/mg creatinine)__________

Biometric Analisis

1. Haemoglobin (g/dl) ______________________
2. Haematocrit (%) ______________________
3. Red Blood Cells (million/L) ______________________
4. Mean Cell Volume (fl) ______________________
5. Mean Cell Haemoglobin (pg) ______________________
6. Mean Cell Haemoglobin Concentration (g/L) __________

Indicators of Iron

1. Serum Iron (µg/dl) ______________________
2. Total Iron Binding Capacity (µg/dl)______________
3. Transferrin Saturation (%) ______________________
4. Serum Ferritin ((µg/L) ______________________

Institute of Biomedical and Life Sciences
University of Glasgow.
Centro de Investigación en Alimentación y Desarrollo, A.C.

24 Hour-Recall

Name (child) ______________ **Code** _______ **Community** ________ **Date** ____________
Responsible ________________________________

Meal	Description of Food	Place	Recipe	Eaten Portion	Grams	Code

Institute of Biomedical and Life Sciences
University of Glasgow.
Centro de Investigación en Alimentación y Desarrollo, A.C.

Responsible ______________________ **Date** ______________

Supervisor ______________ **Community** __________ **Code Number** ______

Criteria Selection Information

Child ______________________________ , ______________
Surname **Name (s)** **Code Number**

I. Current Health Status and Medication

I.1. Presence of the following diseases?

1- Crohn Disease ______________
2- Peptic Ulcer ______________
3- Fever ______________
4- Any surgical Intervention (Specify)
____

5- Pancreatitis ______________

6- Coeliac Disease ______________
7- Metabolic disorder or pathological condition (Specify)
____

Medication or nutrient supplementation.
I.2. Is your child receiving any medication or nutrient supplementation?
1- Anti-parasitic-treatment ______________
2- Iron, Vitamin C, Vitamin A supplementation ______________
3- Other (Specify) ______________
3- Medical treatment or nutrient in the past, when, and how long? (specify).
____

II. Current Clinical Symptoms?

II.1. Clinical symptoms shown by the child today? (Daily visits for one week)

1- Respiratory infections (pneumonitis, asthma, cough)______________

2- Allergies (rush, urticaria) (specify)
____

4- Abdominal Pain ______________

5- Headache ______________

6- Diarrhoea ______________

3- Vomiting ____________________

Other (Specify) ____________________

III. Medical History

III.1. Clinical Symptoms in the past? (record dates when possible)

1- Respiratory infections (pneumonitis, asthma, cough)____________________

2- Allergies (rush, urticaria) (specify)

____

3- Vomiting ____________________

Other (Specify) ____________________

4- Abdominal Pain ____________________

5- Headache ____________________

6- Diarrhoea ____________________

7- Parasitic Infections ____________________

IV. Medical History of the Family's Child

1. Crohn Disease ____________________
2- Peptic Ulcer ____________________
3- Fever ____________________
4- Any surgical Intervention (Specify)

____

5- Pancreatitis ____________________

6- Parasitic infections (Specify)

____

7- Coeliac Disease ____________________

8- Metabolic disorder ____________________ or pathological condition (Specify)

____

Relationship with Child ____________________

Name	Sex	Age	Name of disease and Date

Institute of Biomedical and Life Sciences
University of Glasgow.
Centro de Investigación en Alimentación y Desarrollo, A.C.

Responsible ____________________ **Date** ____________________

Supervisor ______________ **Community** __________ **Code Number** ____

Socio-economic Information

Name ______________________________________ __________

Surname Name (s) Code Number

I Information about the Parents

Mother's Age	1. Unmarried	2. Married	3. Widow	4. Divorced
Father's Age	1. Unmarried	2. Married	3. Widow	4. Divorced

I.I. Information about the mother

1. Current Occupation ______________________________

0- Unemployed ______ 1- Housewife _______ 2- Employed _______

2. Kind of Job? ______________________________

3- Professional ______ 2- Skilled manual with special training or course __________

1- Unskilled Job _______

3. School education ______________________________

0 1 2 3 4 5 6 (Primary) 7 8 9 (Secundary) 10 11 12 (High School)

Primary + Secundary + Technical Training 12
Primary + Secundary+ High School+ Technical Training 13
University and others 14

