PREVENTION OF ROTAVIRUS DIARRHOEA IN GAMBIAN CHILDREN

PHILIP WILLIAM HANLON

Being a thesis submitted for the degree of Doctor of Medicine in the University of Glasgow.

March 1987

Medical Research Council

The Gambia.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to express my thanks to Dr. Brian Greenwood who acted as my supervisor during the three years in which this thesis was in preparation. During this period he was a continuing source of help, advice and encouragement.

I would also like to thank my wife, Dr. Lesley Hanlon, who worked with me in The Gambia and shared the work of project administration and clinical care of the study children. She was a constant source of enthusiasm and common sense and the success of the project owes a great deal to her.

I am also grateful to Dr. Richard Hayes who provided statistical advice during the period of study design, checked the results of my statistical analysis and carried out the multiple logistic regressional analysis. Thanks are due to Dr. Ruth Bishop who advised on laboratory methodology and study design. I am also grateful to Dr. David Carrington who provided invaluable assistance with interpretation and presentation of results.

I am grateful to Dr. Vicki Marsh who worked on the project during parts of 1984 and 1985. I would also like to express thanks to Mr. Peter Byass who supervised the computing aspects of the study and assisted with data analysis.

The difficult task of setting up and carrying out the various laboratory investigations in The Gambia owes a great

deal to the knowledge and experience of Dr. Hilton Whittle and members of his staff, Mr. Ousman Jobe who carried out the rotavirus neutralising antibody assays and Miss Haddy Sillah who performed the polio neutralising antibody assay. I am grateful to them all. I would like to express my thanks to Miss Fiona Shenton and Dr. Musa Hassan-King for their contributions to the detection and characterisation of rotaviruses. I wish to express my thanks to Dr. Ray Sanders who visited The Gambia as a W.H.O. consultant during which time he carried out the R.N.A. electrophoresis. I am grateful to Dr. David Cubitt for performing electron microscopic examinations on a number of stool samples collected during the study.

This project would not have been possible without the general support and infrastructure of the Medical Research Council in The Gambia. In particular I wish to thank the field staff who worked with enormous enthusiasm and commitment. I would also like to thank the Diarrhoeal Diseases Control Programme of the World Health Organisation and its Director, Dr. M. Merson, for providing financial support. I am also grateful to Smith - Kline RIT, who provided all the vaccines.

While in The Gambia I enjoyed the help and assistance of several members of The Gambia Medical and Health Department. In particular I would like to thank Dr. F. Oldfield, Dr. K. Cham, Sister B. M'Boge and all the staff of Bakau health centre.

Finally, I would like to express my gratitude to the people of Bakau for their cooperation with all aspects of these studies. In particular, I would express my thanks to the mothers and children who were recruited to the studies for their patience and good humour. It was a real pleasure and privilege to have known and worked with them.

SUMMARY

Between June 1984 and June 1986 a variety of studies on rotavirus diarrhoea and its prevention were conducted in Bakau, a peri-urban Gambian community. This thesis reports the results of these studies and places them in the context of current knowledge concerning the epidemiology and immunology of rotavirus infection.

Short, well-demarcated epidemics of rotavirus diarrhoea were observed in this study during two consecutive cool dry seasons in The Gambia. This very clear seasonal pattern has now been documented for four consecutive years. During the 1985/86 epidemic transmission was intense with an attack rate for infants of 36%. It was estimated that asymptomatic virus shedding in stools was two thirds as common as symptomatic infection. Rotavirus diarrhoea was more severe than diarrhoea due to all other causes and clinical rotavirus infection was associated with weight loss in the post-infection period. Non - epidemic periods were characterised by very occasional mild cases and asymptomatic virus shedding in neonates. There was a change in R.N.A. electropherotypes from a predominantly long pattern in 1983/84 and 1984/85 to short patterns in 1985/86.

A case-control study was conducted on 92 cases and 92 age and vaccine status matched controls to determine social and environmental risk factors for rotavirus infection. No risk factors were found but there was an association between rotavirus cases and the presence of a dog in the child's compound.

A randomised, double blind, placebo controlled trial of the bovine rotavirus vaccine RIT 4237 was undertaken in young Gambian children. Three doses of rotavirus vaccine were administered, commencing at the age of ten weeks, concurrently with oral or killed poliomyelitis vaccine. Pre-vaccination rotavirus neutralising antibody levels were high. 84/185 infants (45%) showed an increase in neutralising antibody titre after receiving rotavirus vaccination, compared with 20/91 (22%) of unvaccinated infants. During 14 months of observation, clinical rotavirus infection was detected in 24/78 (31%) children in the rotavirus/oral polio group, in 34/83 (41%) children in the placebo/oral polio group, and in 23/92 (25%) children in the rotavirus/killed polio group, giving an overall vaccine efficacy of 33% (95% C.I. 4% to 53%). RIT 4237 did not reduce the severity of clinical infections. No effect from the concurrent administration of oral polio vaccine on the immune response to rotavirus vaccine was observed. Lower type 1 and type 3 polio antibody levels were found in children who received oral polio and rotavirus vaccines than those who received oral polio vaccine and placebo but differences between the two groups were not statistically significant.

A number of possible explanations for the poor efficacy of RIT 4237 are considered. High maternal antibody levels to rotavirus at the time of vaccination may have reduced the immune response to vaccination. Another possibility is that Gambian breast milk contains anti-rotavirus antibody or non-antibody factors which inhibited the vaccine response. In

addition, naturally circulating enteroviruses may have interfered with the immune response to the rotavirus vaccine.

In anticipation of the development of a successful rotavirus vaccine, factors leading to poor vaccination compliance were investigated. The vaccination status of 251 children aged 12 - 18 months living in Bakau was determined from their health cards. Two subgroups were identified: children who were fully vaccinated, and those who had received less than half their vaccinations. The social and environmental circumstances of these children were investigated. In the poorly vaccinated group both parents were less well educated and there was a trend towards poorer literacy. Mothers of poorly vaccinated children had a poorer knowledge of the diseases their children should be vaccinated against and had a more superstitious view of disease causation. Those children who showed poor compliance came from larger families. Mothers of well vaccinated children were more inclined to bring them for non-curative services.

The implications of the above findings for further research into rotavirus and diarrhoeal disease in general are also considered.

ABBREVIATIONS

B.S.A. - Bovine Serum Albumin.

C.H.N. - Community Health Nurse.

C.I. - Confidence Interval.

CO_2 - Carbon Dioxide.

d.f. - Degrees of freedom.

D.N.A. - Deoxyribonucleic Acid.

D.P.T. - "Triple" vaccine against Diphtheria, Whooping Cough and Tetanus.

E.D.T.A. - Ethylenediaminetetraacetic acid.

E.L.I.S.A.- Enzyme-linked Immunosorbant Assay.

E.M. - Electron Microscopy.

E.M.E.M. - Eagle's Minimum Essential Medium.

E.P.I. - Expanded Programme for Immunisation.

Ig G. - Immunoglobulin G.

Ig M. - Immunoglobulin M.

Ig A. - Immunoglobulin A.

M - Molar

M.R.C. - Medical Research Council.

P.A.G.E. - Polyacrylamide Gel Electrophoresis.

P.B.S. - Phosphate Buffered Saline.

P.T.A. - Phosphotungstic Acid.

R.C. - Rotavirus Clinical Form.

R.N.A. - Ribonucleic Acid.

R.S.1. - Weekly Rotavirus Surveillance Form.

R.S.2. - Twice-weekly Rotavirus Surveillance Form.

S.D.S. - Sodium Dodecyl Sulphate.

T - Tween.

$T.C.I.D._{50}$ - 50% Tissue Culture Infecting Dose.

W.H.O. - World Health Organisation.

CHAPTER I

INTRODUCTION

This thesis reports the results of a series of studies concerned with the prevention of rotavirus diarrhoea in The Gambia. The Gambia is a poor country which faces many health and nutritional problems. Government spending on health is low (estimated at less than £3 per person, per year). Such a severe lack of funds inevitably leads to shortages of manpower and equipment but, despite these constraints, much has been accomplished in recent years.

My own work was conducted under the auspices of the Medical Research Council (M.R.C.) which gave me access to some of the resources necessary to carry out a sophisticated research project but it was clearly vital that the my work should be relevant to the needs of the Gambian people. Diarrhoeal disease had been identified in 1980 by the Gambian Government as a health priority and the following year a Diarrhoeal Disease Control Committee was established. The committee's main priority has been to establish oral rehydration as the first line treatment for all episodes of diarrhoea. Initially the M.R.C.'s contributed to the diarrhoea control effort by mounting a major study to investigate the aetiology of diarrhoea. Unfortunately this work proved somewhat unrewarding as the majority of diarrhoeal cases were not associated with pathogens which could be identified with techniques available in the country at that time. The committee, therefore, requested that the

next phase of research should concentrate on aspects of prevention. As a result, research efforts were directed towards rotavirus associated diarrhoea because there was a realistic prospect of preventing this condition through vaccination.

Childhood Diarrhoea in the Developing World.

The impact of childhood diarrhoeal disease throughout the developing countries of Asia, Africa and South America is truly staggering. In several published studies, diarrhoea has been shown to be the major cause of early childhood death, (1,2,3) and in 1982 the World Health Organization (W.H.O.) estimated that in children under five years of age there were 4.6 million deaths a year attributable to diarrhoea (4). These mortality statistics reflect much heavier burdens of morbidity. Children suffer the greater burden of disease as investigations worldwide have found diarrhoea incidence peaking between the ages of 6 months and 3 years, with an annual rate ranging from 2 to 12 episodes, averaging 4 per year in most poor communities (5,6,7,8). The impact of these global statistics at the community level was elegantly illustrated by Chen who calculated that a village health worker, who might commonly be responsible for delivering health care to a target population of 5,000, would have to deal with 8 new cases of gastroenteritis each day and, that on any given day, roughly 75 children would be experiencing diarrhoea (9).

Diarrhoea threatens the lives of children in two main ways. Fluid loss leading to dehydration is the acute threat, but repeated acute attacks or chronic diarrhoea will also lead to weight loss, growth faltering, and malnutrition (9,10,11, 12).

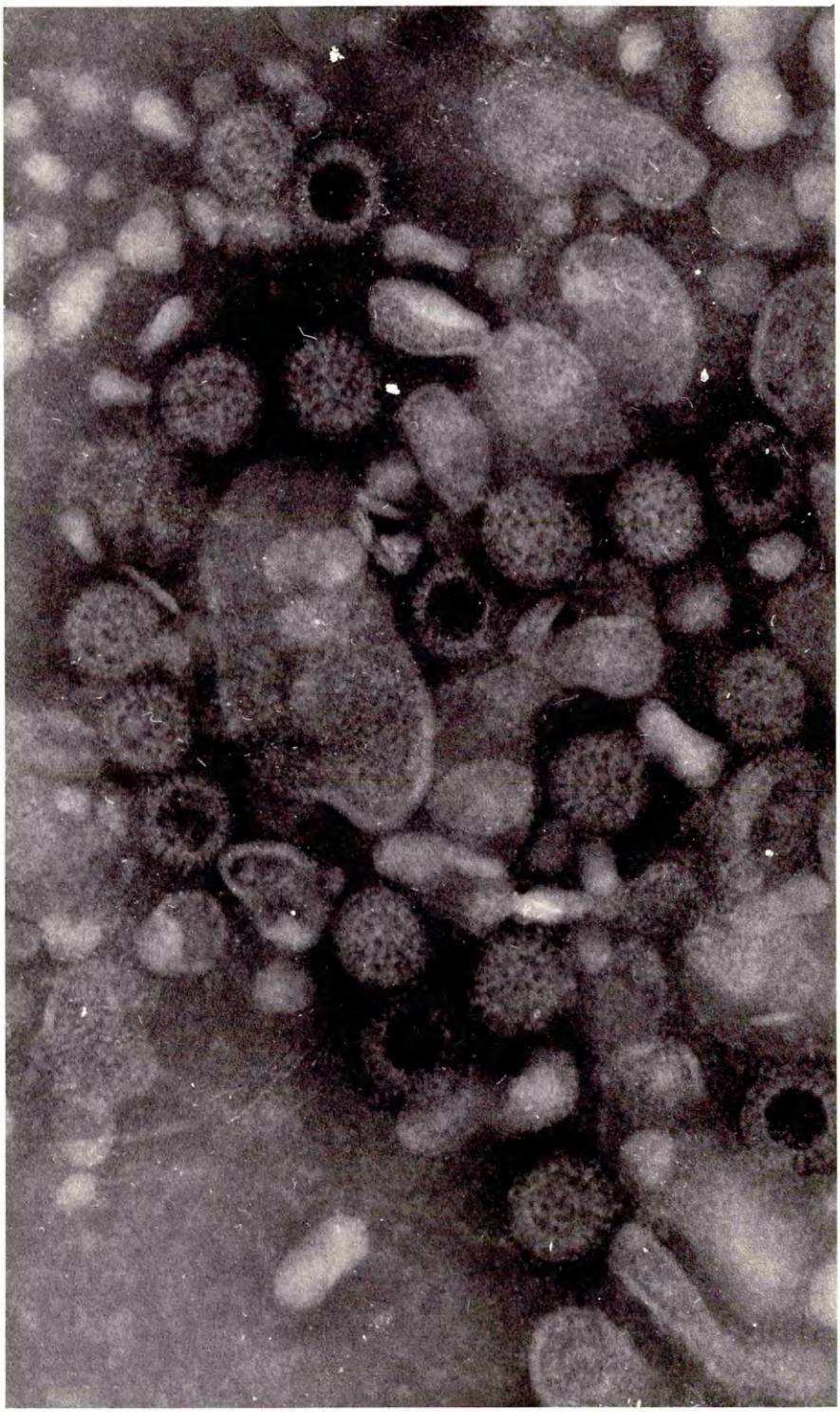
In recognition of the threat posed by diarrhoeal diseases the World Health Organization has initiated a global Diarrhoeal Disease Control Programme, the objectives of which are to reduce diarrhoeal morbidity and mortality. One of the first tasks of this programme was to fund a variety of aetiological studies to establish the major pathogens responsible for gastroenteritis in the Developing World. These studies have shown that rotavirus and enterotoxigenic Escherichia coli (E. coli) are the two major pathogens associated with childhood diarrhoea worldwide (13,14).

Rotavirus and Childhood Diarrhoea.

Before 1972 there was a high index of suspicion, but no direct evidence, that much gastroenteritis in humans was due to viral infections (15). Many large surveys in the more or less "developed" countries showed that as many as two-thirds of all diarrhoeal episodes were of unknown aetiology. In April 1973 electron microscopy revealed virus-like particles in an ultra thin section of an epithelial cell from the duodenum of a child suffering from acute gastroenteritis (16). The new virus was called rotavirus because its capsid structure suggested the spokes of a wheel, and "rota" is the Latin word for a wheel (fig 1). Since then rotavirus has

FIGURE 1

Electron micrograph of rotavirus.



Whose micrograph?

Magnification x 200,000, stain P.T.A., pH 7.

established itself as the single most important childhood diarrhoeal pathogen (17,18,19). Rotavirus is the cause of acute diarrhoea in approximately 40% of children requiring hospital admission in developing countries, (20 - 25) and in 50 - 60% of children admitted to hospital in industrialised countries (26 - 31). Community surveys have been less numerous, but they consistently give lower rates of rotavirus infection than hospital studies, a fact which probably indicates the relative severity of rotavirus compared to other diarrhoeal pathogens (14,32).

The Epidemiology of Rotavirus.

Rotavirus infection has a worldwide distribution, affecting all age groups but symptomatic attacks are largely confined to young children (17,18,33 - 37). Serological surveys indicate that most primary infections occur during the first two years of life in both industrialised and developing countries (33 - 37), but frequent asymptomatic infections occur in older children and adults who come in contact with infected children (38,39).

The attack rate for rotavirus disease is remarkably similar in children in developed and developing countries, despite the fact that the attack rate for diarrhoeal diseases as a whole is up to seven times greater in developing countries (40 - 45). The attack rate for rotavirus diarrhoea in children aged 6 - 24 months has been estimated at 0.3 - 0.8 episodes per child per year in both settings (40,41,42,46,47). This fact is relevant to discussions

concerning the need for a rotavirus vaccine because in countries with a heavier burden of diarrhoeal disease the importance of rotavirus diarrhoea might be underestimated simply because it makes up a smaller proportion of the whole. It is, therefore, more meaningful to discuss age specific clinical attack rates as this gives an indication of the proportion of children who are at risk from the dangers of clinical rotavirus infection and are therefore likely to benefit from vaccination.

This consistency of incidence and attack rates through a wide variety of environments has led some to speculate that the mode of rotavirus transmission is different from other enteric pathogens. Rotaviruses infect the small intestine via the oral route. Multiplication occurs within mature epithelial cells lining the villi of the upper portion of the small intestine, causing lysis of cells and release of infectious particles into the intestinal lumen (48). Infection in man seems to be restricted to the small intestine and there is no direct evidence of infection in the respiratory tract (49). The primary mechanism of spread is probably intra-familial (38,39,50), via faecal-oral contamination, but airborne spread of dust or droplets carrying virus particles is also a possibility, as is infection via contaminated food or water (51).

The incubation period is 24 - 48 hours and the onset of illness is abrupt with watery diarrhoea causing a greater degree of dehydration and fever than with most other enteric

pathogens (17,18,19,32,44). Vomiting, which is a frequent early feature of the disease, increases the degree of dehydration. Most children excrete rotavirus, in amounts detectable by electron microscopy (fig 1), for 5 days following the onset of symptoms.

Seasonal variation in rotavirus infection is complex and presently unexplained. Comparison of monthly incidence in hospitalised children indicates that infection is endemic in most communities. In areas with extreme fluctuations in climate between winter and summer months, rotavirus seems to be epidemic in the winter (27,29,52). In more temperate climates rotavirus peaks in the winter months but is present throughout the year (26,31), while in tropical areas either no seasonal fluctuation is seen (49) or modest peaks associated with cooler dryer months are observed (49,19,24,25). The epidemic nature of rotavirus in The Gambia is striking and will be described in detail later in the text. Recent studies have indicated that atmospheric humidity and temperature have an influence on rotavirus survival on fomites (53). It was found that the Wa strain of human rotavirus survived longer on non-porous surfaces at lower temperatures and lower humidity and it is possible that this phenomenon may partially explain the association between climate and clinical illness.

Rotavirus infection in neonates is common but is usually mild or asymptomatic (44,54 - 66). Infection is particularly common in hospital nurseries where the aggregation of babies and attendant adults seems to enhance the possibility of

spread from infected children. In contrast, neonatal infection is relatively rare in children born at home (42) or at village health centres (19). Antirotavirus antibodies have been demonstrated in cord blood and breast milk but breast feeding seems to provide only partial protection from neonatal rotavirus infection (66,67).

As stated previously, severe clinical disease is largely confined to children aged between 6 - 24 months (34,35,37) but, even in this age group, asymptomatic infection can be common (40,42,67,). In children less than two years old, sequential clinical infections have been observed (42), but it is not clear how frequently this occurs, nor whether reinfection occurs more commonly with heterotypic than homotypic serotypes of the virus (68).

Symptomatic rotavirus infection is rare but can occur in older children and adults (46,49,64,69,70,71).

A number of serologically distinct strains of rotavirus exist and these are described later. However, serological strain typing is performed as a research investigation only in reference laboratories. To date most epidemiological surveys of rotavirus strains have relied largely upon analysis of patterns formed when the 11 segments of double stranded R.N.A. contained in the virus genome are separated by polyacrylamide gel electrophoresis (PAGE) (72). Human rotaviruses show characteristic groups of R.N.A. segments, whereby 1-4, 5-6, 7-9, 10-11 migrate as separate groups.

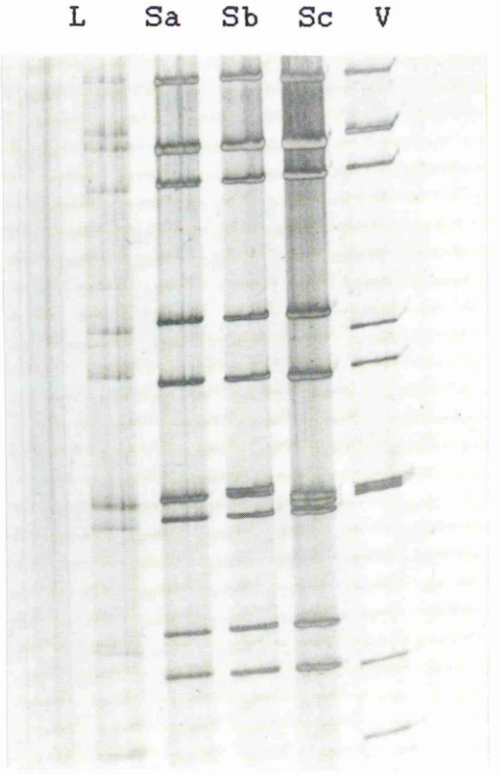
These result in a number of migration patterns ("electropherotypes") which divide conveniently into those which show a short (S), and those which show a long (L) electropherotype. Figure 2 shows the long electropherotype (L) and the the three short electropherotypes (Sa, Sb and Sc) detected during the 1985/86 epidemic. Note that it is the variable migration of the segments 10 and 11 which determines whether the virus has a short or long electropherotype. The photograph also includes the vaccine virus electropherotype which is distinct from human rotavirus isolates, being derived from a bovine rotavirus strain. In general, multiple electropherotypes exist simultaneously in most closely populated communities (73 - 79) but, usually, one or two electropherotypes are dominant in any single outbreak and sometimes the dominant electropherotype is detected in small numbers for several months before it becomes dominant (73,78).

Some Immunological Aspects of Rotavirus Infection.

Rotaviruses belong to the family of viruses known as Reoviridae and are classified as a genus within a family (19,51). The virus particles are made up of two concentric icosahedral protein capsids enclosing 11 segments of genomic double-stranded R.N.A.(80). Until recently, it was thought that all known rotaviruses possessed a common group-specific antigen. However, evidence is now accumulating that there are strains of rotaviruses infecting man, animals, and birds, which do not share this common antigen (81) and are consequently not detectable by the presently available

FIGURE 2

Photograph of the long electropherotype (L) together with the three short electropherotypes (Sa, Sb and Sc) found in rotaviruses detected during the 1985/86 epidemic in Bakau. The vaccine virus electropherotype (V) is also included.



serological tests. The original rotaviruses have now been designated group A. The newer strains are called groups B to E. The vast majority of human infections seem to be caused by group A viruses. Rotaviruses may be further typed by serological methods (81,82,83,84). Subgroup specificity is determined by an antigen located on the inner capsid of the virus particle. Two subgroups (I and II) have been identified. The antigen which determines serotype is found on the outer capsid. This antigen also determines neutralising specificity and, to date, six separate serotypes have been identified (81,85,86). A summary of rotavirus classification is shown on Table I.

The relationship between R.N.A. electropherotype and subgroup/serotype is such that a short R.N.A. pattern is generally found in subgroup I serotype 2 viruses, while a long pattern is associated with subgroup II serotypes 1 and 3. These associations are based on a relatively small number of observations and, at present, can only be described as provisional.

Diagnosis of rotavirus infection is most commonly performed by an Enzyme-Linked Immunosorbent Assay (E.L.I.S.A.) which relies on recognition and binding to the common group antigen (87), or by direct visualisation in the electron microscope.

Immunity to disease associated with rotavirus infection is not fully understood but seems to rely on an amalgamation of

Table I

Classification of rotaviruses.

Method of classification	Determinant for classification	Examples
Group	Common group antigen	A,B,C,D,E.
Subgroup	Inner capsid antigen	I, II
Serotype	Outer capsid antigen	1,2,3,4,5,6.
Electropherotype	R.N.A. migration pattern	long short

passive and active immunity, superimposed on non-specific resistance mechanisms (17,88,89,90). Newborn babies are at least partially protected by maternally derived antibody in the blood together with antibodies and non-antibody factors ingested in breast milk (91,92). Neither mechanism is foolproof, as breast-fed children and those with high levels of maternal antibody can become symptomatic (93). This enigma may be resolved when neutralising antibody in breast milk and serum are titrated and related to the serotype of the infecting virus.

It has been shown in animal hosts and animal models that passively acquired antibody can protect neonates against diarrhoea caused by rotavirus (94), provided that enough specific antibody with neutralising activity is present continuously in the small intestine of the host. Passive immunity is being used with success to prevent rotavirus diarrhoea in calves, through vaccination of the dam to stimulate increased and prolonged secretion of antibody in colostrum and milk (95).

In studies conducted on children, treatment with either human serum gammaglobulin or colostrum and milk globulin derived from cows vaccinated with human rotavirus resulted in only partial protection (96). As with animals, a better protective effect was obtained in infants when rotavirus antibodies were given prophylactically. The efficacy of the therapeutic and prophylactic use of preparations conveying passive immunity in man has not been established but merits further investigation.

Babies infected with rotavirus loose maternally derived and naturally acquired antibodies with time (61) and are not immune to reinfection with rotavirus, although they do suffer less severe clinical infections (67). This indicates that they have retained a degree of immunity to rotavirus which prevents severe clinical disease when reinfection occurs during the first few years of life (67).

It is not clear at present which part of the immune response is the most protective against clinical rotavirus infection. It has been shown in man and various animal models that IgG, IgM and IgA antibodies are produced in response to rotavirus infection (89). Most investigators, however, seem to be agreed that in humans, of all the parameters of immunity to rotavirus, local gut immunity appears to have the greatest protective role (88,89,90). Studies carried out on animals have also pointed to the importance of local gut immunity (93,97,98). This was shown in an experiment where colostrum containing rotavirus antibody was fed to lambs on the first day of life but, when the lambs were challenged on the next day with lamb rotavirus, they developed severe diarrhoea despite the presence of serum rotavirus antibody (acquired from the colostrum) on the day of challenge. In contrast, lambs which were fed colostrum for the first four days of life and challenged with rotavirus on the second day of life did not develop illness (93).

Although the local and systemic immune systems can function relatively independently it is much more convenient to measure the serum response to rotavirus infection than try

to estimate intestinal IgA which would require much more invasive techniques. The disadvantage of measuring serum antibody is that there are a number of different techniques for measuring antirotavirus antibody and not all are equally useful for predicting protection from subsequent infection. Complement fixing antibody does show some correlation with clinical protection (99) but the best results seem to be obtained when neutralising antibody is measured against the four major serotypes known to cause infection in man (68). The antigen which defines the serotype also determines neutralising specificity and antibody levels measured in this way have been shown to predict accurately the level of protection from subsequent homotypic and, to a lesser extent, heterotypic infection (68).

The relationship between the age specificity of clinical rotavirus infection and immune status is also poorly understood. As was stated above, sequential infections do occur in the first few years of life, which may indicate that protective immunity from one infection is imperfect or that it is serotype specific. By the age of three years almost all children have detectable serum antibody to rotavirus (33 - 36) and no longer suffer from clinical episodes of rotavirus infection. This age specificity may not, however, be the simple result of acquired immunity because evidence is emerging, in an animal model, of specific virus receptors on the gut mucosa which are age dependent (100).

Childhood Diarrhoea in The Gambia.

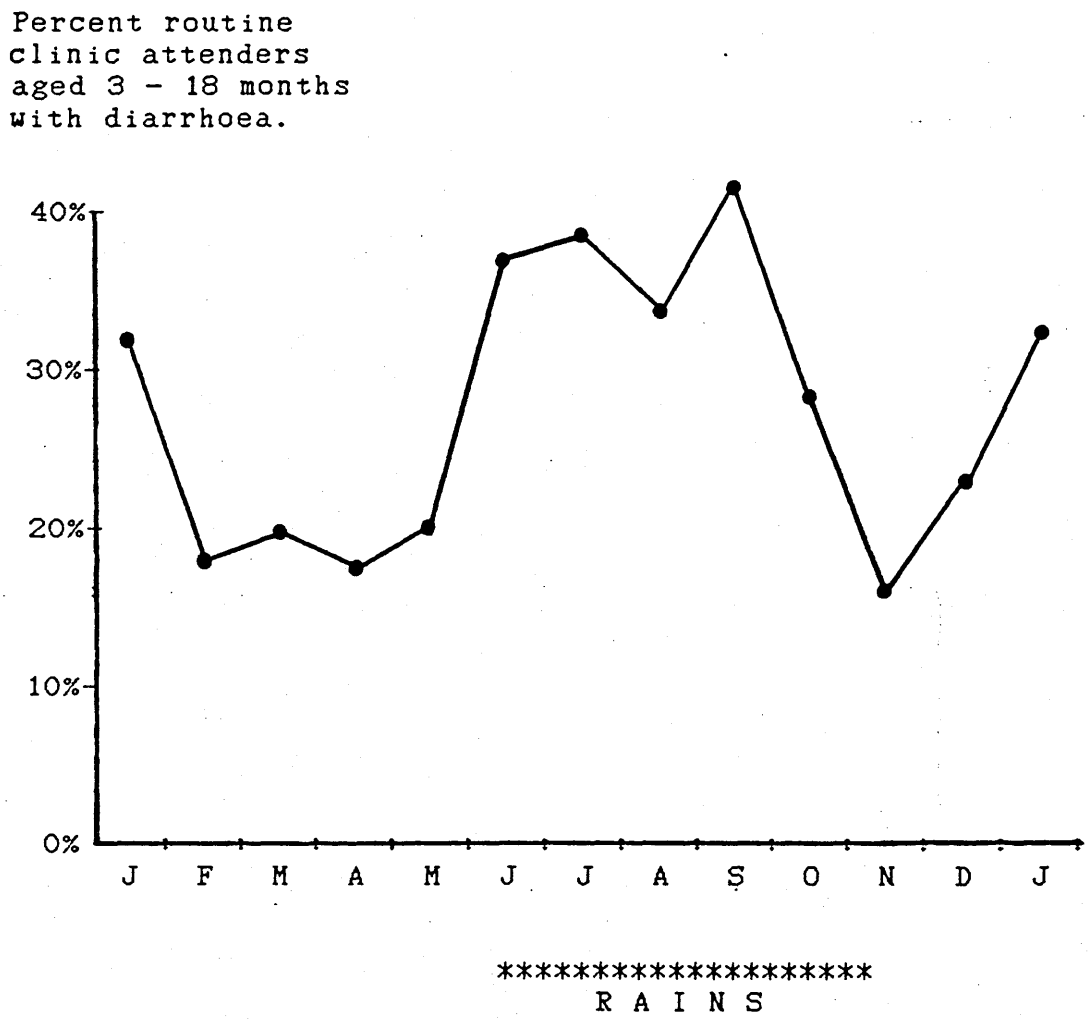
Diarrhoea is an established problem in The Gambia and, as in most developing countries, children less than 5 years of age are most frequently affected (101 - 104). Diarrhoea is common throughout the year but the prevalence of acute and chronic diarrhoea is greatest during the rainy season (fig. 3) (101). There is, however, a smaller peak in acute diarrhoeal prevalence during the dry season.

The importance of diarrhoeal disease is further illustrated by unpublished Gambia Government figures which indicate that 15% of all "under-fives" consultations at government clinics are due to diarrhoea and that, during the rainy season, 30% of children suffer an acute attack of diarrhoea during the course of any given two week period. The equivalent figure for the dry season is 15%. The accuracy of these figures is open to question but a rigorous prospective survey of children under the age of 2 years has shown just how common diarrhoea is, in as much as children under the age of two years were found to suffer from an average of 7 - 8 episodes per year (102).

As might be expected with such a common condition most attacks are mild and it is the exceptional case which requires hospital admission or is life threatening. More severe episodes do, however, occur. This is evident from the fact that 8% of paediatric hospital admissions are caused by diarrhoea (104). Further evidence of the importance of diarrhoea was discovered during a recent survey of childhood

FIGURE 3

Monthy variation in clinic attendance due to diarrhoea at Keneba clinic, The Gambia (101).



deaths conducted in rural Gambia during which diarrhoea was shown to be the third most common cause of death, after malaria and acute lower respiratory tract infection (105).

Diarrhoea in The Gambia is also almost exclusively responsible for the non-dietary element in failure to grow (10,106 - 108). Consequently, any intervention which succeeded in reducing the prevalence of diarrhoeal disease would reduce fatalities associated with marasmus.

Diarrhoea is, therefore, important for three main reasons. Firstly, it is exceedingly common and accounts for a large proportion of the clinical load placed on the child health services. Secondly it is a major cause of childhood mortality and thirdly, it also contributes to the even larger problem of malnutrition.

Preventing Diarrhoea in The Gambia

The search for effective preventive measures for the control of diarrhoea in The Gambia has not been an easy one. Historical evidence from Europe shows that the prevalence of diarrhoea falls as living standards rise. The provision of clean water and good sanitation are obviously vital but there is little prospect of these improvements coming to The Gambia in the near future. Others have tried to identify single interventions which would reduce the spread of diarrhoea. To this end, an investigation was undertaken to determine the relationship between social and environmental variables and diarrhoea in an urban area of The Gambia

(109). The results showed a considerable variation between individual children with respect to the frequency of diarrhoea, but none of the social and environmental variables recorded showed a significant relationship with diarrhoea prevalence. This study suggested that single public health interventions were not likely to improve health status but rather highlighted the need for a general rise in living standards, education and access to health care. However, one weakness of this study was the fact that diarrhoea was treated as a single disease entity rather than a symptom which could be caused by a wide variety of pathogens.

A more fruitful approach to the prevention of diarrhoea might be to identify the important aetiological agents and concentrate efforts on the control of specific pathogens. The problem with this approach in The Gambia has always been the lack of knowledge concerning the aetiology of diarrhoea.

The Aetiology of Diarrhoea in The Gambia.

Efforts had been made between 1970 and 1975 to discover the aetiology of diarrhoea in The Gambia (101). These studies, which were conducted in rural Gambia, resulted in remarkably low yields of diarrhoeal pathogens (5.6% of cases and 3.5% of controls). One of the main reasons for this was the lack of facilities which would have allowed the investigators to test for toxin producing E. coli or rotavirus. They did, however, highlight an epidemiological observation which was to prove important in the study of rotavirus. Peak diarrhoea

prevalence coincided with the period of highest rainfall, but a smaller, cool, dry season epidemic was also observed. During this dry season outbreak severe acute dehydration was a feature (110). Retrospective scrutiny of records made some 10 to 15 years earlier indicated that this second peak was not just a recent phenomenon.

The first study to search systematically for E. coli and rotavirus was conducted during 1981 and 1982 in Bakau, a peri-urban community (102). Table II summarises the important findings from this study. From these data it can be seen that toxigenic E. coli and rotavirus were the two most commonly identified pathogen. Indeed they were the only pathogens which could be positively associated with diarrhoea. The bacteria and or viruses which were identified were associated with diarrhoea in only 27.5% of cases while control stools had pathogens in 19% of the samples. In symptomatic children less than six months of age, pathogens were identified in diarrhoeal stools in less than 10% of cases. It would not be unreasonable to argue from these figures that the aetiology of diarrhoea in this Gambian community is largely unknown.

This state of ignorance leaves ample scope for speculation as to the aetiology of diarrhoea in the majority of cases. Diarrhoeal episodes of unknown aetiology are almost certainly a mixed bag of infections due to a variety of organisms as well as some cases of diarrhoea which result from infection elsewhere in the body (e.g. malaria). Of these possibilities, diarrhoea viruses are of particular

Table II

Isolation of individual enteropathogenic bacteria and viruses in diarrhoea (D) and control (C) specimens, collected from children aged 0 - 24 months in Bakau, The Gambia.

Goh et al. 1985 (102).

	Morbidity Status	
Organisms Identified	D	C
Toxigenic E.coli	51	41
Rotavirus	30	10
Campylobacter	22	48
Salmonella	6	18
Shigella	8	6
Mixed	25	9
Total Examined	516	695

importance to this study as a number of other viruses have now been associated with diarrhoea. These include small round structured viruses such as Norwalk agent, astrovirus and calicivirus as well as coronavirus and adenovirus. With the exception of the "fastidious" faecal adenoviruses type 40,41, these other viruses are less well established as major diarrhoeal agents in man. It is clear that a comprehensive assessment of "infectious" diarrhoea would require a tremendous technical effort outside the scope of many developing countries. For my own study, there were logistic and financial problems which made a more comprehensive assessment impossible. I was, however, able to set up a small collaborative exercise with Dr. David Cubitt which allowed some samples to be examined by E.M. in London.

Although such practical difficulties should not, in theory, influence academic decisions, the choice, in reality, lay between adopting the policy of only testing the stools for rotavirus or not doing the study at all. There were, however, major problems associated with my acceptance of this compromise. In a community in which diarrhoea is common and asymptomatic episodes of rotavirus infection are known to occur the presence of rotavirus in a diarrhoeal stool does not in any way prove causation. As a consequence, in this presentation, the term rotavirus diarrhoea denotes rotavirus associated diarrhoea. In addition, the compromise I adopted meant that I would have no knowledge of the frequency with which mixed infections occurred and I would be able to say very little about the microbiological background against which the vaccine trial was performed.

I do, however, feel that the decision can be defended on academic as well as simply pragmatic grounds. Firstly, rotavirus is now well established as a pathogen and, as will be seen later in the text, the clinical episodes associated with rotavirus in The Gambia were distinctive in terms of their severity and timing. Secondly, the design of the rotavirus vaccine trial allowed for a comparison between groups such that mixed infections (although undetected by my methodology) were probably equally represented in vaccinated and control groups. My inability to calculate vaccine efficacy with and without the inclusion of mixed infections was a handicap but this was somewhat offset by the fact that it was decided from the outset that it would only be practical to introduce rotavirus vaccination into countries like The Gambia if the vaccine were to have a very high efficacy so any minor miscalculation of vaccine efficacy would not be of major practical importance.

One further problem was the fact that it is almost certainly the case that children in The Gambia have a considerable load of infection with enteroviruses. Concurrent enterovirus infection is thought by some to have a detrimental effect on the response to the oral polio vaccine and so, by the same logic, might have a similar effect on the oral rotavirus vaccine. This important issue will also be discussed in more detail later in the text.

Rotavirus in The Gambia.

It is easy to both overestimate and underestimate the

importance of rotavirus infection in The Gambia. There is a local tradition which speaks of severe diarrhoea occurring during the cool dry season and experienced clinicians had for many years observed a peak in admissions due to dehydrating diarrhoea during the dry season but it was not until a longitudinal study was conducted on 126 children in Bakau during 1981-82 that rotavirus was for the first time defined as an important pathogen in The Gambia (44). During this study well defined epidemics were observed during two consecutive cool dry seasons. During this study cases were almost entirely confined to infants after the age of 1 month.

This study also showed that rotavirus was the second most commonly identified cause of diarrhoea. However, since the incidence of diarrhoea due to all causes is so high, it is only if an organism is capable of infecting the same child several times in the same year that its frequency as an aetiological agent would rise much above 10%. With that in mind, it is probably true to say that rotavirus diarrhoea is as common in The Gambia as diarrhoea due to any single pathogen is likely to be.