I.2. Information about the Father ______________________________

1. Current Occupation

0- Unemployed 1- Housewife _______ 2- Employed _______

2. Kind of Job? ____________________

3- Professional ______ 2- Skilled Manual with special training or course ______
1- Unskilled Job ______

3. School education ____________________

0	1 2 3 4 5 6	7 8 9	10 11 12
	Primary	Secundary	High School

Primary + Secundary + Technical Training 12
Primary + Secundary + High School+ Technical Training 13
University and others 14

II. Family Income

II.1. Family Monthly Income?
1. <$ 1013.7 1176.4 (1.0 minimal wage), **2.** $ 1,520.55-1764.67 (1.5 minimal wage),

II.2. $ 2027.4 -2352.9 (2 minimal wage), **4.** $ 2534.2 - 2941.1 (2.5 minimal wage),

5. $ 3041.1 – 3529.3 (3 minimal wage), **6.** $ 4054.8 – 4705.8 (4 minimal wage).

II.3. Time receiving this Income?
0- <1 Month 1. 6 Months 2- >6 Months

II.4. Economical Support, amount, and source ____________________
II.5. Expenditure in food per week ____________________

III. Household conditions

III.1. Numbers of rooms of the house including kitchen?

0- One ______ 1- Two ______ 2- Three ______ 3- >Three ______

III.2. Material of walls

7. Cement and Brick
6. Cement and Adobe (hardened mud)
5. Cement and Block
4. Cement, Brick, and Adobe.
3. Asbestos and wood.
2. Metal laminate and wood.
1. Board laminate and wood.

0- Other materials (specify) ________________________________

III.3. Material of roof

8. Concrete
7. Concrete and Asbestos
6. Metal laminate and concrete
5. Board laminate and concrete
4. Tile and wood
3. Asbestos
2. Tile
1. Metal laminate and wood
0. Board laminate

1II.4. Material of floor
2- Cement
1. Bare-earth
Other materials (specify) ________________________________

III.5. Presence of piped water?

1- Outside ______ 2- Inside ______ 0- No (specify) (Boiled or purified water)

III.6. Presence of sewage system in the house

0- No ______ 1-Yes ______ Other (Specify) (latrine or simple hole)

III.7. Presence of electricity in the house?

0- No______ 1- Yes ______

IV. Health Service

0- None, 1- Public, 2- Private

APPENDIX 6

The Lower and Higher Prevalence of Malnutrition on the Basis of the Z Scores of the Ratios H/A, W/A, and W/H for each Mexican State in Pre-school Children in Communities with 500 to 2500 inhabitants (Avila-Curiel, *et al.*, 1998).

Malnutrition Status	Mexican States and Prevalence of Malnutrition	
Z H/A Malnutrition	Lower Prevalence (%)	Higher Prevalence (%)
Mild	Baja California Sur (10.0)	Morelos (26.8)
	Sonora (10.9)	Tlaxcala (26.7)
	Baja California Norte (12.0)	Tabasco (26.0)
	Aguascalientes (15.0)	San Luis Potosi (25.1)
	Sinaloa (21.4)	Oaxaca (24.0)
Moderate		
	Baja California Norte (6.0)	Yucatan (26.6)
	Nuevo Leon (9.1)	Campeche (25.3)
	Sinaloa (9.4)	Guerrero (24.8)
	Chihuahua (9.4)	Quintana Roo (24.9)
	Jalisco (9.8)	Oaxaca (24.0)
Severe		
	Sonora (2.9)	Chiapas (28.0)
	Baja California Norte (4.3)	Yucatan (25.6)
	Sinaloa (4.3)	Oaxaca (22.8)
	Tamaulipas (5.4)	Guerrero (22.7)
	Jalisco (5.6)	Campeche (21.6)
Z W/A Malnutrition		
Mild	Sonora (8.5)	Yucatan (32.9)
	Baja Californa Norte (10.5)	Tlaxcala (32.6)
	Baja California Sur (13.4)	Oaxaca (31.3)
	Durango (16.5)	Campeche (31.0)
	Sinaloa (19.1)	Morelos (30.8)
Moderate		
	Baja California Norte (2.5)	Guerrero (22.2)
	Sonora (4.21)	Yucatan (21.6)
	Sinaloa (5.3)	Oaxaca (18.6)
	Jalisco (5.8)	Puebla (17.6)
	Baja California Sur (6.0)	Campeche (14.9)
Severe		
	Sonora (0.2)	Guerrero (10.1)
	Durango (0.6)	Puebla (7.7)
	Morelos (0.8)	Yucatan (7.0)
	Coahuila (0.9)	Hidalgo (6.2)
	Sinaloa (1.9)	Oaxaca (5.0)

Z W/H Malnutrition	Mexican States and Prevalence of Malnutrition	
Mild	**Michoacan (6.7)**	**Colima (18.8)**
	Baja Californa Norte (7.2))	**Guerrero (16.7)**
	Chiapas (8.0)	**Guanajuato (16.5)**
	Campeche (8.5)	**Nayarit (15.7)**
	Sinaloa (10.9)	**Oaxaca (11.1)**
Moderate		
	Morelos (1.4)	**Colima (11.8)**
	Baja California Norte (2.1)	**Nuevo Leon (8.8)**
	Queretaro (2.7)	**Tlaxcala (6.1)**
	Oaxaca (4.3)	**Guanajuato (6.0)**
	Sinaloa (5.8)	**Zacatecas (5.2)**
Severe		
	Campeche (0.6)	**Nuevo Leon (6.6)**
	Coahuila (1.1)	**Colima (5.5)**
	Oaxaca (1.0)	**Chihuahua (4.8)**
	Sinaloa (1.1)	**Yucatan (4.1)**
	Queretaro (1.2)	**Puebla (4.1)**

APPENDIX 7

The Prevalence of Intestinal Parasitic Infections in 341 Rural School Children and the Prevalence Observed for each Rural Community in the State of Sinaloa.

General Mean Prevalence (%)									
	A.lumbricoides	*T.trichiura*	*G.lamblia*	*H.nana*	*E. vermicularis*	*E.coli*	*E.nana*	*I.butschlii*	*C.mesnili*
Rural Area of Sinaloa (n=341)	2.9	12.3	23.4	20.5	1.5	52.8	41.9	2.9	6.2
Prevalence by Rural Community									
Imala n=37(82.7)	2.6	13.2	36.8	21	0	71	47.4	5.3	0
Las Puentes n=68 (78%)	2.9	8.8	29.4	14.7	0	38.6	42.6	5.8	0
Pueblo Nuevo n=72 (40%)	0	6.9	25	27.7	2.7	51.3	43	2.7	25
El Treinta n=19 (47.5%)	5.2	5.2	36.8	15.7	5.2	52.6	0	10.5	10.5
Paraiso n=21 (70%)	0	0	9	18.1	0	27.2	18.1	9	0
D.Arango n=23 (40%)	0	0	26	17.4	0	52.1	47.8	0	0
Higueral n=101(46.%)	5.5	22.7	15.5	20.9	0.9	55.4	42.5	2	0.9

APPENDIX 8

The Prevalence of Intestinal Parasitic Infections in 151 School Children and the Prevalence observed by Community in the State of Oaxaca.

General Prevalence							
	A.lumbricoides	*T.trichiura*	*G.lamblia*	*H.nana*	*E.coli*	*E.nana*	*I.butschlii*
Oaxaca (N=151)	6.6	10.6	31.1	21.9	40.4	35.1	16.6

Prevalence by Community							
Suburban							
Lomas San Jacinto n=62 (21.7%)	0	5	17.5	21.9	10	0	2.5
Rural							
La Era n=33 (67.3%)	3	0	24.2	12.1	39.4	30.3	30.3
Lobera n=30 (52.6%)	3	0	30	26.4	50	50	10
Pluma Hidalgo n=22 (21.5%)	9.0	27.3	50	0	54.5	41	22.7
Sta. Ma. Magdalena n=26 (47.2%)	23	30.70	46.1	30.8	65.4	73	23

APPENDIX 9

Prevalence of Different Intestinal Parasitic Infections in the General Population from Different Communities of the State of Sinaloa from 1987 to the Present Study.