At about the same time that rotavirus was first shown to be an important cause of diarrhoea in The Gambia, results were published from a trial of a new bovine rotavirus vaccine. This trial had been carried out in Finland (111) but, despite the apparent success of the Finnish trial, it was clearly important to establish that the new vaccine would have an equally protective effect in developing countries

where the infecting dose of virus would be higher and the environment so different. Therefore, a clinical trial of this new rotavirus vaccine, conducted in the tropics, became an important priority of the WHO's Diarrhoeal Disease Control Programme. Consequently a rotavirus vaccine trial became the centrepiece of a series of studies relating to rotavirus in The Gambia. These studies are the subject of this thesis.

Possible Approaches to Vaccination.

It is clear from the evidence presented above that an effective and inexpensive rotavirus vaccine would make a considerable contribution to the control of severe diarrhoeal disease in the Third World. In industrialised countries, a case can also be argued for rotavirus vaccination (17). As evidence accumulated showing the importance of rotavirus as a pathogen, so efforts were directed increasingly towards the development of an effective rotavirus vaccine.

Efforts to develop an effective vaccine have, however, been hampered by a number of difficulties. Initially, rotavirus could not be cultivated in the laboratory but this problem has now been overcome. The second problem was lack of information about rotavirus immunology and epidemiology. In particular, information was lacking with respect to (a) the epidemiological and immunological characteristics of the four main rotavirus serotypes; (b) the age, geographical, and temporal distribution of rotavirus infections, as well

as the immunological interactions between the various subgroups and serotypes, including the nature and degree of cross-protection; and (c) the mechanisms of active immunity.

Despite the lack of these basic facts relating to the immunology and epidemiology of rotavirus infection, various groups embarked on the development of rotavirus vaccines and a number of promising rotavirus vaccine candidates are now at various stages of development. This state of affairs has led to criticism from some quarters that the pace of vaccine development is outstripping knowledge of the disease but, in striving for the goal of an appropriate vaccine, it is surely desirable to pursue two parallel courses simultaneously, that is the further investigation of epidemiology and the development of suitable vaccine candidates. As will be seen in Chapters IV and VI, this parallel approach was adopted in our Gambian studies.

The first approach to developing a rotavirus vaccine in humans was to use live attenuated human rotaviruses (112). The possiblity of using this approach developed from the successful cultivation of the Wa strain of human rotavirus in primary African green monkey kidney cells, following prior passages in piglets. This cell culture adapted strain, which is a serotype 1 virus, had not gained in virulence during passage, and there was a suggestion that it had become attenuated. Following numerous in vitro and in vivo safety tests, the strain was administered to 12 adult volunteers in the U.S.A.. None developed a diarrhoeal

illness. One-half demonstrated serological evidence of infection. Further studies were, however, suspended when it was discovered that three volunteers had developed low-level elevations of serum transaminase ten days after challenge (113,114).

More recently, the successful cultivation of each of the four main distinct human rotavirus serotypes has offered possibilities for development of a vaccine by conventional tissue culture methods employing a cultivable human rotavirus strain.

Adaptation of human rotavirus strains to low temperatures also holds promise as another approach to the development of an attenuated vaccine. In Japan, a temperature sensitive strain of human rotavirus serotype 1 was obtained by a step-wise reduction of cultivation temperature from 37 °C to 25 °C. A serotype 2 virus is undergoing similar studies and both are being evaluated for safety in piglets (115).

A second strategy for vaccine development involves the use of an animal rotavirus strain that can infect man and produce cross-protective immunity without inducing illness. Various animal strains including the Nebraska calf diarrhoea virus (NCDV), U.K. bovine strain, and the rhesus monkey strain are now being considered for this purpose.

Animal experiments had shown that infection of gnotobiotic piglets by a single serotype did not confer protection against an infection by another serotype, although it did

confer very good protection on challenge with the homologous serotype (116). It was, therefore, slightly surprising when it was shown that that a bovine rotavirus candidate (RIT 4237) seemed to protect colostrum deprived piglets against infection by a human rotavirus strain (117). This information resulted in a variety of trials in Finland which are described in detail in the next section. The apparent success of these Finnish trial led directly to the field trials of this vaccine in The Gambia.

When our studies began in The Gambia, the bovine U.K. strain was at the stage of volunteer studies in U.S.A., while progress with the rhesus monkey strain was being hampered by febrile reactions in some adult volunteers (118).

The third approach to vaccine development is to produce reassortant viruses that take advantage of the characteristic of reoviridae to undergo genetic reassortment with efficiency during mixed infections in cell culture (119,120). Reassortants have now been developed between wild-type bovine or rhesus rotaviruses and each of the four human serotypes. These viruses show promise as potential vaccine candidates but testing is still at a relatively early stage (120).

The final approach to developing a suitable vaccine is the least well advanced but may in time give the best result. This approach employs recombinant DNA technology to clone the rotavirus genome. Cloned DNA copies of the rotavirus

double-stranded RNA genomic segments corresponding to subgroup and serotype genes have been isolated and the DNA sequences determined (121,122). In time, it may be possible to prepare antigenically-reactive peptides by chemical synthesis or to incorporate rotavirus genes into appropriate live viral or bacterial expression vectors.

With each potential vaccine candidate development has to pass through several phases. Once the candidate is identified it is tested for immunogenicity, safety and efficacy in animals. Next, these same characteristics are evaluated in man. For ethical reasons, such studies are undertaken in seropositive and then seronegative adult volunteers. Once immunogenicity and safety have been established in adults, progressively younger groups of seropositive and then seronegative children can be studied. In contrast to animal and adult human studies, efficacy studies in children must be undertaken under natural conditions, in which children are not challenged experimentally with rotavirus, but rather are evaluated for protection against naturally occurring disease.

At the time of writing, only two candidate rotavirus vaccines (the attenuated bovine rotavirus vaccine - RIT 4237, and the rhesus monkey virus vaccine) have reached the stage of field trials. Of the two, testing of RIT 4237 is furthest advanced.

Development and Testing of RIT 4237.

The RIT 4237 vaccine strain is a viral clone obtained from high-passage level of the Lincoln isolate of the Nebraska calf diarrhoea virus in primary bovine kidney cells (123). The virus is subgroup I and has a non-human serotype (124). In 1983 Zissis published evidence that colostrum-deprived piglets would produce antibody to RIT 4237 either given intramuscularly (twice) or once intragastrically and once intramuscularly (125). He then went on to show the protective effect of these vaccination schedules against two strains of human origin, evaluated by artificial challenge.

The virus was then produced in high titre in primary monkey kidney cells, which are a suitable substrate for human vaccine production (123). Immunogenicity and safety in man was then established (126). In adults the vaccine did not cause clinical symptoms and a booster response in rotavirus serum antibodies was seen in 2/20 subjects. The second group of vaccinees consisted of 20 children around two years of age. The vaccine did not cause detectable rotavirus excretion in the stools and did not produce gastrointestinal or constitutional symptoms. It did, however, induce seroconversion in 13/19 (68%) of children initially found to be seronegative by neutralisation assay (126).

Shortly afterwards RIT 4237 was the subject of a randomised, double-blind, placebo controlled trial carried out in Tampere, Finland (111). 178 infants aged 8 to 11 months received one dose of the vaccine at the beginning of the

epidemic season. The majority of the children were seronegative at the time of vaccination. 50% of the seronegative vaccine recipients seroconverted and a booster response to vaccination was detected in 40% of those seropositive at the time of vaccination.

In this study vaccination with RIT 4237 resulted in a 50% reduction in rotavirus illness (loose stools or diarrhoea) and an 88% reduction in rotavirus-associated diarrhoeal episodes of at least 24 hours duration. It was, therefore, concluded that RIT 4237 gave better protection against severe rotavirus disease (defined as symptoms lasting for more than 24 hours) than rotavirus infection. It should be noted that mild episodes lasting less than 24 hours were more common than severe cases although, from a clinical viewpoint, the latter were more important.

Another significant and encouraging observation made in the course of this trial was that, while RIT 4237 was produced from a subgroup I rotavirus, the naturally occurring infection was caused by a subgroup II rotavirus. This supported the probability of heterologous protection. It would have been even more encouraging if the ability to protect against a wide variety of serotypes had also been demonstrated but at that stage the serotype of the virus which caused the outbreak in Finland was not determined.

At the same location the following year clinical efficacy was again measured in a larger cohort of 331 infants aged 6

to 12 months (127). On this occasion, however, two doses of vaccine were administered before the epidemic. Seroconversion was 53% and the clinical protection rate was 82% using a similar definition of clinically significant rotavirus diarrhoea. The epidemic on this occasion was caused by a subgroup II serotype 1 rotavirus, but protection was also evident against the small minority of cases caused by serotype 2 and 3 viruses.

These children were followed through a second rotavirus epidemic and, although the number of cases were small (1 in the vaccinated group and 6 in the control group), there was evidence that RIT 4237 could protect children through a second epidemic season.

When our studies in The Gambia began only the first of the Finnish trials had been completed but it is now clear that the second study had essentially similar results.

Another important fact which emerged from the Finnish studies was that the vaccine "take" could be enhanced, if it was administered with an antacid substance like milk (128). The presumed explanation for this is that rotavirus is sensitive to gastric acidity. Comparisons were made of vaccine response in breast-fed and bottle-fed infants. Although the response to the vaccine in bottle-fed children (86% serconversion) was better than in breast-fed children (71% seroconversion), both response rates were considered satisfactory. The amount of anti-rotavirus antibody in the breast milk was not measured and it is still not clear

whether breast milk antibody can compromise vaccination "take".

In response to these promising trials conducted in Finland, W.H.O. sponsored two further studies of RIT 4237 in the developing world, one in Peru and the other in The Gambia.

Study Aims in The Gambia.

The major objective of the studies we carried out in The Gambia was to find effective ways of controlling rotavirus diarrhoea. This could only be achieved by coming to a more complete understanding of several related issues. These were:

1. The epidemiology of rotavirus in The Gambia. One of the main aims of my study was to increase understanding of the epidemiology of rotavirus in The Gambia as this information would be used to determine future rotavirus vaccination policy.

2. The social and environmental context of rotavirus disease. I wanted to understand the social and environmental context in which rotavirus infection occurred as very little was known about this aspect of rotavirus infection in the developing world but such information might lead in time to effective envionmental interventions.

3. Prevention of rotavirus disease by vaccination. The central aim of my research was to assess the protective

effect provided by the bovine rotavirus vaccine RIT 4237 in a Gambian environment.

4. To investigate vaccine compliance in the rapidly expanding urban areas of The Gambia since this knowledge would be relevant to any future vaccination policy.

CHAPTER II

THE GAMBIA AND THE COMMUNITY

Geography.

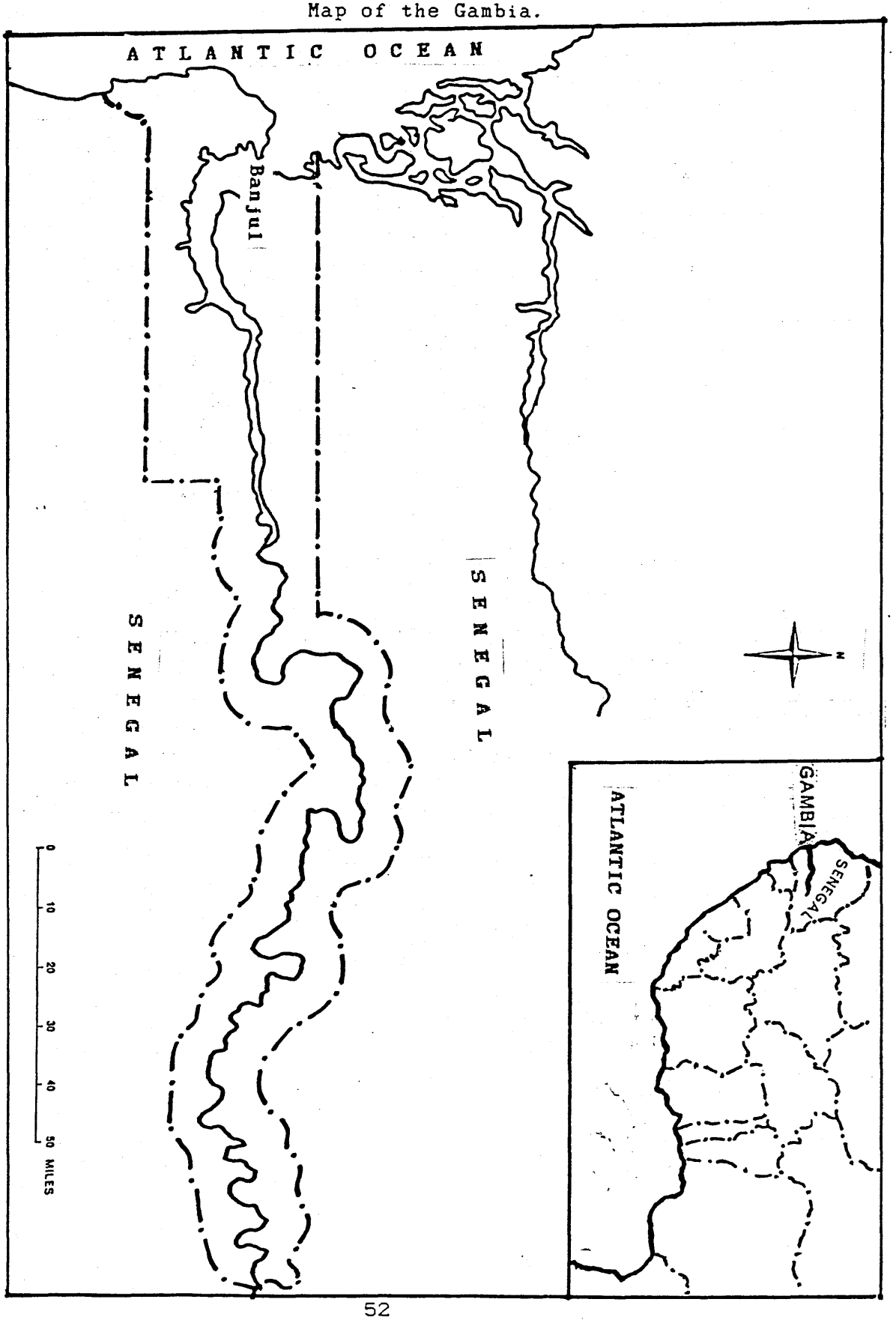
The Gambia is a small country on the west coast of Africa. It lies between latitudes 13° 3′ 00″ and 13° 49′ 00″ N and longitudes 16° 48′ 00″ and 13° 47′ 00″ W (fig. 4.). The country is made up of two narrow strips of land on either side of the Gambia river, after which the country is named. The short Atlantic coastline is only 48kms long while the eastern extremity of the country is 487kms from the coast, giving a total land area of 11,607 square kms, making it one of the smallest countries in Africa. The entire country is low-lying, nowhere rising more than 90 metres above sea level.

Historically, the Gambia was of interest to various colonial powers because the river was one of the best natural waterways on the west coast of Africa which was a major centre for trafficking in slaves. The river was disputed by the Portuguese, French and British in turn and the somewhat strange shape of the country stems from the fact that the British declared the area on either side of the river a British colony, thereby forming an enclave within Senegal which was at that time part of French West Africa.

The river is, therefore, the major geographical feature of the country, providing as it does a natural means of

FIGURE 4

Map of the Gambia.



communication and transport. In its non-saline upper reaches, it provides a source of fresh water for rice irrigation. Nearer the coast the river becomes salty and low lying swamps are found on either side beyond which lies a low sandstone plateau with laterite outcrops.

The vegetation is a mixture of savanna grass- and wood-land, with the exception of the mangrove swamps which line the lower reaches of the river.

Since independence was gained in 1965 The Gambia has been a parliamentary democracy. The country has six administrative divisions but villages have retained their traditional political organization with village leaders (Alkalos) and elders.

Climate.

The Gambia has a tropical climate, with wet and dry seasons (129). The entire annual rainfall (average 800 mm per year) occurs within the rainy season which starts in June and continues till September or October. Towards the end of the rainy season rainfall decreases while humidity stays high, but, by the beginning of December, there is a marked fall in humidity and a slight fall in mean temperature (26 ^{o}C in the wet season and 24 ^{o}C in the dry season). As will be argued later in the text, this change in climate may well have a profound influence on the epidemiology of rotavirus in The Gambia. Inland, the moderating influence of the sea is not felt and variation in temperature is more extreme. The

climate remains dry until the return of the rains the following June but temperatures do tend to rise as the next rainy season approaches. The rotavirus epidemic season (December - February) is, therefore, associated with relatively cool and dry weather.

In common with all of sub-Saharan Africa, The Gambia has suffered from a series of poor rainy seasons over the past 10 - 20 years. This change in rainfall has had a severe impact on the water table level and has decreased the amount of swampland suitable for rice cultivation.

Population.

A national census was carried out in 1973 and again in 1983, but results are not yet available from the most recent census (130). In 1973 the country had a population of 493,499, giving a population density of 47 per square kilometer, making it one of the most densely populated countries in the African continent. Unofficial results from the 1983 census indicate an annual growth rate of approximately 2.5%, with a continuing trend towards rural - urban population drift.

In 1973 the infant mortality rate was 217 deaths per 1,000 live births, making it one of the highest in Africa, matched only by Chad, Upper Volta and Mali (131). By 1983 the infant mortality rate had fallen to 160 deaths per 1,000 live births but this still compares unfavourably with most other

African rates. The second year mortality rate is also relatively high with 41 deaths per 1,000 surviving infants. These high rates are reflected in the life expectancy figure which was last calculated as 41 years (132).

The largest ethnic group in The Gambia are the Mandinka, who comprise over 40% of the total population. The remainder is made up of a number of ethnic groups, the largest of which are the Fula (18%) and the Wolof (16%). Over 90% of the population are Muslim, while a small minority are Christian or follow a traditional religion.

Economy.

The majority of the population live in rural areas, growing rice, maize, sorghum and millet for their own consumption, and groundnuts for export. There is no manufacturing industry and the groundnut crop is the country's main source of foreign currency. The salaried sector is extremely small, but a large number of people make their living through trade, largely operating outside the formal economy. Tourism is limited to the coastal region and makes a small contribution to the economy. In the peri-urban areas around the capital Banjul, a larger proportion of the population are wage-earners but, even in these circumstances, family income is often supplemented by agriculture or trading.

Education.

Government efforts have been concentrated on providing

universal primary education which is provided free for children aged eight years or above. Places in the secondary schools are awarded on a competitive basis, and an estimated 40% of those completing primary school go on to secondary education.

Health Services.

Public health care in The Gambia is the responsibility of The Ministry of Health and Social Welfare. It is provided free of charge to all children but some adults are charged for consultation and treatment. Health services are provided at four levels: (i) sub-dispensaries; (ii) dispensaries; (iii) health centres; and (iv) hospitals. In 1980, the Gambian Primary Health Care Action Plan was implemented which has resulted in village health services being established in 230 villages with populations greater than 400 people (133). It is estimated that over 90% of the population now have access to basic health services, and the country has achieved an enviable level of vaccination coverage (133).

Administration and delivery of maternal-child health services vary between the urban and rural areas. This aspect of health care is dealt with in more detail in Chapter VII.

In addition to the medical facilities provided by the government, a variety of non-western, traditional forms of prevention and treatment are widely available. Many people

use a combination of traditional and western medicine, often selected in a seemingly haphazard manner.

Despite the undoubted success of many aspects of health care in The Gambia, the population, in particular children, bear a heavy burden of morbidity and mortality. In children, malaria, lower respiratory tract infection, malnutrition, and diarrhoeal disease are the major problems. In addition measles, tuberculosis, schistosomiasis, glomerulonephritis and a large variety of skin infections are seen commonly. Adults continue to suffer from malaria but most are semi-immune. Respiratory infections, venereal disease, tuberculosis and hepatoma are among the major diseases which cause adults to present to the Medical Research Council clinic (134,135).