Parasite	Community and Year							
	Salado 1987 n=516	Navolato 1989 n=302	El Batallon 1990 n=103	Argentina 1991 n=137	Villa Juarez 1994 n= 416	Toledo Corro 1994 n=136	Mirador 1994 n=264	Present Study n=341
	Prevalence (%)							
A. lumbricoides	7	3	3.9	2.9	20.2	4.4	17.8	3
T. trichiura	7.9	8.9	15.5	3.6	13.2	16.9	17.8	12.7
G. lamblia	28.2	35.4	18.4	24.8	24	44.1	29.5	24.1
E. coli	59.5	58.3	70.9	54.8	67.3	62.5	63.3	54.3
E. nana	70.3	85.1	86.4	63.5	69.9	75	68.6	43.2
H. nana	22.3	22.2	28.2	24.1	32.9	30.1	27.7	20.5
E. vermicularis	3.9	1.7	3.9	7.3	4.8	2.9	3.8	1.5
I. butschlii	14.5	16.2	9.7	5.1	12.5	4.4	9.1	5.7
Hookworm	1.7	0.7	5.8	6.5	10.1	0.7	0.8	0
S. stercoralis	1.7	3.3	3.9	1.4	6.2	1.5	1.9	0

APPENDIX 10

Prevalence of Different Intestinal Parasitic Infections in the General Population from Different Communities of the State of Oaxaca from 1993 to the Present Study.

Parasite	Community, size and age group of Population and Year			
	Jamiltepec n=499 (2-14 years) 1993	Ejutla n=250 (0-12 years) 1998	Nopala General Population 1999	Present Study n=151 (6-10 years)
	Prevalence (%)			
A. lumbricoides	42	21.2	8.12	6.6
T. trichiura	0	10.8	6.8	10.6
G. lamblia	4	7.2	16.4	31.2
E. coli	11	3.6	11.3	40.4
E. nana	0	13.2	4.4	35.1
H. nana	0	1	1.4	21.9
E. vermicularis	0	0	0.6	0
I. butschlii	0	0	2.8	16.6
Hookworm	0	0	0	0
S. stercoralis	0	0	0	0

APPENDIX 11

The Nutritional Status According to Z-Score for the ratios H/A, W/A, and W/H in 137 School Children from the State of Oaxaca

Nutritional Status (Z score)	Lomas San Jacinto			La Era			La Lobera			Pluma Hidalgo			Sta. Maria de Magdalena		
Severe Malnutrition (<-3)	H/A	W/A	W/H	H/A	W/A	W/H	H/A	W/A	W/H	H/A	W/A	W/H	H/A	W/A	W/H
Moderate Malnutrition (-3, -2)	5	0	0	3	0	0	2	0	1	1	0	0	0	1	1
Mild Malnutrition (-2,-1)	12	4	0	7	3	0	7	5	0	10	9	0	8	0	1
Normal (-1,+1)	10	12	1	14	13	1	7	11	3	7	6	6	8	17	11
Obesity degree 1 (>+1,+2)	12	20	25	6	14	22	9	9	20	2	5	14	10	8	9
Obesity degree 2 (>+2,+3)	0	2	9	0	0	7	0	0	1	0	0	0	0	10	0
Severe Malnutrition (<-3)	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0
Total	n=39	n=39	n=39	n=30	n=30	n=30	n=25	n=25	n=25	n=20	n=20	n=20	n=26	n=26	n=26

APPENDIX 12

The Average Daily Intake of Nutrients Estimated from the 24-hr Recall Method for 190 School Children from the States of Sinaloa and Oaxaca