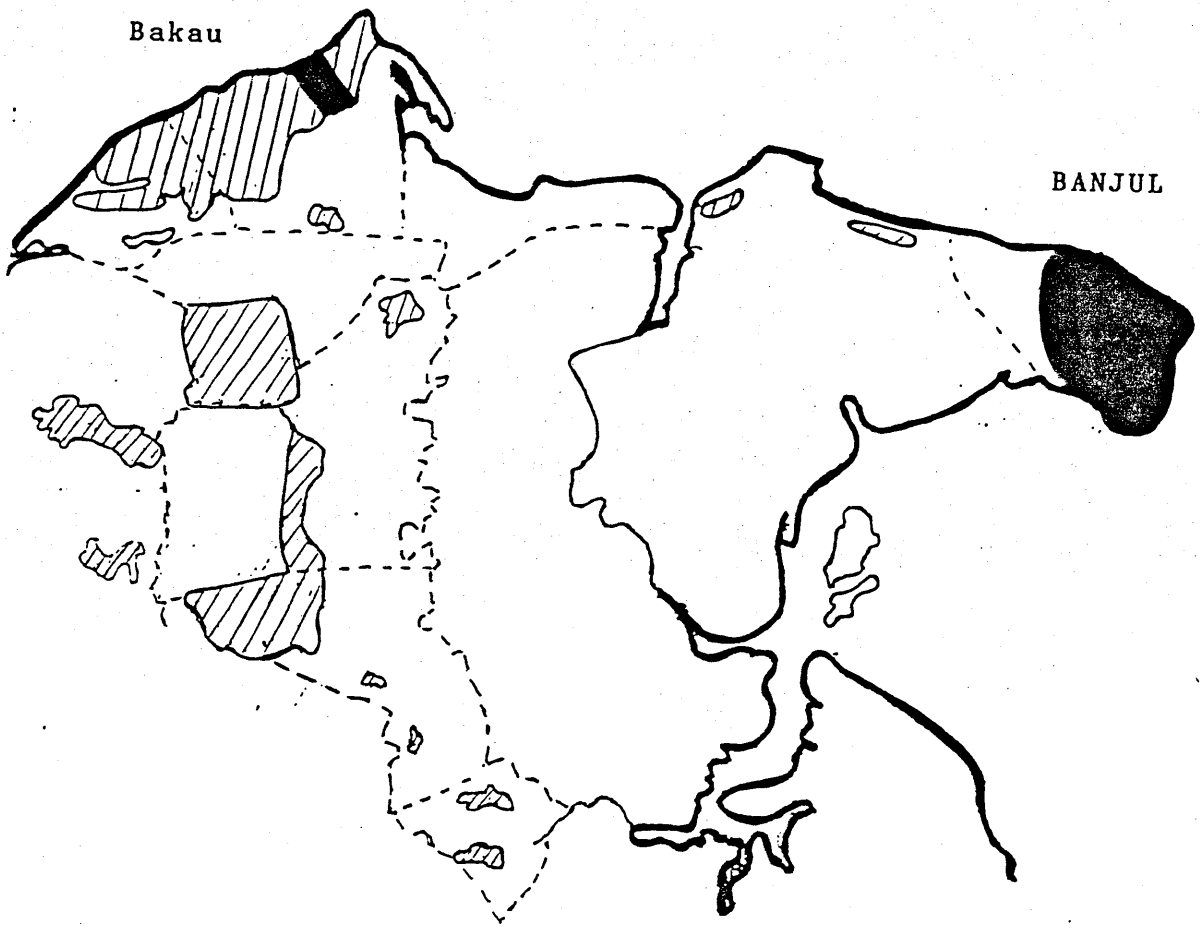
Bakau.

Bakau is a peri-urban community situated on the coast within 15 kms of the capital Banjul (fig. 5). The population of Bakau have co-operated with various M.R.C. studies since 1981, and it was the site of all the studies described in this thesis.

The first settlers in Bakau were three Mandinka families who moved to the area in the late eighteenth century (136) and their descendants still hold dominant positions within the village hierarchy. In recent years there has been a considerable increase in the population of Bakau, as rural people have moved to the urban areas seeking employment.

FIGURE 5

Bakau and other settlements near to Banjul.



= other urban areas

Despite this, the community has retained many of the features of a traditional village.

The population at the time of the study was approximately 14,000. A full census of the area was made by the M.R.C. in 1981 and I updated and extended the census in 1984 for our own purposes. A proportion of the population commute each day to the capital, where most administrative, commercial and industrial work is concentrated. Bakau is itself a commercial centre, although all enterprises are on a very small scale. In addition, many of the women grow their own vegetables in small plots for sale in the market. Income is difficult to estimate accurately. Although all families are poor by western standards there is little doubt that the people of Bakau enjoy a higher living standard than their rural counterparts.

Residents of Bakau, when asked where they lived, always referred, not to their house, but to the compound in which their house was situated. Compounds are spatially discrete, areas surrounded by makeshift fences. They vary considerably in size but usually contain several houses, which open onto a common courtyard. Bakau contains a total of 710 compounds, the largest of which houses almost 200 residents.

The majority of houses are built with mud bricks, and have corrugate iron roofing and cement floors. Housing standards do, however, vary considerably from grass huts to European style houses with glass windows.

A chlorinated water supply runs to a small number of public standpipes. Water is collected in clay or plastic containers and carried to the homes. Public services are not extensive and there is no mains drainage or sewage disposal. Consequently, household sullage can cause unpleasant pools of stagnant water in some parts of the town. There are a number of refuse collection points and a private cleansing firm clears these sites every three to four days. The same company is responsible for cleaning the two public latrines and collecting and emptying night soil from those compounds that use pails. The most commonly used sanitation method is the pit latrine but very young children are often allowed to defaecate on the ground.

The health facilities available to the people of Bakau consist of the main government hospital in the capital, the Medical Research Council outpatient clinic and ward, situated one kilometer from the edge of Bakau, and the Bakau Health Centre. The M.R.C. clinic is very popular because it offers a wider range of investigative and curative services, but all vaccinations and maternal-child health services are provided at the health centre. For this reason very young children will visit the health centre frequently, but not exclusively, during the first year of life.

Before the present study began meetings were held with the elders of Bakau to explain the aims of the various trials and subsequently each mother gave informed consent. The level of cooperation given by the population of Bakau was exceptional.

CHAPTER III

METHODOLOGY

EPIDEMIOLOGICAL AND CLINICAL ASPECTS.

The Role of Field Assistants.

A total of 15 field assistants worked on various aspects of these studies. Their major contribution was within the community itself, where they administered questionnaires, collected stool samples, paid reminder visits, and acted as interpreters. In addition, they also acted as interpreters and assistants in the clinics. Most of the field assistants were recruited in 1984 before the census of Bakau was performed and each one was given training in the various tasks and skills which they were required to perform. I also maintained a system of continuous in-service training so that the range of tasks they could be asked to do was constantly expanded as the project progressed.

Surveillance of 1984/85 Outbreak.

The method of surveillance employed during the 1984/85 rotavirus outbreak in Bakau was very different from that of the 1985/86 outbreak, described below. Longitudnal surveillance had not been established at the onset of the 1984/85 outbreak, so the epidemic was monitored by a system of case finding within the "at risk" age group. I examined all children less than three years of age presenting to the M.R.C. clinic or the Bakau health centre with diarrhoea

during December, January and February. Clinical details were recorded and a stool sample collected. Stool samples were then screened for rotavirus by ELISA. This method of surveillance almost certainly failed to detect less severe episodes of rotavirus associated diarrhoea, which were treated at home by the mothers, but it did allow us to gather some information about the timing and duration of the epidemic.

Selection of Vaccination Study Cohort.

All children born in Bakau during 1985 were invited to participate in the rotavirus vaccine trial. In the absence of rigorous official vital registration, it was necessary for field assistants to find and record all new births to mothers resident in the study area whether they occurred at Bakau health centre, the government hospital or at home. Table III shows the total number of births and those excluded from the final analysis. Mothers were interviewed during the first few days of the child's life at which time the study was explained and informed consent obtained. Children who migrated from the study area during the course of the study were considered lost to follow-up and have been excluded from the analysis of vaccine efficacy but their serological results have been included when available. Only children who had received a minimum of one vaccination, at least one month before the onset of the rotavirus epidemic, were included in the final analysis of vaccine efficacy, but the one hundred younger children who were excluded because their vaccinations occurred around the time of the epidemic

Table III

1985 birth cohort from Bakau: status with respect to rotavirus vaccine trial.

1985 births	482
Stillbirths	20
Refusals	15
Neonatal deaths	14
Total recruited	433
Outmigrations	65
Away during epidemic	15
Denominator for cases	351

Children who received at least one dose of rotavirus vaccine at least one month before the onset of the epidemic and were resident in the study area during the epidemic = 253.

were followed through the 1985/86 epidemic and they have been included in calculations for attack and incidence rates.

Vaccination Trial: Study Design.

The rotavirus vaccine study was designed to measure the efficacy of RIT 4237 in a Gambian environment and to demonstrate any mutual interference between the oral rotavirus vaccine and oral polio vaccine when both were administered together. The latter was important because delivery cost would be decreased, and compliance increased, if both vaccines could be administered together but mutual interference would be undesirable for the rotavirus vaccine and unacceptable for polio. For this reason the rotavirus vaccine schedules were timed to coincide with the Gambia Government's recommended times for polio vaccination (table IV).

At recruitment, children were randomly allocated to one of three groups (table V). Group A received three doses of oral rotavirus and oral polio vaccines, group B three doses of oral placebo and oral polio vaccine and group C three doses of oral rotavirus and intramuscular (I.M.) polio vaccines. Comparison of polio serology in groups A and B would indicate any adverse effect on the response to oral polio caused by the rotavirus vaccine, while comparison of the rotavirus antibody response in groups A and C would reveal any detrimental effect on the rotavirus vaccine caused by oral polio.

Table IV

Recommended vaccination timetable for Gambian children at the time of the study.

VACCINATION	AGE OF CHILD
B.C.G.	After Birth
First polio and D.P.T.	10 weeks
Second polio and D.P.T.	14 weeks
Third Polio and D.P.T.	18 weeks
Measles and Yellow Fever	9 months

Table V

Rotavirus vaccine trial.

Vaccination: group allocation and timetable.

Group	Group A	Group B	Group C
Vaccines	Rotavirus + Oral Polio	Placebo + Oral Polio	Rotavirus + I.M. Polio
Doses of Vaccine	10, 14, 18 weeks old	10, 14, 18 weeks old	10, 14, 18 weeks old
Blood Samples	10, 14, 22 weeks old	10, 14, 22 weeks old	10, 14, 22 weeks old

The vaccinations were administered by a member of the study team but incorporated into the Gambia Government's vaccination programme at Bakau health centre. Gambian children normally receive vaccines at the ages of ten, fourteen, and eighteen weeks but, in the case of defaulters, a period of four weeks was always allowed to elapse between each vaccination.

Capillary blood samples were taken before the first and second vaccinations, and one month after the third vaccination. These samples were analysed for polio and rotavirus neutralising antibody.

Rotavirus vaccine and placebo preparations were presented in identical precoded vials ensuring that the investigators remained blind with respect to the randomisation between groups A and B. Since group C received killed intramuscular polio vaccine it was not possible to remain fully blind with respect to this group although, in practice, any given child's group allocation was not known during clinical assessment, surveillance or laboratory analysis.

Field Surveillance.

Surveillance began during the first week of life, at which time a stool was collected from each child. Field assistants completed morbidity questionnaires on a weekly basis, increasing to twice weekly during the rotavirus epidemic season (December - February). The weekly morbidity

questionnaire (RS1 - fig. 6) and twice weekly questionnaire (RS2 - fig. 7) were similar apart from the period of recall required by the mother. Mothers were asked standardised questions about the child's diet and symptomatology since the previous visit. Her answers were then recorded on the RS1 or RS2 surveillance form by the field assistant. Information was also collected concerning any other symptoms the child had suffered, and a rectal temperature was recorded. If the child had suffered from diarrhoea since the previous visit and had not already submitted a stool specimen at the clinic, the field assistant was instructed to collect a specimen at that visit. Each form was then checked by a more senior member of the study team, after which the data was entered into a microcomputer. In this way a complete morbidity record for each child in the cohort was compiled.

Clinical Surveillance.

We provided a clinical service for the study children in the form of a daily clinic held in the health centre, and an out of hours service at the M.R.C. ward, situated nearby. Whenever a study child presented with an episode of diarrhoea, details of weight, length, temperature, symptomatology, diagnosis and treatment were recorded on a precoded rotavirus study clinical (RC) form, illustrated in figure 8. In this way a full clinical record was made, and a stool collected, during most diarrhoea episodes.

FIGURE 6

R.S.I.

ROTAVIRUS STUDY

Name: Health Card No: 1 ☐☐☐☐☐☐☐

D.O.B. Sex: Patient No: 7 ☐☐☐☐☐☐☐

Mother: Date: 13 ☐☐☐☐☐☐☐

Father: Date of last questionnaire: 19 ☐☐☐☐☐☐☐

Compound:

Village:

---

1. Is the mother the respondee? Yes; No 25 ☐

   If No, is the respondee Guardian; Nursemaid; Relative; Other 26 ☐

2. Was the child with the respondee? Yes; No 27 ☐

   If No, was the child Dead; Hospital; Clinic; Visiting; Moved away; Other 28 ☐

3. What is the child being fed?

Breast	Yes; No	29 ☐
Other milk feeds	Yes; No	30 ☐
Weaning foods	Yes; No	31 ☐
Sharing adult foods	Yes; No	32 ☐
Other. Specify	Yes; No	33 ☐

4. Health. Record whether the child has had any of the following symptoms during the past week. Use Yes; No; Don't know

		Today	1	2	3	4	5	6	7
Day of week									
1. Diarrhoea	34								
2. Vomiting	42								
3. Fever	50								
4. Fresh cold/upper respiratory	58								
5. Chest infection/lower respiratory	66								
6. Other	74								

P.T.O.

5. Examine the health card

   Has the child had an episode of diarrhoea during the last week? Yes; No

6. Record the child's temperature 83 |__|__|.|__| 8

7. Has stool sample been collected? Yes; No

   If Yes, attach Brady No. [ ] 88 |__| 89 |__|__|

8. Comments:

Field workers signature

R.S.2.

FIGURE 7

ROTAVIRUS STUDY

Name: Health Card No: 1 |_|_|_|_|_|_|_|

D.O.B. Sex: Patient No: 7 |_|_|_|_|_|_|_|

Mother: Date: 13 |_|_|_|_|_|_|_|

Father: Date of last questionnaire: 19 |_|_|_|_|_|_|_|

Compound:

Village:

---

1. Is the mother the respondee? Yes; No 25 |_|

   If No, is the respondee Guardian, Nursemaid; Relative; Other 26 |_|

2. Was the child with the respondee? Yes; No 27 |_|

   If No, was the child Dead; Hospital; Clinic; Visiting; Moved away; Other 28 |_|

3. What is the child being fed?

Breast	Yes; No	29 |_|
Other milk feeds	Yes; No	30 |_|
Weaning foods	Yes; No	31 |_|
Sharing adult foods	Yes; No	32 |_|
Other. Specify	Yes; No	33 |_|

4. Health. Record whether the child has had any of the following symptoms during the past week. Use Yes; No; Don't know

		Today	1	2	3	4
	Day of week					
1. Diarrhoea	34					
2. Vomiting	39					
3. Fever	44					
4. Fresh cold/upper respiratory	49					
5. Chest infection/lower respiratory	54					
6. Other	59					

70

P.T.O.

5. Examine the health card.

   Has the child had an episode of diarrhoea recorded during the last week? Yes; No 6

6. Record the child's temperature 65 | | | . | |

7. Has stool sample been collected? Yes; No 6

   If Yes, attach Brady No. 70 | | 71 | | | |

8. Comments

Field Worker's signature 75

FIGURE 8

ROTAVIRUS STUDY R.C.

Name: Health Card No: 1 |_|_|_|_|_|_|

D.O.B. SEX: Patient No: 7 |_|_|_|_|_|_|

Mother: Date: 13 |_|_|_|_|_|_|

Father: Called: Yes/No |_|

Compound:

Village:

Anthrops

Weight kg: 20 |_|_|.|_|_|

Length mm: 25 |_|_|_|_|

Temperature °C: 29 |_|_|_|_|

HISTORY

Diarrhoea

Duration: 33 |_|_|

Frequency: 35 |_|_|

Character: 37 |_|_|

Vomiting

Duration: 39 |_|_|

Frequency: 41 |_|_|

Fever

Duration 43 |_|_|

URTI

Duration 45 |_|_|

Other

Examination

Dehydration. 1.0-5%; 2/5-10%; 3. 10% and over 47 |_|_|

Other

Investigations

1. Stool Brady No. 48 |_| 49 |_|_|_|_|

2. Blood Brady No. 53 |_| 54 |_|_|_|_|

Severity 58 |_|_|

F.T.O.

Diagnosis

60 ___
62 ___
64 ___
66 ___

Treatment

68 ___
70 ___
72 ___
74 ___

Has R.C. form been completed for an earlier presentation of this illness? Yes/No 76 ___

On what date was that form completed? 77 ___

Clinician: 83 ___

A vaccination clinic was held weekly and mothers were paid a reminder call when vaccination or blood sampling was due.

Anthropometric Measurements.

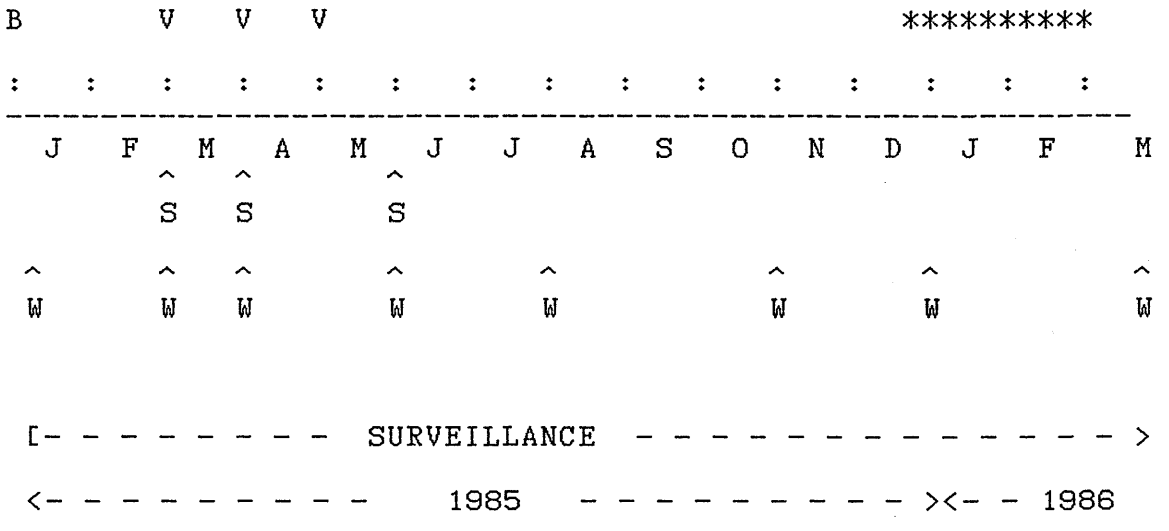
Anthropometric data (weight and length) were also collected from each child at the ages of 10,14,22,30,39,65,and 78 weeks. In addition, the child's weight was measured whenever the presenting complaint was diarrhoea. These data were recorded on the child's own clinical record, but a separate computerised record was also kept. From these data it was possible to compare the weight and pattern of growth before and after clinical episodes of diarrhoea.

For clarity, the timetable of events for a child born on 1st January 1985 and followed till April 1986 is shown in figure 9.

Diarrhoea was defined as the passage of three or more loose stools in any 24 hour period but for children less than two months of age any change in stool consistency of concern to the mother was included. Following W.H.O. recommendations, a case was considered severe if its duration was greater than 24 hours and associated with one or more of the following: passage of six or more stools in 24 hours, vomiting, rectal temperature $> 38\ ^{o}C$, clinical dehydration. Using these criteria almost all the cases examined at the clinic were defined as severe. A more sensitive means of assessing severity was therefore devised. This method was based on the

FIGURE 9

Timetable of trial events for a child born on 01.01.85 and followed till 30.03.86.



B - Birth

V - Vaccination

S - Blood sampling

W - Weight and length

**** - Epidemic of rotavirus

TABLE VI

Severity score for clinical features of diarrhoea.

	Score		
	1	2	3
Symptom			
Diarrhoea frequency	3 - 4	5 - 8	> 8
Vomiting frequency	1 - 3	4 - 6	> 6
Dehydration	< 5%	5 - 10%	> 10%
Pyrexia (rectal)	< 38 °C	38 °C to 39 °C	> 39 °C

The total score is calculated by summing the individual scores for each symptom.

total of scores awarded for stool frequency, vomiting frequency, degree of dehydration, and level of pyrexia, having allocated individual scores according to the system illustrated in table VI.

Micro-Computer Management of the Vaccine Trial.

Computerised data processing was highly desirable because of the relatively large amount of data being generated by the trial. Similarly, it was necessary to incorporate day-to-day management of the trial within the overall data system, rather than dealing with the data on a purely retrospective basis since the management of age-related events longitudinally in the continuously recruited cohort would otherwise have been very complex. In The Gambia mainframe and large mini computers are not a realistic proposition (137) so there was clearly a need to devise a suitable microcomputer based system.

Torch Computer's enhancement of the BBC microcomputer was used in conjunction with the dBase II database management system, running under the CP/M operating system.

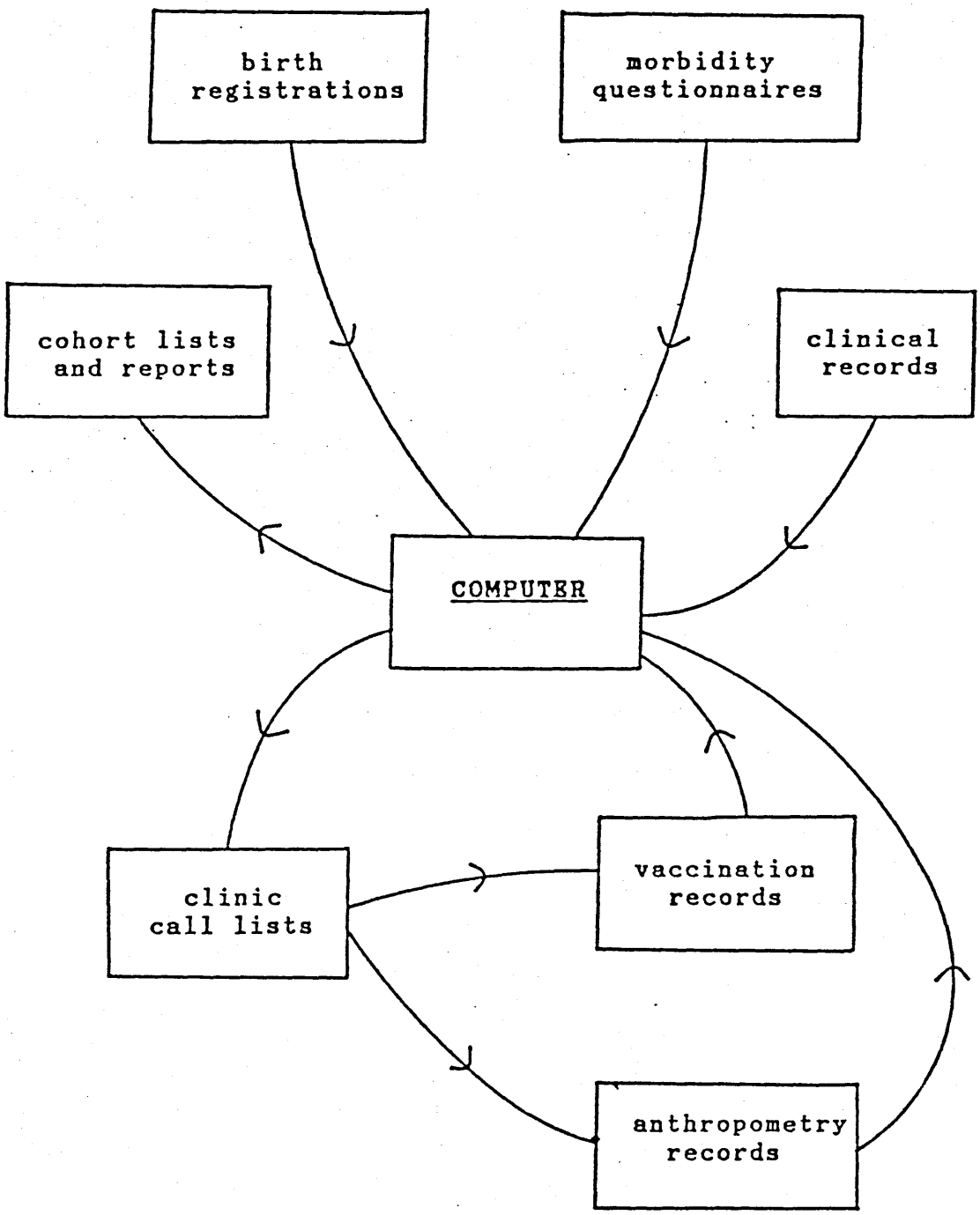
During 1984 field assistants updated the Bakau census. Each person within Bakau was then given a six-digit identification number, made up of three digits relating to the compound of residence and three digits as a personal identifier within the compound. These data were then entered into a census database. When a new birth was identified the next available census number for the appropriate compound

was then allocated to the infant. From the field assistant's registration of births, records of identification number, name, date of birth, sex and field worker assigned to the case were compiled for each infant. Thus, the computer system was required to accept new subjects in this format on an on-going basis throughout the study, together with subsequent data relating to vaccinations, anthropometry, morbidity and clinic visits.

The system used these data to generate information vital to the continued smooth running of the trial. Clinic call lists were generated for each weekly vaccination clinic indicating which children were due for vaccination, blood sampling and anthropometry. The list contained details of each child as well as the initials of the field worker responsible for each case. Each fieldworker then took a copy of the list, identified his or her own cases from it and made home visits asking mothers to bring their children to the clinic. Once the clinic had been completed, the identification numbers of those children who attended were entered into the computer. In this way defaulters were automatically identified and called for the following week's clinic, while those who attended were recalled at the appropriate time for the next stage of the trial. The inter-relationships of these requirements are shown conceptually in figure 10.

FIRURE 10

Conceptual Data System.



Cross-Sectional Studies.

Cross-sectional studies were undertaken to determine the point prevalence of rotavirus infection (symptomatic and asymptomatic) among the vaccine study cohort during the wet season (July 1985) and the dry season (January 1986). These surveys were extended to include a random sample of older Bakau children up to the age of 5 years. Diarrhoea morbidity data and a stool sample were collected from each of these children.

Surveillance for Vaccination Side Effects.

Morbidity data were collected daily for a seven day period to detect adverse reactions to rotavirus vaccination from a subset of 164 children following 164 vaccinations. Rectal temperatures were recorded at each visit to detect any febrile reaction to vaccination.

Post-Vaccination Vaccine Virus Excretion Surveillance.

An attempt was made to collect a stool sample each day for seven days following vaccination to detect evidence of post-vaccination excretion of the vaccine virus. The visit for this purpose coincided with the visit for vaccination side effects surveillance.

Social and Environmental Risk Factors

A search was made for any relationship between social and

environmental variables within Bakau and the risk of rotavirus associated diarrhoea. 92 children who suffered a proven clinical episodes of rotavirus associated diarrhoea during the 1985/86 epidemic and 92 controls, who had not suffered a clinical rotavirus infection, were investigated. Controls were strictly matched for age, sex and rotavirus vaccination status. A pre-tested questionnaire (Appendix I) was used which sought information on 55 social and environmental variables under the broad headings of child's compound, child's house, water supply, sanitation, hygiene, animal contacts, ethnic and family background, parental education, family wealth and income, and the child's feeding pattern. The questionnaire was administered to the child's mother with the aid of a Gambian interpreter. Although it was not possible to be strictly blind with respect to the case/control allocation of each child, in practice the status of any given child was not known at the time of the interview.

The information was then entered on to the pre-coded questionnaire and the paired case/control responses analysed using conventional statistical techniques.

Vaccination Compliance Study.

During August 1985, I collected vaccination compliance data retrospectively for 154 children aged 12 - 18 months living in Bakau. The vaccination record was copied from the "road to health card" which was kept by the mother and contained a

record of the child's weight gain, vaccination dates, clinic attendances and clinical records. The vaccination status of each child, as it had been on its first birthday, was then determined. A Gambian child should have received 9 vaccinations (table IV) by the age of one, so a score of 0 - 9 was calculated for each child reflecting the number of vaccinations received. The children were then ranked according to their vaccination score and two groups selected for further investigation. The first group contained 42 children who were fully immunised and so had scores of 9, while the second was made up of 23 poorly immunised children with scores of 5 or less. Data on 29 socioeconomic and attitudinal variables were collected by means of a pre-tested questionnaire which was administered to the children's mothers by a Gambian investigator who remained blind to the child's vaccination status (Appendix II).

Ethical Approval.

Each separate aspect of this project was passed by the joint Gambian Government/Medical Research Council Ethical Committee which acts as the national ethical committee and includes Gambian lay and professional representatives.

LABORATORY METHODS.

The assays used were approved by the Diarrhoeal Disease Research Committee of the World Health Organisation. Dr. Ruth Bishop visited our laboratories as a W.H.O. consultant and, in consultation with Dr. Hilton Whittle the Deputy

Director of the M.R.C. in The Gambia, decided on the methodologies used. The laboratory work was performed by Mr. O. Jobe, Miss H. Sillah and Miss F. Shenton although I was involved in, and assisted with, these tests throughout the period of evaluation. The rotavirus ELISA is a well established technique, kits for which were supplied by Dr. T. Flewett of the W.H.O. rotavirus reference laboratory in Birmingham. The methodology for the rotavirus neutralising assay was supplied to Dr. Whittle by Dr. Bishop. The polio neutralising antibody assay had been used by Dr. Whittle in The Gambia for several years and employed standard techniques. R.N.A. electrophoresis was carried out by Dr. Ray Sanders of the W.H.O. reference laboratory in Birmingham who visited The Gambia as a W.H.O. consultant.

Vaccination and Placebo Preparations.

Rotavirus vaccine RIT 4237 came in monodose vials from the manufacturer Smith - Kline - RIT. The vaccine lot used in this trial (L1207) had a high titre $10^{7.8}$ per monodose vial. The placebo preparation was made from uninfected primary monkey kidney cells. Both vaccine and placebo were freeze dried and stored at -20^{o}C. In the case of groups A and B each vial was reconstituted before use with 0.5ml of oral polio vaccine, while 0.5ml of distilled water was used in group C. Reconstituted vaccine was then administered orally via a tuberculin syringe from which the needle had been removed. As the antibody response to rotavirus vaccination is enhanced by giving an antacid substance like milk with

the vaccine (128), mothers were encouraged to continue their normal habit of breast feeding their children immediately before and after vaccination. Oral Sabin polio vaccine and intramuscular Salk vaccine were also supplied in monodose vials by the manufacturer of the rotavirus vaccine Smith - Kline - RIT.

Laboratory Methods - Detection of Rotavirus in Stool.

All stools collected were examined for rotavirus using the W.H.O. double sandwich ELISA (87). This method required a 10% suspension of faeces in phosphate buffered saline which was clarified by low speed centrifugation (3,000g for 10 - 15 minutes). The supernatant was then removed and this formed the 1/10 dilution which was used for the ELISA test. The deposit was discarded.

A 1/10,000 dilution of buffered hyperimmune rabbit serum was prepared and 0.1ml of this preparation dispensed into each well of a microtitre plate. The plate was then covered and incubated at either 37°C for 2 hours or overnight at 4°C. The contents of the wells were then discarded and the plates washed six times in buffer. 0.075ml of phosphate buffered saline (PBS) with 0.1% v/v Tween 20 and 0.01M Ethylenediaminetetraacetic acid (EDTA) pH 7.2 was then added to each well with the exception of the 2 pairs of plate control wells.

Two wells were used per faecal sample. 0.025ml of faecal extract was added to each of the two wells while the last

four wells on the plate received substrate only (two wells) and conjugate and substrate (two wells). These four wells functioned as ELISA plate controls. The plates were then covered and incubated overnight at 4°C. The microtitre plate was then washed six times with PBS. A 0.075 ml volume of PBS with 0.1% v/v Tween 20 and 0.01 M EDTA was then added to all wells except the four plate control wells. Three wells were reserved for the addition of (i) rotavirus negative faecal extract (negative control) (ii) rotavirus positive control (iii) 1/10 dilution of the rotavirus positive control in PBS/EDTA.

0.1ml of 1/10,000 (in PBS/T/BSA) hyperimmune guinea-pig anti-rotavirus serum was added to each well of the plate (excluding the plate control wells). The plate was again covered and incubated at 37°C for 2 - 3 hours. The contents of the wells were discarded after which the plate was washed in PBS. A 0.1ml volume of alkaline phosphatase conjugated anti-guinea-pig IgG (diluted 1:500 in PBS/T/BSA) was then dispensed into each well, including two plate control wells, after which the plate was covered and incubated for a further 2 hours at 37°C. The conjugate was then discarded and, after washing with PBS, 0.1ml of substrate was added to each well (including the two pairs of plate control wells) and the plate again incubated for approximately 20 minutes at 37°C. The reaction was stopped by the addition of 0.05ml of 3 molar sodium hydroxide when the 1/10 dilution positive control showed yellow colouration. The results could easily be read by eye but a

more accurate measure was obtained by light absorbence at 405 nanometres in an automated photometer.

A direct blocking assay was then used to confirm that positive results were due to specific binding of rotavirus antibody to rotavirus antigen in the faecal extract. Each faecal sample requiring confirmation was then incubated overnight at 4 °C with pre-immune rabbit serum and a hyper-immune rabbit anti-rotavirus serum in separate wells and the test repeated as before. Immunological binding with hyper-immune serum prevented antigenic binding to the solid phase resulting in a negative reaction. In the well with pre-immune serum no rotavirus blocking took place and the antigenic binding to the solid phase took place with a positive reaction as previously seen. The optical density of true positives incubated with post-immune serum showed at least a 50% reduction compared with wells incubated with pre-immune serum.

Laboratory Methods - R.N.A. Electrophoresis.

Where possible, positive stool samples had their rotavirus R.N.A. electropherotype determined using an adaptation of a silver staining polyacramide gel technique (138). A minority of ELISA positive samples had insufficient volume of stool present in the pot to allow this test to be carried out.

R.N.A. was first extracted from the faecal samples. A suspension of faecal sample was prepared in distilled water at a concentration of 10 - 20% (wt/vol). A 0.45ml volume of

suspension was added to 0.05ml of 1 M sodium acetate, pH 5.0, containing 1% sodium dodecyl sulphate (SDS). The suspension was mixed for a few seconds and then incubated at 37°C for 15 minutes. A 0.5 ml volume of a 3:2 (vol/vol) phenol - chloroform mixture was added to the faecal suspension, mixed for 30 seconds and then incubated at 56°C for a further 15 minutes (Phenol consisted of a mixture of 500g phenol, 70g m-cresol and 200g of water containing 0.5g 8-hydroxyquinoline). The emulsified mixture was then centrifuged for 3 minutes in an Eppendorf centrifuge (model 5412) at 1,500g, and the resulting upper aqueous layer removed, carefully avoiding the intermediate white layer. R.N.A. was precipitated from the aqueous layer by the addition of one-tenth volume of 3 M sodium acetate and two volumes of cold ethanol. After incubation at -20°C for 2 hours the R.N.A. was then pelleted and dried and finally resuspended in buffer consisting of 0.125 M Tris-HCl pH 6.8, 10% glycerol, and 0.1% bromophenol blue. After centrifugation for 3 minutes at 1,500xg for 3 minutes the supernatant was used for electrophoresis.

Samples (0.025 mls) were then loaded on to 10% polyacrylamide slab gels. Electrophoresis was performed at room temperature for 5 hours at 40 mA. R.N.A. visualisation was obtained by silver staining according to Herring et al. (138). Gels were photographed by illumination from below using Polaroid MP.4 land camera, and Polaroid type 667 black and white film.

Laboratory Methods - Rotavirus Neutralising Antibody.

Rotavirus neutralising antibody was measured using a standard technique except that, in our case, we used the RIT 4237 strain of rotavirus as test antigen. This vaccine strain was cultivated in MA 104 monkey kidney cells and live virus was harvested in 1ml aliquots when required for the neutralising assay. Microtitre plates were then prepared by adding 0.2ml of media containing 2×10^5 MA 104 cells/ml to each well, and incubating at 37°C in a CO_2 incubator for three days, by which time a confluent monolayer of cells had formed on each well.

Individual serum samples were then diluted on a separate microtitre plate starting with a dilution of 1:10 and progressing through doubling dilutions to 1:1280. The 1ml aliquot containing rotavirus at 10,000 T.C.I.D.$_{50}$ was then diluted 1:100 with minimum essential medium and 0.2ml added to each serum dilution. Positive control wells, containing virus and media only, and negative control wells containing serum and media only were also prepared. The neutralisation plates were then incubated at 37°C for 16 -18 hours in a CO_2 incubator.

The MA 104 cells were then added to the neutralisation plates, each sample being tested twice in parallel. In addition each test plate contained a positive and negative control. Known standards were also included after every five samples. The plates were then incubated for 20 hours at 37°C

in a CO_2 incubator, at which time culture medium was removed and the cells fixed with formalin.

Cytopathic effect was then detected by indirect immunofluorescence. Rabbit anti-rotavirus antibody was added to each well and the plates incubated for 45 minutes. After washing with PBS, conjugated sheep anti-rabbit IgG was added and the plates again incubated for 45 minutes. Cells were again washed and then examined under an ultraviolet light microscope for fluorescent foci of rotavirus infected cells.

Titres of neutralising antibody were expressed as the last dilution of serum to neutralise the virus and thus inhibit fluorescence. When a sample, tested twice in parallel, showed a discrepant final titre the geometric mean titre was calculated.

Laboratory Methods - Polio Neutralising Assay.

In this assay doubling dilutions of sera were used to neutralise polio virus type 1, type 2 and type 3 at standard dilutions. Neat serum was diluted 1:10 with Eagles minimum essential medium and each sample was tested twice in parallel. Doubling dilutions of each sample were then made in each row of a flat bottomed, low evaporation microtitre plate. For each polio virus in each batch of sera tested, the following were included: (i) the appropriate polio standard serum (ii) a positive control (i.e. +ve virus and -ve serum) and (iii) a negative control (i.e. -ve virus and

-ve serum). Each strain of polio virus was then diluted from frozen stock with cold medium and kept at 4^{o} C. Dilutions of virus were as follows: -polio 1, a 1:2 dilution of stock virus with a concentration of 1×10^{6}, polio 2, stock virus with a concentration of 1×10^{5}, polio 3, a 1:3 dilution of stock virus with a concentration of 1×10^{5} related to 100 $T.C.I.D._{50}$ by earlier evaluations.