Nutrient	Mean Daily Intake	Standard Deviation	SE	Minimum	Maximum	Range	Confidence Limits (95%)
Energy (Kcal)	1532.9	517	37.7	517.5	3308	2790.5	1458.5-1607.3
Energy (Kj)	6416.4	2183.4	159.2	2165	13653	11488.6	6102.3-6730.6
Protein (g)	52.9	26.4	1.9	14.4	149.3	135	49-56.7
Fat (g)	42.4	25.6	1.9	1.3	154.2	153	38.7-46.1
Carbohydrates (g)	252.6	81.4	5.9	102.3	491	389	240.9-264.4
Na (mg)	973.9	699.4	51	56	6437.3	6381	873.3-1074.5
K (mg)	1204.9	783.5	57.2	71.7	7031.3	6959.5	1092.2-1317.7
Ca (mg)	609.4	303.2	22.1	130	1882	1752	565.8-653
Mg (mg)	351.9	178	12.9	90.5	1193.9	1103.4	326.3-377.5
P (mg)	691.3	337.2	24.6	147	2374	2227	642.7-739.8
Fe (mg)	13.6	6.3	0.47	4	49	45.1	12.7-14.6
Zn (mg)	8	5.6	0.4	0	61.2	61.2	7.3-8.9
Vitamin A (µg)	446.8	525.2	38.3	0	2554.8	2554.8	371.2-522.3
Tiamin (mg)	1.1	0.52	0.04	0.27	4.7	4.5	0.99-1.14
Riboflavin (mg)	0.9	0.8	0.05	0.2	8.9	8.7	0.85-1.07
Niacin (mg)	8.2	4.8	0.3	1.9	43.9	42	7.5-8.9
Vitamin C (mg)	90.5	79.4	5.8	0.3	426	425.7	79.0-101.9
Vitamin E (mg)	10	9	0.7	0.8	62.4	61.6	8.7-11.3
Piridoxine (mg)	1.8	1	0.07	0.23	8.2	8	1.6-1.9
Vitamin B12 (µg)	1.1	1.9	0.14	0	12.6	12.6	0.83-1.37
Folic Acid (µg)	160.7	149.9	10.9	0.7	948.3	948	139.1-182.3
Panthotenic Acid (mg)	2.4	1.6	0.12	0.2	12.4	12.2	2.14-2.6

The Average Daily Intake of Nutrients Estimated from the 24-hr Recall Method for 52 School Children from the State of Sinaloa.

Nutrient	Mean Daily Intake	Standard Deviation	SE	Minimum	Maximum	Range	Confidence Limits (95%)
Energy (Kcal)	1826.5	593	82.3	799	3308	2509.3	1661.4-1991.6
Energy (Kj)	7689.3	2486.8	344.8	3341.4	13653.7	10312.3	6996.9-8381.6
Protein (g)	59.6	22.8	3.1	21.7	124.3	102.5	53.3-66
Fat (g)	62.5	29.5	4	25.3	154.2	128.9	54.3-70.7
Carbohydrates (g)	276.8	89.5	12.4	108.6	491.5	382.9	251.9-301.7
Na (mg)	1474.9	911.5	126.4	457.9	6437.3	5979.3	1221.2-1728.7
K (mg)	1614.3	857.5	118.9	451.2	4754.2	4302.9	1375.6-1853.0
Ca (mg)	721.9	342.4	47.5	230.1	1582.4	1352.3	626.5-817.2
Mg (mg)	400	194.7	27.0	144.7	1053.0	908.3	345.9-454.4
P (mg)	815.0	424.3	58.8	212.7	2374.0	2161.3	697.0-933.2
Fe (mg)	14.8	7.3	1.0	6.2	36.6	30.4	12.2-14.2
Zn (mg)	9.8	4.6	0.64	3.5	25.1	21.6	8.5-11.0
Vitamin A (µg)	514.3	517.5	71.7	8.2	2554.8	2546.6	370.2-658.4
Tiamin (mg)	1.3	0.5	0.07	0.4	2.7	2.3	1.08-1.4
Riboflavine(mg)	1.1	0.4	0.05	0.4	2.2	1.8	1.02-1.24
Niacin (mg)	11.0	4.3	0.6	4.2	23.3	19.0	9.9-12.3
Vitamin C (mg)	85.6	68.5	9.5	9.8	277.3	267.5	66.5-104.7
Vitamin E (mg)	11.2	7.8	1.0	1.6	41.5	40.0	9.02-13.4
Piridoxine (mg)	2.2	1.3	0.2	0.7	8.2	7.5	1.80-2.54
Vitamin B12 (mg)	1.4	1.2	0.16	0	5.4	5.4	1.02-1.67
Folic Acid (mg)	222.8	194.1	26.9	40.5	948.3	907.7	168.6-276.8
Panthotenic Acid (mg)	3.0	1.8	0.25	0.7	12.4	11.6	2.5-3.45

The Average Daily Intake of Nutrients Estimated from the 24-hr Recall Method for 138 School Children from the State of Oaxaca