A 0.05 ml volume of virus dilution was then added to each well (except for the negative control) on the microtitre plate. The plate was then covered and kept at $+4^{o}$ C overnight. The following morning vero cells were stripped with trypsin, washed and suspended in warm medium at a dilution of 2×10^{5} cells/ml. A 0.1 ml volume of this cell suspension was then added to each well starting with the negative control wells, to avoid the danger of splash back.

The plates were then covered and incubated in a 5% CO_2 incubator at 37^{o} C. The plates were examined for cytopathic effect on the seventh day.

The titre of polio neutralising antibody was taken as the last dilution to neutralise the cytopathic effect of the virus strain. Each serum sample was tested twice in parallel against each polio strain. Where the duplicated test did not give identical results the geometric mean of the two titres was taken as the final titre. Negative results were expressed as any titre < 1:10.

Laboratory methods - electron microscopy.

A pilot study was conducted to explore the possibility of sending stool preparations through the post for examination by electron microscopy in the United Kingdom. It should be stressed that this was a pilot study. Results have only been included because they have some relevance to the virological background against which these studies were conducted.

Carbon formvar coated copper grids were supplied by Dr. David Cubitt. Faecal samples were mixed with phosphotungstic acid (with added Amphotericin B) and then placed on the copper grids. Excess fluids was blotted off until the grid was dry. The grids were then sent by post to Dr. Cubitt at the Central Middlesex Hospital in London. 15% of the grids were either damaged in transit or poorly prepared but results are available for the remaining 296 samples. I used samples from the wet and dry season cross sectional surveys. It was not possible to examine all these samples so stools from children with diarrhoea were selected along with control samples which were very roughly matched for age of child providing the sample. I also included some other samples which had been collected outwith the cross sectional survey but were known to be positive for rotavirus when tested by ELISA. The latter have been excluded from the presentation of results but they did demonstrate a 93% correlation between the ELISA results in The Gambia and Dr. Cubitt's E.M. findings. It must be pointed out that the majority of samples came from the vaccine trial cohort and only a random sample of older children in the community were

sampled so the age distribution is strongly weighted towards younger children.

CHAPTER IV

ROTAVIRUS EPIDEMIOLOGY IN BAKAU

INTRODUCTION.

In The Gambia research into the epidemiology of rotavirus has been in progress since 1982 and the major findings from these earlier studies have already been discussed in Chapter 1 (44, 102). In these earlier studies it was found that rotavirus gastroenteritis occurred in short, well defined, annual winter epidemics which had their maximum impact on infants after the age of one month. Sporadic infection was also observed in neonates who were often asymptomatic. In the two consecutive years studied there was a major change in subgroup, serotype and R.N.A. electropherotype of the dominant rotavirus. Rotavirus was clearly an important pathogen during the dry season but thoughout the rest of the year it had no clinical impact on the population. There was anecdotal evidence that rotavirus caused a more severe form of gastroenteritis than was usually encountered in The Gambia but this was clearly a matter which required more investigation. It was also important that I should gather as much descriptive data concerning rotavirus in The Gambia as was possible because this information would be required in the future to facilitate rational decision making about the value of more widespread use of a rotavirus vaccine.

As described in Chapter III, a variety of techniques were employed between 1984-86 to further increase our understanding of rotavirus epidemiology in Bakau. These were

(a) case finding during the 1984/85 outbreak, (b) longitudinal surveillance of the 1985 birth cohort, and (c) two cross sectional studies of children aged less than five years.

RESULTS.

Seasonal Variation.

We confirmed the seasonal pattern of rotavirus infection in the Gambia, as clearly defined outbreaks of clinical rotavirus infection occurred in the cool dry seasons of two further successive years.

During the dry season month of January 1985, 33 children presented with acute diarrhoea associated with rotavirus but no cases were detected in either the month before or after this outbreak. These cases occurred in children with ages ranging from 1 to 24 months, mean 9.7 months.

The 1985/86 outbreak, which occurred during the equivalent cool dry season of the following year, was documented more fully. The epidemic began in the last week of December 1985 and lasted seven weeks. A total of 93 cases were detected of which 13 occurred in December, 77 in January and 3 in February. The attack rate among 115 children who had not received rotavirus vaccine was 36%.

As surveillance was confined to children aged less than 1 year the cases detected had an age range of 1 to 12 months,

with a mean of 8.1 months. Sex distribution of cases was approximately equal with 49 males and 44 females.

Outside the epidemic period only three clinically mild episodes of rotavirus associated diarrhoea were detected in the vaccine study cohort. These infections all occurred in children aged between 4 and 8 weeks.

Dry Season Cross-Sectional Survey.

A cross-sectional survey of the rotavirus vaccine study cohort was conducted during January 1986, a time of high rotavirus transmission. A total of 337 samples were collected from 351 children aged 0 - 12 months (Table VII). Of 79 children with diarrhoea, 20 children had rotavirus antigen detected in their stool and 14 had an asypmtomatic rotavirus infection. 7 were asymptomatic on the day of sampling but rotavirus positive because they were in the recovery phase of a clinical rotavirus episode which had already been detected. Stool samples were also collected from 194 of a random sample of 200 children aged 13-60 months. Of 44 children with diarrhoea, 5 had rotavirus detected in their stool sample, while 2 had asymptomatic rotavirus infections. All rotavirus associated cases were less than 3 years old.

Rainy Season Cross-Sectional Study.

A similar cross-sectional survey was undertaken during the 1985 rainy season. 169 samples were collected from 210

Table VII

A. Cross-sectional study conducted during one week in January 1986 at a time of high rotavirus transmission on a cohort of children aged 0 - 12 months.

337 stools collected 78% of the cohort.
79 children had diarrhoea - 23%
20 had rotavirus associated diarrhoea - 6%
14 had asymptomatic rotavirus infections - 4%
7 had asymptomatic rotavirus excretion, but were known cases in the recovery phase - 2%

B. Cross-sectional study of a random sample of 205 children aged 13 - 60 months, conducted during one week in January, 1986 at a time of high rotavirus transmission.

194 Stools Collected.
44 had diarrhoea - 21%
5 had rotavirus associated diarrhoea - 2.5%
2 had asymptomatic rotavirus associated diarrhoea - 1%

vaccine study children who were all at that time less than 7 months old. 26 children were suffering from diarrhoea but none had rotavirus detected in their stools. Only 2 asymptomatic cases were detected, both in children less than 2 months old. 220 stools were collected at the same time from a random sample of 250 children aged 8 - 60 months. 46 children had diarrhoea but rotavirus was not detected in any of their stools. Only 1 case of asymptomatic rotavirus infection was detected.

Neonatal Rotavirus Infection.

During 1985 a stool specimen was collected during the first week of life from 410 newly born children. The vast majority of children were asymptomatic when the sample was taken. This single stool specimen was collected on the day that the child was recruited to the study so the age (in days) of each child on the day of sampling varied according to how quickly the new birth had been detected by the field assistant. Only 4 (1%) children were found to be excreting rotavirus and none were symptomatic.

Electron Microscopic Examination of Stools.

Some stools collected from children during the wet and dry season cross-sectional surveys were sent for examination by electron microscopy (tables VIII and IX). Stools from children with diarrhoea were selected and matched with an equal number of stools from control children who were asymptomatic on the day of sampling. The sample was weighted

Table VIII

Electron microscopic examination of stools collected in the wet season (July 1985). Age range of children 0 - 60 months but sample heavily weighted towards children < 12 months.

	Children with diarrhoea	Children without diarrhoea
Number of children	62	64
Small round viruses (enterovirus)	0	2
Small round structured viruses	0	1
Coronaviruses	8	1
Rotavirus	0	0

Table IX

Results from electron microscopic examination of stools collected during the dry season rotavirus epidemic (January 1986). Age range of children 0 - 60 months but the sample was heavily weighted towards children < 12 months.

	Children with diarrhoea	Children without diarrhoea
Number of children	95	75
Small round viruses (enterovirus)	1	0
Coronavirus	0	1
Rotavirus	21	12
Astrovirus	0	2
Coronavirus	0	1
Adenovirus	1	0
Coronavirus + small round virus (enterovirus)	0	1
Rotavirus + astrovirus	2	0
Rotavirus + coronavirus + adenovirus	0	1

towards children aged less that 12 months and a disproportionate number of control samples were lost or damaged in transit but tables VIII and IX are of some value because they contain the only available data concerning the viruses circulating in the community during the wet and dry seasons of my study. The large number of rotaviruses found in the dry season sample was expected and simply confirms the results shown on Table VII but asymptomatic carriage rate appears higher here. It is clear that other viruses were circulating during the dry season but they were not likely to be major pathogens in this study.

Rotavirus Severity.

To assess severity we devised a system of scoring which is discussed in Chapter III and summarised in table VI (page 74). The system of severity assessment recommended by W.H.O. was inadequate because, although it is useful as a means of standardising severity assessment for international comparison, almost all the cases in our study fell into the severe category so that it failed to discriminate between mildly severe and very severe cases. Episodes of rotavirus diarrhoea were significantly more severe than episodes of diarrhoea due to all other causes (X^2 = 86, 6 d.f., $p < 0.001$) (Table X). 66% of all children who attended the clinic with diarrhoea had severity scores of 4 or less. To achieve such a low score these children typically presented with mild diarrhoea which was not associated with vomiting, dehydration or pyrexia. In contrast only 31% of all rotavirus cases who came to the clinic fell into this very

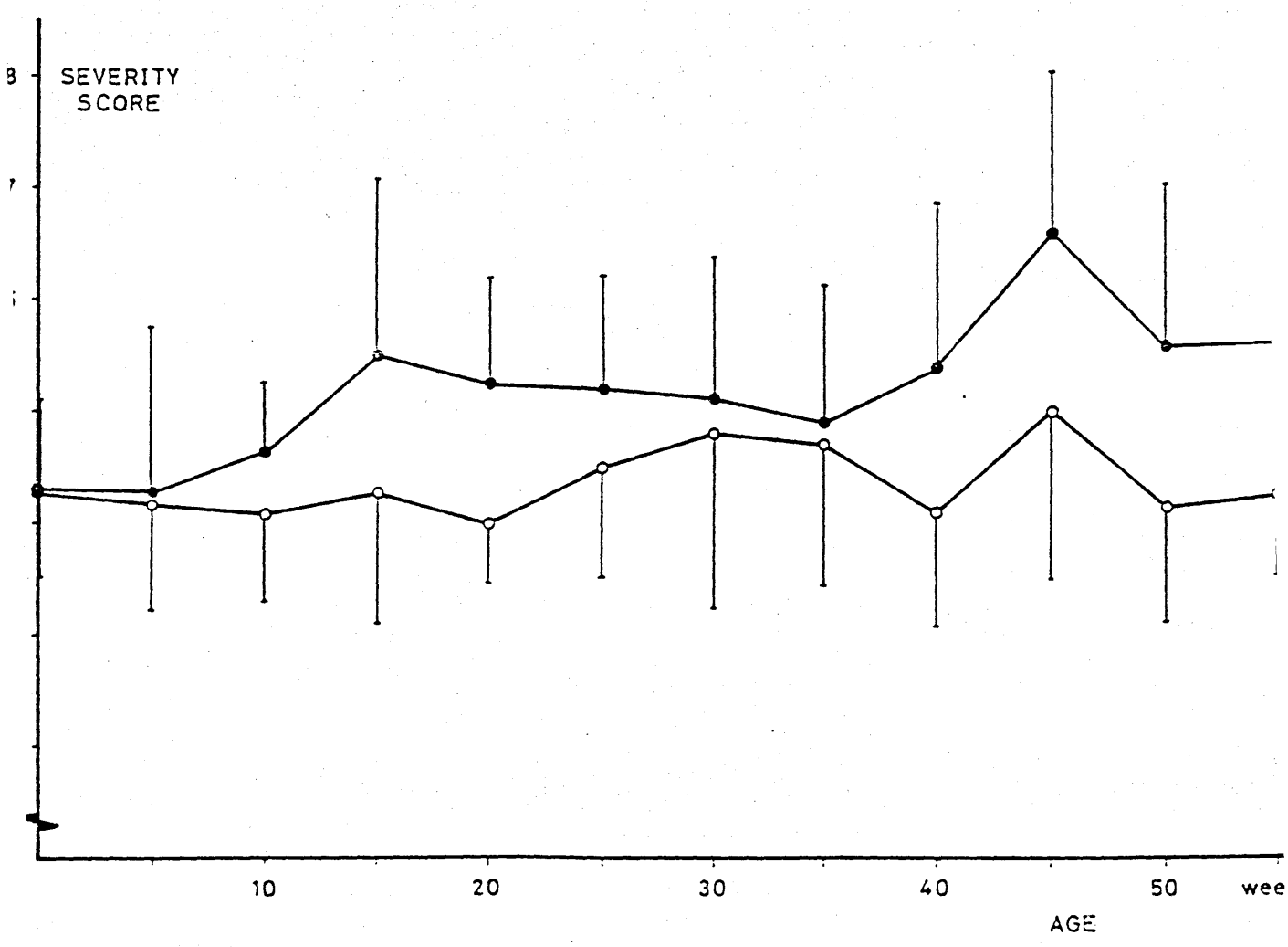
Table X

Comparison of severity scores for 81 cases of rotavirus diarrhoea with 1018 diarrhoea presentations due to other causes.

Severity score	All diarrhoea		Rotavirus cases	
3	217	21%	5	6%
4	462	45%	20	25%
5	211	21%	17	21%
6	89	9%	24	30%
7	27	3%	10	12%
8	9	1%	2	2%
9	3	<1%	3	4%

Figure 11

Severity scores, by age, for rotavirus cases (●) and cases of diarrhoea due to all other causes (o).



mild category. As rotavirus has an age related incidence, these data were reanalysed allowing for age at presentation, using a two way analysis of variance. This analysis again showed rotavirus episodes to be more severe than those due to other causes ($t = 2.95$, $p < 0.01$) (Fig. 11). Rotavirus diarrhoea was also more commonly associated with dehydration than other types of diarrhoea. Only 15 out of a total of 926 cases presenting to the clinic had greater than 10% dehydration, but 8 of these were subsequently found to be rotavirus infections ($X^2 = 32.6$ 1 d.f. $p < 0.001$).

Post-Infection Weight Patterns.

Clinical rotavirus infection was associated with loss of weight in the post-infection period. Examination of 166 weight charts (85 clinical cases and 81 age and vaccine status matched controls) showed acute weight loss during or immediately after the epidemic in 41 cases and 13 controls ($X^2 = 18.1$, 1 d.f., $p < 0.001$). There was, however, no difference in the pre-infection weights or weight patterns of cases and controls. An example of a weight chart from a rotavirus case is shown in figure 12.

R.N.A. Electropherotype.

There was a major change in R.N.A. electropherotype from one year to the next. Table XI shows the results of R.N.A. electrophoresis for 73 of the rotaviruses detected in cases from Bakau during the 1985/86 epidemic. 67/73 (92%) showed a short electropherotype (Illustrated in figure 2). Table XI

Figure 12

Sample weight chart from a child who suffered acute weight loss following an episode of rotavirus diarrhoea. The timing of the rotavirus episode is marked with an arrow.

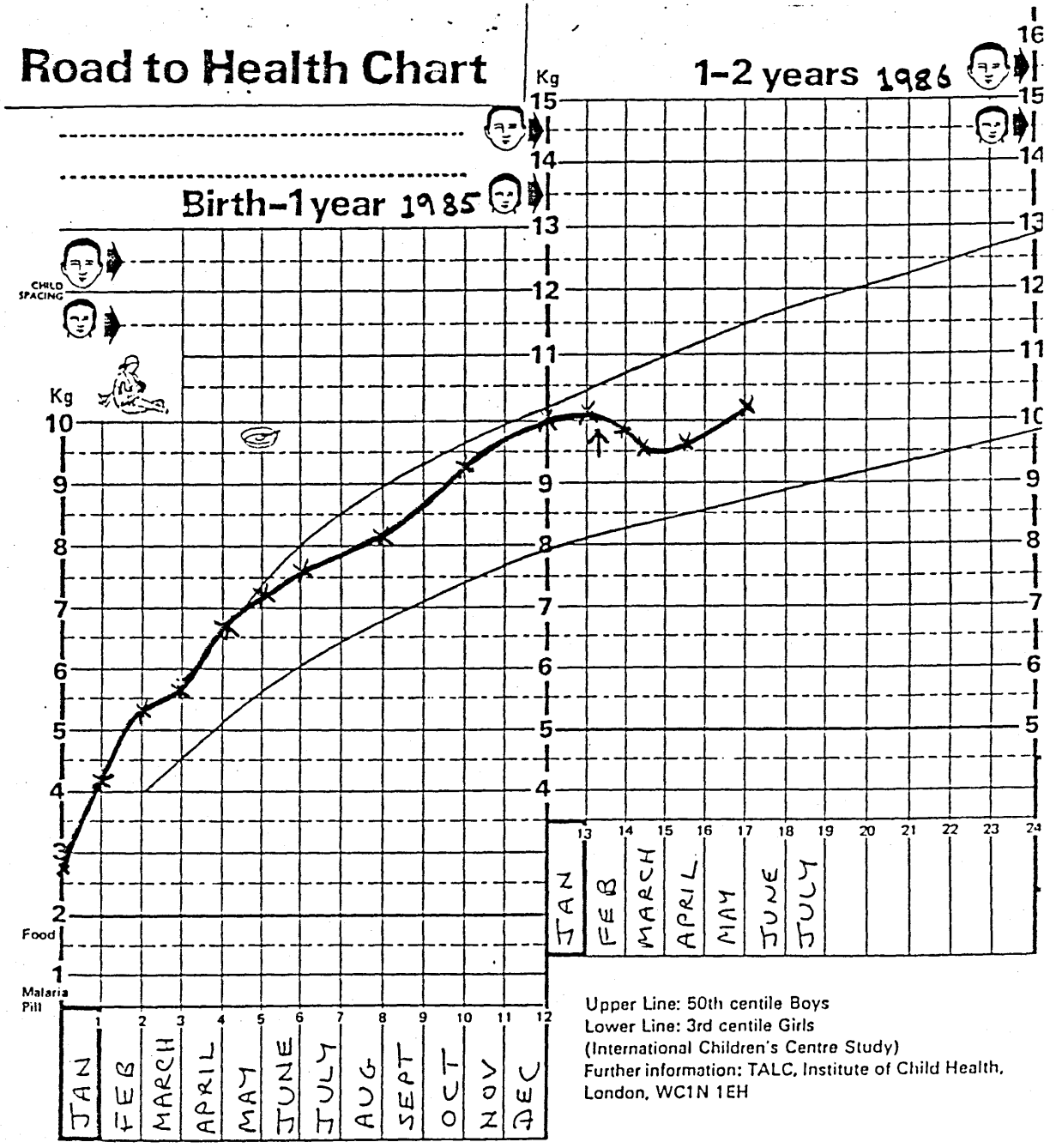


Table XI

R.N.A. Electropherotypes in Bakau during 1982/86

Analysis of 73 cases of rotavirus occurring in Bakau. Short pattern electropherotypes showed minor variations in migration patterns which were designated Sa, Sb, and Sc.

SHORT ELECTROPHEROTYPES			LONG ELECTROPHEROTYPES
Sa	Sb	Sc	La
17	23	27	6

67/73 (92%) of cases had a short electropherotype.

R.N.A. electropherotype by epidemic season, in Bakau.

	R.N.A. Electropherotype.	
	No. Short	No. Long
Year		
1982/83 *	5	1
1983/84 *	0	8
1984/85	6	22
1985/86	67	6

Note

* Rowland et al (44)

also contains data from 1982 - 86, some of which has been published previously (44). Cases from the 1984/85 outbreak shared the same long R.N.A. electropherotype as samples collected during the previous year but, by 1985/86, a short migration pattern predominated. Six samples collected from the intervening non epidemic period also showed a predominantly short pattern. Other samples collected during the 1985/86 outbreak from a community 6 miles away show a similar short pattern.

Although these rotaviruses were sent to the W.H.O. reference laboratory in Birmingham to be subgrouped and serotyped the technique failed because of high levels of alkaline phosphatase in the stool samples. It is, however, reasonable to assume that the short electropherotypes were subgroup I serotype 2 and the long electropherotypes subgroup II serotype 1 or 3.

DISCUSSION.

The very marked seasonal pattern of rotavirus infection has now been observed in The Gambia for four consecutive years. Epidemics occur during the cool dry season months with the most intense transmission in January. During the rest of the year rotavirus infection is almost entirely confined to mild or asymptomatic infections in neonates and very young children. At the end of December or early January, however, an epidemic, the onset of which can be accurately pinpointed to the day, suddenly begins. At present we can only speculate as to the cause of this very striking feature in

the epidemiology of rotavirus in The Gambia.

In most temperate countries rotavirus infection is endemic but more common during the winter months while studies from those tropical countries which experience seasonal variation show an association between rotavirus infection and cool dry weather (26,27,29,31,52,139,140). This is particularly striking in a recent study from Gabon (141) where a bimodal dry season corresponded to a bimodal epidemic. Some recent laboratory studies have suggested a possible mechanism which might account for this observation. The Wa strain of human rotavirus was found to survive longer on non-porous surfaces in conditions of low temperatures and low relative humidity (53). Other studies have shown improved survival of a bovine rotavirus at low temperatures and either low or very high, but not intermediate, humidity (142). There seems to be no obvious behavioural factor associated with the seasonal nature of rotavirus infection in Bakau so an association between rotavirus survival on fomites and environmental factors, like temperature and humidity, might be one possible explanation for the seasonal pattern which has been described.

During the epidemic of 1985/86 transmission was intense, and almost completely confined to children less than two years of age. The clinical attack rate for infants was 36%, and 40% of all diarrhoeal episodes in infants presenting to the clinic were due to rotavirus. A cross sectional study carried out at the height of the epidemic showed that the

point prevalence of rotavirus associated diarrhoea in children aged 0 - 12 months was 6%, while the survey of children aged 13 - 60 months showed that clinical infection was less intense in older children and did not occur beyond the age of 36 months.

Hospital studies have shown that in certain circumstances the level of asymptomatic rotavirus infection can be very high (143). Other studies have now established that asymptomatic shedding of rotavirus is common in neonates (54 - 66) but the evidence from community studies is less uniform, with an enormous range of asymptomatic infection rates reported . In our studies we found a 1% asymptomatic neonatal infection rate but this was almost certainly an underestimate as only one sample was collected from each child during the first week of life. Rotavirus shedding in neonates only lasts for approximately 48 hours and can occur at any time during the first week or two of life so very frequent sampling is required if accurate rates of asymptomatic infection are to be determined. The results from the non epidemic cross sectional survey suggests that, in older children, there is a very low level of asymptomatic shedding throughout the year, while the epidemic season cross sectional survey suggests that at times of high transmission asymptomatic rotavirus shedding is common. On the day of sampling the point prevalence of asymptomatic shedding was two thirds that of symptomatic infection (4% and 6% respectively).

One of the difficulties raised by the high rate of

asymptomatic infection during the rotavirus epidemic was the interpretation of stool ELISA results. During this study I limited the analysis of stool specimens to the detection of rotavirus. There was, however, always the danger that other organisms were causing diarrhoea and rotavirus was present in the stool as an asymptomatic passenger. The frequency with which this happened would obviously depend on which other pathogens were circulating in the community, but it should be stressed that the simple detection of rotavirus in the stool did not prove that it is causing diarrhoea. In this respect the results of the samples sent for E.M. are helpful. No firm conclusions can be drawn from such a small set of results but there was no evidence that other viruses were circulating in significant numbers during the rotavirus epidemic and the studies carried out during 1981 - 82 in Bakau did not show a significant number of mixed rotavirus/bacterial infections. When this limited evidence is added to the fact that rotavirus associated attacks were clinically distinct it is likely that, in the majority of cases, clinical episodes of diarrhoea associated with rotavirus were caused by this agent.

Data have been collected on the predominant R.N.A. electropherotypes responsible for rotavirus outbreaks in four successive years in Bakau. Short patterns were observed in 1982/83 while in the two successive years long patterns predominated. In 1985/86 the patterns were predominantly short. Only 5 samples are available from the 1985 wet season of which 4 show a short R.N.A. electropherotype similar to

the pattern found in the rotaviruses which predominated in the following epidemic. Although the number of samples in some years is small, it does seem that in any single epidemic one strain of the virus predominates. In general, multiple electropherotypes exist simultaneously in most closely populated communities but, usually, one or two electropherotypes are dominant in any single outbreak. Sometimes the dominant electropherotype is detected in small numbers for several months before it becomes dominant. Observations will have to be made in subsequent epidemics to determine whether the cycle of changing strains which seemed to be emerging during 1982 - 86 continues as variations of this type might play an important role in determining the response to different rotavirus vaccines.

Episodes of rotavirus associated diarrhoea were more severe than those due to other causes. I was, however, struck by the fact that the vast majority of diarrhoea episodes due to all causes were very mild. A child typically presented with 3 - 6 loose stools per day and no other symptoms or signs. Dehydration was uncommon but, when it did occur, the majority of cases with significant dehydration were found to be associated with rotavirus. Oral rehydration with home made sugar salt solution has been promoted energetically in Bakau for several years and each child had early access to our treatment facilities, therefore, dehydration as the only index of severity could have been misleading. To overcome this difficulty we devised a scoring system with separate scores for frequency of diarrhoea and vomiting, degree of dehydration and level of pyrexia (Table VI). Children with

rotavirus infections who visited the clinic had significantly higher severity scores than all other clinic cases presenting with diarrhoea with allowance made for age at presentation.

The relative severity of rotavirus infection is reflected in the fact that the clinical cases showed evidence of acute associated weight loss which seemed to persist for some weeks after the attack. This potentially important phenomenon will require further investigation.

The results reported in this chapter confirm the importance of rotavirus as an aetioligical agent of diarrhoea in The Gambia. Its importance lies in its severity. The formal data reported above was corroborated by the fact that all 14 children who required admission for rehydration to the small M.R.C. children's ward during January of 1986 had rotavirs detected in their stools. The potential benefits of vaccination should not, however, be overestimated. If the clinical severity of rotavirus infection could be prevented by vaccination deaths from acute dehydration would be reduced and this, together with the prevention of weight loss, would be significant. However, the impact on diarrhoeal disease as a whole is likely to be modest.

CHAPTER V

SOCIAL AND ENVIRONMENTAL RISK FACTORS FOR ROTAVIRUS DIARRHOEA

INTRODUCTION.

Throughout the developing world, health planners are increasingly coming to realise that effective strategies for the control of disease require co-operation among a variety of agencies and Government departments. As a consequence, the concept of integrated development has increasingly become the orthodoxy of the day. The integrated development approach seeks to attack problems from several standpoints, so that the combined skills of agriculturalists, nutritionists, water engineers and doctors might be brought to bear on, for example, a project designed to increase the nutritional level and food production of a community with a high level of childhood marasmus and kwashiorkor. This approach has a strong intellectual appeal but in practice it has had notable failures as well as successes. Integral to this approach has been the need to be sensitive to the wishes of those people the project was designed to help. Increasing emphasis has been placed on low cost and low technology solutions which will be sustained by the community once the initial project has come to an end.

These ideas are relevant to any strategy for the control of rotavirus in The Gambia. Improved sanitation, housing and water supply might make as much of an impact on the disease as an effective vaccine, and would have many other

beneficial effects. Clearly, such measures are not in competition with vaccination but any integrated approach to disease prevention should employ all effective means. Unfortunately, The Gambia is a poor country with many problems and limited funds must be spent in the most cost - effective way. For that reason we decided to investigate particular social and environmental factors within Bakau which might be associated with a high risk of rotavirus infection. If such factors could be discovered, it might then be possible to intervene in specific and less expensive ways to decrease the prevalence of rotavirus disease.

In Europe and North America the reduction of infant and child mortality which took place throughout much of last century was closely associated with major improvements in living conditions. These included the provision of piped water, safe sewage disposal, rising standards of housing, improved nutrition and universal education. Specific medical interventions seem to have played a small part, and largely arrived on the scene long after the downward trend in mortality figures had been established (144). Such comprehensive improvements are, however, a long term goal in most developing countries. In an attempt to achieve more immediate results Governments and aid agencies have often tried to focus on single interventions.

Although the provision of safe drinking water and adequate sanitation has been stressed in many programmes aimed at reducing diarrhoeal diseases , the results have often been

disappointing. Some studies have shown that if mothers are given specific health related instruction, such as the importance of hand washing (145) or information in the hygienic use of latrines (146), then health benefits do accrue. Other studies have highlighted the value of education in general (147,148) which seems to have a less direct but more profound effect on disease. It is, however, difficult in such studies to avoid the difficulties of confounding variables, as those with better education often also have higher incomes, smaller families and better homes.

The fact that the incidence rates and attack rates for rotavirus have been broadly similar, irrespective of the country in which these statistics have been gathered, suggests that environmental factors may be less important in determining the prevalence of rotavirus disease than they are in diarrhoea caused by other common enteric pathogens.

Despite these potential difficulties, I considered it a worthwhile exercise to investigate the social and environmental background of 92 cases and an equal number of age, and vaccine status matched controls. The variables I tested for are listed in Appendix I.

RESULTS.

The Child's Compound - Table XII.

In Bakau, compounds vary considerably in size and housing

Table XII

Variables concerning the compounds and dwellings occupied by 92 cases of rotavirus diarrhoea and 92 controls.

Compound

	CASES	CONTROLS
No. of buildings in compound (mean)	3.7	4.6
No. of people in compound (mean)	38	45
No. of children less than 3 years(mean)	5.6	6.8
No. of children less than 2 years(mean)	3.2	3.2
No. of European style houses	7	16
No. of Cement/corrugate house	82	113
No. of Mud/corrugate houses	242	281
No. of Mud/thatch houses	12	12

Dwelling

	CASE	CONTROL
No. sharing house (mean)	6.0	7.2
No. eating out of common bowl (mean)	6.6	7.2
No. under three years (mean)	1.6	1.7
No. under two years (mean)	0.9	0.9
No. sharing bedroom (mean)	3.8	2.8
No. sharing bed (mean)	2.8	2.8
No. living in European style house	3	6
No. living in Cement/corrugate house	28	33
No. living in Mud/corrugate house	57	50
No. living in Mud/thatch house	4	3

type. In the older part of the town, compounds are large with nearly every resident related to one-another in some way. In the newer parts of the town compounds are ethnically more mixed and tend to be smaller. We were, however, unable to demonstrate any difference in the average number of people living in the compound, the average number of buildings, or the type of buildings. The most popular type of dwelling was a simple house with one or two rooms, constructed from mud bricks, with a corrugate iron roof. Poorer forms of housing (mud walls, with thatch roofing) were uncommon but occurred in approximately equal numbers in the compounds of cases and controls.

A crude crowding index, calculated by dividing the number of compound occupants by the number of houses, showed no significant difference. As rotavirus is a disease of young children, we counted the number of children under the age of two and three years respectively living in the compound but no significant difference was detected between the groups.

The Child's Dwelling - Table XII.

It might be argued that the particular dwelling occupied by the cases or controls was more relevant to the risk of disease transmission than the whole compound but we were unable to detect any significant difference in housing type between cases and controls. The total number of people sharing the dwelling, the number of people who fed from the same bowl of communal food and the number of people sharing the child's bed were not significantly different. The number

of children less than three years old sharing the house, sharing the bedroom and sharing the bed, was also very similar in both groups.

Water Supply - Table XIII.

Bakau's chlorinated water supply runs to approximately 40 communal standpipes. Mothers and older children collect water in large plastic, clay or metal containers which are then carried to the house and used as a communal source of water for the occupants of each house. The usual procedure is to have separate containers for washing and drinking water but this is not universal.

We were unable to detect any difference in the average distance cases and controls lived from the nearest standpipe. There was no significant difference in the type of containers used, the frequency with which they were refilled, the frequency with which they were cleaned or the number of people who used water from them.

Sanitation - Table XIV.

Sanitation in Bakau was provided principally by pit latrines but a few compounds used pails while some houses had flush toilets. Approximately 10% of compounds were without any sanitation facilities. Very small children were, however, frequently allowed to defaecate on the ground the faeces then being covered with a little soil. More hygiene-conscious mothers provided their children with a small pot.

Table XIII

Water supply and storage for 92 cases and 92 controls.

	CASES	CONTROLS
Mean distance to standpipe (metres)	76	84
No. storing in clay jars	75	68
No. storing in metal pots	25	33
No. storing in plastic buckets	49	57
No. storing in glass bottles	17	20
No. of people using water (mean)	6.0	6.6
No. of children using water (mean)	1.6	1.3
No. of times/day water collected (mean)	1.7	1.9
No.of times/day containers cleaned(mean)	1.6	1.7

Table XIV

Sanitation facilities and behaviour for 92 cases and 92 controls.

	CASES	CONTROLS
No of children using pot	61	64
No. of children using the ground	18	15
No. of children using a nappy	10	12
No. of compounds with a latrine	83	78

We were not able to detect any difference in the sanitation facilities or toilet habits between the compounds of cases or controls. Defaecation habits and means of disposal were not significantly different for cases or controls.

Hygiene, Cleanliness and Animal Contact - Table XV.

It seemed likely that the mother's personal hygiene might influence disease transmission but there was no significant difference in handwashing activity, childcare activities or general cleanliness. We asked, in particular, whether a study child was allowed to play in the soil outside the compound, which seemed a potentially hazardous activity because of the danger of contact with faecal material. There was no difference in the proportion of children allowed to do this.

Mothers were advised to prepare fresh weaning food for the child before each meal, but working mothers would often prepare food in the morning which was left standing all day. There was no difference in the frequency of this behaviour between the two groups.

Animals frequently live within the compounds and sometimes within the homes. Little is known about the frequency with which animal rotaviruses infect humans but animal contact is a potential risk factor. There was no significance difference between the groups in the number or range of animals with which cases and controls had effective contact

Table XV

Hygiene and cleanliness in 92 cases and 92 controls.

	CASES	CONTROLS
No. of times swept daily	74	82
Regular hand washing by mothers	92	92
Prepares fresh food for each meal for the child	39	32
Prepares fresh food daily	46	53
Prepares fresh food for child less than daily	7	7
Mean cleanliness/tidyness score allocated by field assistant on a 0 - 5 scale	3.3	3.4
Child allowed to sit on the ground	44	45
Child eats sand	33	33
Same broom used for inside and outside the house	33	29
No. in contact with cows	0	1
No. in contact with goats	18	26
No. in contact with sheep	50	61
No. in contact with pigs	0	2
No. in contact with dogs	48	31
No. in contact with cats	86	82
No. in contact with chickens	90	90

except for dogs which were more commonly found in the compounds of cases. This difference just reached statistical significance ($X^2 = 5.7$, 1 d.f. $p < 0.02$). This finding is difficult to explain and is discussed later. Cows and pigs are the most likely source of animal rotaviruses but both these animals were uncommon in Bakau as the population is Muslim and does not keep pigs while cows are largely confined to rural areas.

Family Background - Table XVI.

Although there was considerable diversity in family structure and ethnic background within Bakau, these factors were similar for cases and controls. Ethnicity, marital status, and employment status of both parents were similar in each group. These results are not surprising because, although ethnic stereotypes did exist, there was very little ethnic segregation. The vast majority of compounds were ethnically mixed and over one third of the marriages were between different racial groups.

No significant differences were detected in family structure. The mother's position in the order of wives, the number of children she had borne, the number of live children she had and the number of deaths and stillbirths were all similar for cases and controls.

Parental Education and Attitude to Disease - Table XVII

As discussed earlier in this chapter, parental education often correlates with various measures of childhood health

Table XVI

Family background of 92 cases and 92 controls.

	CASES	CONTROLS
No. of Mandinka mothers	35	42
No. of Wollof mothers	11	8
No. of Jolla mothers	23	14
Other tribal groups	23	14
No. of Mandinka fathers	39	41
No. of Wollof fathers	8	11
No. of Jolla fathers	12	8
Other tribal groups	13	22
No. of divorced mothers	0	1
No. of unmarried mothers	10	9
No. of married mothers	82	82
No. of fathers living in compound	68	70
Mothers in paid employment	17	27
Mean number of sibs	2.9	2.9
No. of stillbirths/abortions(mean)	0.3	0.4
No. of deaths in sibs (mean)	0.5	0.5

status. In Bakau, however, we found that the the number of years of formal education and the level of schooling reached were similar for both the mothers and fathers of cases and controls. We also enquired about their belief with respect to the cause of disease. Answers were grouped according to whether they represented a more superstitious or more scientific view of disease. There was no difference in the replies given by the mothers of cases and controls.

Parental Income and Wealth - Table XVIII.

It was not easy to estimate income or wealth in Bakau. The father's occupation was a poor indicator of true wealth, while other possible indicators, like housing type, ownership of consumer items, were equally unreliable. Having a white collar job and a refrigerator were probably more indicative of an identification with Western values, than a true index of wealth itself. Despite these difficulties, we had to make some measure of wealth and so we relied on the reported combined monthly income, the amount spent per day on food and the ownership of consumer items. No significant differences were detected between the two groups.

Feeding and Nutrition.

Almost all children in Bakau are exclusively breast-fed for the first 3-6 months of life. A weaning food called "pap" is then introduced into the diet. This is made from pounded grain or rice which is boiled with a little sugar and salt.

Table XVII

Parental education and attitude to disease in 92 cases and 92 controls.

	CASES	CONTROLS
Education.		
Years of maternal education(mean)	2.1	1.8
No. of mothers finishing primary	18	17
No. of mothers finishing secondary	11	13
Years of paternal education (mean)	4.4	3.7
No. of fathers finishing primary	11	3
No. of fathers finishing secondary	30	33
Attitude to disease.		
No. of mothers with superstitious understanding of disease	8	4

Table XVIII

Parental income and wealth for 92 cases and 92 controls.

	CASES	CONTROLS
Mean amount (in Dalasis) spent on food per person per day	1.6	2.2
Estimated cash income per month (mean)	1100D	785D
No. with paid maid	27	17
No. with cash salary	55	68

till at the age of 9 - 12 months adult foods are shared with the child. Breast feeding usually continues well into the second year of life. Although this is the general pattern there is a wide variation. We were not, however, able to detect any difference in feeding practices of cases and controls.

DISCUSSION AND CONCLUSIONS.

This study has shown that the risk of clinical rotavirus infection is not strongly associated with social and environmental factors. Methodological problems certainly existed. Factors like wealth, hygiene, attitudes and cleanliness were difficult to define and measure but, despite these problems, it is disconcerting that none of the measured factors correlated with the risk of clinical rotavirus infection.

There have been very few studies published which have looked for rotavirus infection risk factors. The only one of any consequence (149) found that rotavirus infection on a North American Indian reservation was associated with the presence of a domestic dog in the household. We also found an association between dogs and rotavirus cases. This association is not easy to explain as dogs are not known to suffer from clinical rotavirus infection but one might speculate that they may play a part in transmission of the virus as they often lie in faecally contaminated soil and have close contact with children. It is also possible that the association between dogs and rotavirus cases has arisen

by chance. This is always a possibility when so many variables are being investigated. The study carried out on the Indian reservation also associated rotavirus infection with a household contacts under the age of two years. It was argued that children less than two years old, often being in nappies, would also be at high risk of symptomatic and asymptomatic rotavirus infection and would, therefore, be potential sources of infection. Almost all the children in our survey had household contacts aged less than two years, and the mean number of contacts was approximately 3 in both groups. The risk of exposure to other sources of faecal contamination was also very high given the sanitation facilities available in Bakau. It might, therefore, be argued that contact with other very young children and exposure to faecally contaminated substances were ubiquitous risk factors for all Bakau children.