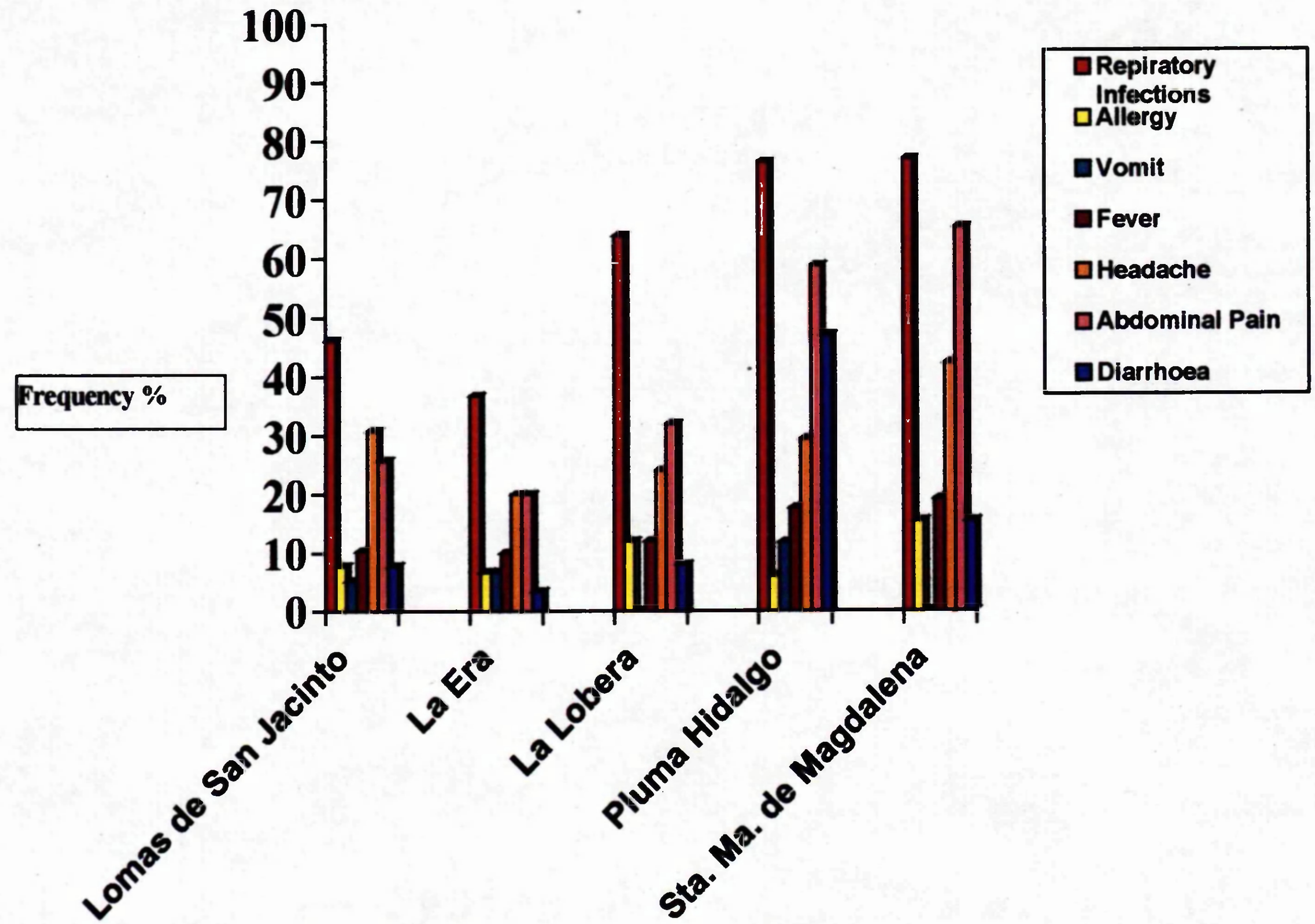
Nutrient	Mean Daily Intake	Standard Deviation	SE	Minimum	Maximum	Range	Confidence Limits (95%)
Energy (Kcal)	1420.6	437.5	37.5	517.5	2561.0	2043.6	1346.4-1494.8
Energy (Kj)	5930.0	1845.2	158.2	2165.0	10715.6	8550.5	5616.8-6242.6
Protein (g)	50.3	27.3	2.3	13.4	149.3	135.0	45.63-54.9
Fat (g)	34.8	19.2	1.6	1.3	96.3	95.0	31.5-38
Carbohydrates (g)	243.4	76.4	6.6	102.3	487.9	385.6	230.5-256.4
Na (mg)	782.4	479.5	41.1	56.0	3346.6	3290.6	701-863.7
K (mg)	1048.4	695.3	59.6	71.8	7031.3	6959.5	930.5-1166.3
Ca (mg)	566.4	276.2	23.7	129.9	1881.9	1752.0	519.5-613.2
Mg (mg)	333.4	168.2	14.4	90.5	1193.9	1103.4	304.9-361.9
P (mg)	643.9	285.1	24.4	146.9	1796.4	1649.4	595.6-692.3
Fe (mg)	13.2	5.9	0.5	4.0	49.0	45.1	12.2-14.2
Zn (mg)	7.4	5.8	0.5	0	61.2	61.2	6.44-8.4
Vitamin A (µg)	420.9	527.7	45.2	0	2428.6	2428.6	331.4-510.4
Tiamin (mg)	1.0	0.5	0.04	0.3	4.7	4.5	0.92-1.09
Riboflavin (mg)	0.9	0.8	0.07	0.2	8.9	8.7	0.55-0.88
Niacin (mg)	7.0	4.5	0.4	1.9	43.9	42.0	6.31-7.82
Vitamin C (mg)	92.3	83.3	7.1	0.3	425.9	425.9	78.2-106.5
Vitamin E (mg)	9.5	9.5	0.8	0.8	62.4	61.7	7.93-11.2
Piridoxine (mg)	1.6	0.8	0.07	0.2	5.8	5.5	1.45-1.73
Vitamin B12 (µg)	1.0	2.0	0.2	0	12.6	12.6	0.65-1.36
Folic Acid (µg)	136.9	121.8	10.4	0.7	927.6	926.9	116.3-157.6
Panthotenic Acid (mg)	2.1	1.5	0.12	0.2	8.5	8.4	1.9-2.4

APPENDIX 13

Proportion of School Children who did not meet the Recommended Daily Intakes for each Nutrient Estimated and for each Community of the State of Oaxaca.

	Lomas de San Jacinto (total=39)		La Era (total=30)		La Lobera (total=25)		Pluma Hidalgo (total=20)		Sta. Maria de Magdalena (total=26)	
Nutrient	Children (%)	Mean (%)	Children (%)	Mean (%)	Children (%)	Mean (%)	Children (%)	Mean (%)	Children (%)	Mean (%)
Energy	89.7	69.7	80	74.4	84	68.2	85	54.9	100	61
Protein	10.2	77.3	16.6	85.3	12	68.4	15	61	23	86.1
Fat	82	53.4	90	52.6	92.3	46	85	32.7	96.1	42.4
Carbohydrates	48.7	73	36.6	78.6	49.5	77.4	60	72.9	61.5	70.5
Calcium	82	59.6	76.6	56.5	72.1	71.9	85	52.9	92.3	55.4
Phosphorus	89.7	61	80	56.1	48	74	65	67.8	69.2	75
Iron	20.5	69	26.6	72.5	40	80	40	74.4	19.2	78.9
Zinc	84.6	59.3	70	62.2	84.1	67	85	52	92.3	54.6
Riboflavin	84.6	60.8	73.3	56.2	84.1	67.4	85	51.4	92.3	59.6
Niacin	89.7	44.7	86.6	54.3	96.1	49.4	85	42.8	100	49.7
Vitamin E	46.1	60	53.3	71.3	48	76	60	64.6	46.1	74.7
Vitamin A	97.4	19.7	90	25	92.1	18	50	34	26.9	53.8
Vitamin B6	46.1	64	36.6	63.5	40	70.3	50	60.9	50	76.9
Vitamin B12	74.3	30.3	76.6	38.3	80	24.3	80	15.4	100	21.9
Folate	43.5	60.8	36.6	66.4	44	65.4	54	64.3	50	69.2
Pantothenic Acid	94.9	43.8	86.6	56.4	96.1	44.5	85	28.6	100	34.3

APPENDIX 14



Frequency of Clinical Symptoms in 140 School Children for each Community of the State of Oaxaca.