Rotavirus has been detected in the handwashings of attendants of children with diarrhoea (150), so one might have expected standards of hygiene and water storage practices to be related to the risk of rotavirus associated diarrhoea. We were, however, unable to demonstrate any such association.

It must, however, be emphasised that, in this community, symptomatic and asymptomatic rotavirus infections are both very common during the epidemic season. As stated previously, the attack rate in Bakau infants for clinical rotavirus infection was 36% and there is evidence to show

that asymtomatic infection is at least two thirds as common as symptomatic infection. Further evidence pointing to the high incidence of rotavirus infection in young Gambian children comes from unpublished serum surveys which have shown that by the age of two years most children have antibodies to rotavirus. It is, therefore, possible that many of the control children had suffered asymptomatic rotavirus infections during the epidemic.

My hypothesis is that, during the rotavirus epidemic period, almost all children within the at risk age group in Bakau came into contact with the wild virus. Some children developed rotavirus associated diarrhoea while others became asymptomatic carriers. Host factors, like immune status, will have been far more important than environmental factors in determining the child's response to rotavirus. Environmental factors will probably have influenced the frequency of exposure and the infecting dose of virus but, even if this did occur, the effect was not large enough to be detected by my methodology.

Improvements in housing, sanitation etc. are clearly important for the people of Bakau, but it seems that if they come, such improvements are unlikely to have a major impact on the burden of rotavirus disease.

CHAPTER VI

A TRIAL OF ROTAVIRUS VACCINE RIT 4237

INTRODUCTION.

As was discussed in Chapters I and III, we conducted a randomised, double blind, placebo controlled trial of the attenuated bovine rotavirus vaccine RIT 4237. This part of the study had two main aims. Firstly, to measure vaccine efficacy and secondly to demonstrate any mutual interference between the oral rotavirus vaccine and oral polio vaccine when both were administered together.

RESULTS.

Compliance.

During 1985, 433 children were recruited into the study and randomly allocated to one of three groups. 80 of these children left the study area before follow-up was completed and so have been excluded from the analysis of vaccine efficacy. 100 children, born in the latter part of the year, have been excluded because they received their first vaccine within one month of the onset of the epidemic. The remaining 253 children had received at least one vaccine dose, one month or more before the epidemic, and were under surveillance for the full duration of the trial.

Table XIX

Vaccination Compliance.

	1st call	within 1 month
1st vac	81%	98%
2nd vac	81%	100%
3rd vac	76%	99%
Follow-up bleed	74%	99%

Vaccination compliance was such that almost every child received each vaccination within one month of the expected date (table XIX) and the majority (80%) on the expected date. Of the reported cases of diarrhoea, 86% had stools collected and 88% were examined by a clinician and clinical records made.

Vaccine Side Effects.

No major vaccine side effects were detected among 248 infants given one or more doses of rotavirus vaccine. Morbidity data was collected for a seven day period after 164 separate vaccinations on 164 children, comprising 51, 51 and 52 children respectively from each vaccination group A, B and C. There were no significant differences in mean temperature or reported symptoms between the groups.

Post-Vaccination Rotavirus Excretion.

There was no apparent post-vaccination excretion of the vaccine virus. 276 stools collected from 104 rotavirus vaccine recipients during the seven days following vaccination were all negative for rotavirus when tested by E.L.I.S.A.

Rotavirus Neutralising Antibody Levels.

Mean neutralising antibody titres to rotavirus were high in all groups at the time of first vaccination (table XX). None of the children were seronegative. In the controls

Table XX

Rotavirus neutralising antibody titre before and 1 month after the administration of 3 doses of rotavirus vaccine or placebo.

		Pre-vaccination		Post-vaccination	
Group	N	Geometric Mean Titre	95% CI	Geometric Mean Titre	95% CI
A (rotavirus and oral polio)	86	138	111-171	162	127-206
B (placebo and oral polio)	91	155	126-190	87	73-103
C (rotavirus and killed polio)	99	162	135-194	162	130-202

(group B) the mean antibody level fell significantly (paired $t = 3.96$, $p < 0.001$) between pre- and post-vaccination samples. In the vaccinated groups, A and C, post-vaccination titres were significantly higher than those of the control group ($t = 4.13$, $p < 0.001$ in both cases). The proportion of children who showed a rise in titre of one dilution or more was significantly higher in the vaccinated group (84/185 (45%)) than in the control group (20/91 (22%)) ($X^2 = 13.2$, 1 d.f., $p < 0.001$) (Table XXI). Only 48/185 (26%) of vaccinated children boosted their rotavirus antibody titres by two or more dilutions. The proportion of children showing a rise in titre was higher among those with low pre-vaccination titre (table XXII) but a similar trend was observed in controls, suggesting that this may have been attributable to regression to the mean. When the mean change in log titre was analysed, the effect of vaccination, as assessed by the difference between vaccinees and controls, was unrelated to pre-vaccination titre.

There was no evidence that the response to rotavirus vaccination was compromised by the simultaneous administration of oral polio vaccine, for geometric mean post-vaccination rotavirus antibody levels were identical in groups A and C (Table XX).

Vaccine Efficacy.

The distribution within groups of clinical cases of rotavirus associated diarrhoea, all of which occurred during a well demarcated epidemic which began in late December 1985

and finished in February 1986 (Chapter IV), is shown in Table XXIII. The proportion of infants who became cases in the vaccinated groups (A + C) was significantly lower than in the control group (X^2 = 3.95, 1 d.f., $p < 0.05$) and the vaccine efficacy was estimated as 33% (95% C.I. 4% to 53%). The results were not materially altered when the analysis was restricted to children who had received all three doses of vaccine one month or more prior to the onset of the epidemic.

Vaccination and Severity.

The rotavirus vaccine had no effect on the severity of attacks (table XXIV). Following W.H.O. recommendations a case was considered severe if the duration of diarrhoea was greater than 24 hours and if it was associated with one or more of the following: passage of six or more stools in 24 hours, vomiting, rectal temperature > 38 ^{o}C or clinical dehydration. By this definition almost all cases which we examined were severe. Cases which were not brought to the clinic were probably less severe but, since we have no firm data on these cases, they have been excluded from analysis. The proportions of severe cases in the vaccinated (40/43) and the control groups (21/26) were not significantly different (X^2 = 1.3, n.s.). Table XXIV shows a comparison of mean severity scores (using the scoring system described in Chapter IV) between the groups. There was no significant difference in mean score between groups.

Table XXI

Changes in titre of rotavirus neutralising antibody one month after the administration of 3 doses of rotavirus vaccine.

GROUP	N	INCREASE [1]	STATIC [2]	DECREASE [3]
A (rotavirus and oral polio)	86	38 (44%)	5 (6%)	43 (50%)
B (placebo and oral polio)	91	20 (22%)	10 (11%)	61 (67%)
C (rotavirus and killed polio)	99	46 (46%)	9 (9%)	44 (45%)
A + C (combined rotavirus vaccine groups)	185	84 (45%)	14 (8%)	87 (47%)

NOTES

1. Post-vaccination titre one or more dilutions higher than pre-vaccination.
2. Post-vaccination titre same dilution as pre-vaccination.
3. Post-vaccination titre one or more dilutions lower than pre-vaccination.

Table XXII

Relationship between pre-vaccination rotavirus neutralising antibody level and subsequent serological response to vaccination.

Pre-vaccination titre	Number showing increase in titre		Mean change in titre[1]		
	Vaccinees	Controls	Vaccinees	Controls	Diff
<80	35/41(85%)	9/17(53%)	+0.603	+0.310	0.293
80 or 112	23/38(61%)	9/28(32%)	+0.233	-0.097	0.330
160 or 224	18/53(34%)	2/18(11%)	-0.082	-0.302	0.220
> 224	8/53(15%)	0/28(0%)	-0.456	-0.724	0.268
Total	84/185(45%)	20/91(22%)	+0.027	-0.254	0.281

Notes.

1. Titres analysed as $\log_{10}$ (titre).

Table XXIII

Clinical cases of rotavirus associated diarrhoea and their severity in recipients of rotavirus vaccine and in controls.

GROUP	N	CASES	MILD	SEVERE	NOT EXAMINED[1]
A (rotavirus and oral polio)	78	24 (31%)	2	20	2
B (placebo and oral polio)	83	34 (41%)	5	21	8
C (rotavirus and killed polio)	92	23 (25%)	1	20	2
A + C (combined rotavirus vaccine groups)	170	47 (28%)	3	40	4

NOTES

1. These children had a laboratory diagnosis of rotavirus but were not examined by a clinician.

Table XXIV

Comparison of severity scores in cases of rotavirus associated diarrhoea by vaccination group.

GROUP	NUMBER	MEAN	S.D.
A (rotavirus and oral polio)	22	5.77	1.57
B (placebo and oral polio)	26	5.11	1.34
C (rotavirus and killed polio)	21	5.71	1.31

Post-vaccination Antibody and Clinical Protection.

There was no convincing evidence that clinical protection could be correlated with post-vaccination rotavirus antibody levels. Analysis was confined to 115 vaccinees and 55 controls who had their post-vaccination samples taken at least 4 weeks before the onset of the epidemic. There was no significant difference between the mean antibody level of those who subsequently became cases and those who did not (t = 0.79. n.s.) and the proportion of children showing an increased antibody titre in response to vaccination was similar in both groups (X^2 = 0.52, 1 d.f., n.s.) (Table XXV). There was no significant trend towards lower attack rates in those with higher post-vaccination titres (Table XXVI).

Polio Neutralising Antibody Levels before and after Vaccination.

Polio neutralising antibody levels were measured after administration of 1 and 3 doses in children who received oral polio vaccine and rotavirus vaccine (group A), or placebo (group B). No difference was observed in the mean antibody response, or the number of vaccine failures (defined as a titre of less than 1:10) after 1 dose of vaccine. Mean antibody levels for polio types 1 and 3 were lower, and the number of vaccine failures higher in infants who received polio vaccine with three doses of rotavirus vaccine than in those who had received placebo (table XXVII) but these differences did not reach statistical significance.

Table XXV

Mean titres and changes in titre of rotavirus neutralising antibody in response to 3 doses of rotavirus vaccine in children who subsequently became cases and those who did not.

Group	N	Mean post-vaccination titre	95% C.I.	Increase[1]	Static[2]	Decrease[3]
Cases[4]	40	139	100 - 194	14	0	26
Non-cases	75	166	128 - 215	33	8	34

Notes

1. Post-vaccination titre one or more dilutions higher than pre-vaccination.
2. Post-vaccination titre same dilution as pre-vaccination.
3. Post-vaccination titre one or more dilutions lower than pre-vaccination.
4. Cases and non-cases constitute 115 children from groups A+C with post-vaccination samples taken one month prior to the onset of the epidemic.

Table XXVI

Rate of clinical rotavirus cases by post-vaccination rotavirus neutralising titres in 115 vaccinees and 55 controls who had post-vaccination blood samples taken at least one month prior to the onset of the epidemic.

Attack Rates

Titre	Vaccinees	Controls
< 80	8/24 (33%)	10/27 (37%)
80 or 112	10/29 (34%)	7/12 (58%)
160 or 224	11/29 (38%)	6/12 (50%)
> 224	11/33 (33%)	1/4 (25%)

Table XXVII

Polio neutralising antibody: geometric mean titres and and vaccine failures in samples taken one month after three vaccinations with either rotavirus vaccine and oral polio vaccine of placebo and oral polio vaccine.

Group	Number of Vaccinees	Number of Vaccine Failures1	Geometric Mean Titre	95% CI
POLIO 1				
rotavirus/polio	87	19 (22%)	281	172-461
placebo/polio	95	16 (17%)	398	256-618
POLIO 2				
rotavirus/polio	87	5 (6%)	977	693-1377
placebo/polio	95	5 (5%)	977	713-1339
POLIO 3				
rotavirus/polio	87	42 (48%)	69	44-108
placebo/polio	95	38 (40%)	120	75-192

Notes.

1. Vaccine failures defined as titres < 1:10.

Serological Comparisons of Oral and Intramuscular Polio Vaccines.

Table XXVIII shows a comparison of serological response to oral polio vaccine (groups A and B combined) and the I.M. polio vaccine (group C). The polio 3 antibody response to three doses of I.M. polio vaccine was significantly higher than that to three doses of oral vaccine ($t = 3.3$, $p < 0.001$), and the number of titres less than 1:10 (considered to be vaccine failures) was significantly lower ($X^2 = 38.6$, 1 d.f., $p < 0.001$). There was no significant difference in polio 1 antibody titres but a significantly higher number of children vaccinated orally failed to respond ($X^2 = 5.1$, 1 d.f., $p < 0.05$). In the case of polio 2, children vaccinated orally had higher antibody levels ($t = 7.25$, $p < 0.001$) with a significantly lower failure rate than children given I.M. polio ($X^2 = 6.3$, 1 d.f., $p < 0.02$).

The relative value of a booster dose at the age of approximately one year, of either vaccine in both groups, was then analysed. For polio types 1, 2 and 3 the boost achieved by the I.M. vaccine was better than the oral vaccine boost, irrespective of the initial vaccine given, although not at a statistically significant level for polio 2.

R.N.A. Electropherotype of Rotaviruses.

As was reported in Chapter IV, rotaviruses from the 73 cases with an adequate volumes of stool sample had their R.N.A. electropherotype determined. The majority of cases (67/73

Table XXVIII

The antibody response to three doses of oral or I.M. polio vaccine

in 280 Gambian children and their responses to a single booster dose of oral or I.M. vaccine.

INITIAL THREE DOSES					BOOSTING DOSE					
group	n	geometric mean titre	95% CI	failures *	boost	n	geometric mean titre	95% CI	failures *	oral/I.M. comparison
POLIO 1										
oral	182	339	244- 470	35(19%)	oral	52	661	351-1243	10(19%)	t = 2.1, p < 0.05
					IM	58	1585	930-2702	7(12%)	
IM	98	251	184- 342	8(8%)	oral	29	468	220- 994	4(14%)	t = 2.7, p < 0.01
					IM	33	1514	983-2332	0(0%)	
POLIO 2										
oral	182	977	776-1231	10(5%)	oral	52	1778	1253-2525	1(2%)	t = 1.8, n.s.
					IM	58	2692	2013-3598	1(2%)	
IM	98	209	144- 304	15(15%)	oral	29	912	391-2126	3(10%)	t = 0.6, n.s.
					IM	33	1230	811-1866	0(0%)	
POLIO 3										
oral	182	93	69- 126	80(44%)	oral	52	102	56- 188	23(44%)	t = 1.9, p ≃ 0.05
					IM	58	245	129- 466	18(31%)	
IM	98	234	173- 318	7(7%)	oral	29	263	110- 629	6(21%)	t = 2.5, p < 0.02
					IM	33	891	543-1462	1(3%)	

* Post-vaccination antibody titre < 1:10.

(92%)) were caused by rotaviruses with short R.N.A. patterns, made up from three distinct electropherotypes (numbering 17, 23 and 27 respectively). Samples from older children collected in Bakau, the Medical Research Council clinic, and a neighbouring community during the epidemic confirm that these short pattern R.N.A. electropherotypes were the dominant rotaviruses during the 1985/86 epidemic. Subgrouping and serotyping of these viruses was attempted but proved impossible for technical reasons.

DISCUSSION.

The major finding in this study was that administration of the bovine rotavirus vaccine, RIT 4237 to Gambian infants gave only limited protection against clinically significant rotavirus associated diarrhoea, a finding similar to that recently reported from Rwanda (151). The vaccine efficacy was estimated to be 33% and I am reasonably confident that this figure gives an accurate measure of the performance of RIT 4237 in The Gambia. My confidence stems from the fact that we were able to detect diarrhoea and collect stools from the study children in nearly 90% of all diarrhoeal episodes so it is unlikely that many cases of rotavirus associated diarrhoea escaped our attention. The lack of information relating to other viruses or bacteria which might have been causing diarrhoea was a weakness in this study but, as I argued earlier in the text, clinical cases of rotavirus associated diarrhoea were distinctive both in the way they presented and the severity of symptoms reported

and such data as I was able to collect gave no indication that mixed infections were common. For these reasons it seems reasonable to propose that the majority of the rotavirus associated cases of diarrhoea were caused by that agent. A further safeguard was the comparability of vaccine and control groups such that diarrhoea associated with rotavirus, as distinct form diarrhoea caused by rotavirus, should have been equally represented in each group. It is also perhaps of interest that during a recent trial of a rhesus rotavirus vaccine candidate, carried out in Venezuela, laboratory investigation of the stool samples was similarly confined to the detection of rotavirus antigen by ELISA (152).

The low efficacy of the RIT 4237 vaccine in The Gambia was, however, a disappointment. Widespread use of such a vaccine would not be cost effective and, with the development of other rotavirus vaccine candidates, it would seem prudent to wait for a more effective vaccine to become available. With the apparent success of the RIT 4237 vaccine in Finland there are important questions to answer in relation to this study. These questions are, however, difficult to answer because our study was not designed to look at these complex issues but was kept deliberately simple so that an unequivocal answer could be found to the question of vaccine efficacy. As a consequence, much of what follows might best be described as speculative.

The Finnish studies (111, 127) used one and two doses of RIT 4237 respectively but the results were essentially similar.

Children aged 6 - 12 months were vaccinated. The majority were seronegative at the time of vaccination and 50% seroconversion rates were achieved. The vaccine gave poor protection against rotavirus infection, but greater than 80% protection was achieved against clinically significant rotavirus episodes. The majority of cases were caused by a subgroup II serotype 1 rotavirus, but the second study (127) also gave some evidence of protection against infection by a subgroup I serotype 2 virus. The vaccine itself is of subgroup I, and has a non human serotype (124).

There are a number of possible explanations for the difference in efficacy of RIT 4237 in Finland and The Gambia. Firstly, there may have been important differences between the vaccinated children in the two countries, such as their pre-vaccination immune status and breast feeding practices. Secondly, there may be important differences in rotavirus epidemiology and disease severity between the two environments. Thirdly, the strain of wild virus causing the outbreaks may have been different. Finally, the microbiological backgrounds against which the two studies were conducted were almost certainly very different.

In The Gambia, rotavirus has its maximum clinical impact on infants so vaccination was carried out early and the children were younger than the vaccine recipients in Finland. In Finland the majority of children were seronegative when they came for vaccination. In The Gambia all pre-vaccination blood samples showed detectable levels

of neutralising antibody. High levels of antibody in very young children have been reported in previous studies and the assumption is that they are of maternal origin (153,154). It is well known that with some other vaccines (e.g. measles) the presence of maternal antibody can have a deleterious effect on vaccine efficacy (155) so it is possible that the high levels of pre-vaccination antibody to rotavirus, in the Gambian children, contributed to the relatively poor performance of RIT 4237.

Maternal antibody is not the only potential factor which may have inhibited antibody response. At present we can only speculate on the possible effect breast feeding in The Gambia may have had on rotavirus vaccination. It has been shown that RIT 4237 may lose infectivity if exposed to gastric acidity (156). When an antacid substance like milk is given with the vaccine the serologic response rate is increased. In Finland breast and formula milk have been shown to be almost equally suitable (128) but it is possible that Gambian breast milk contains substances harmful to the vaccine virus. Such factors may be IgA or other non-specific inhibitors of virus growth. Breast milk has been shown to contain antibody to rotavirus (157) and non-antibody factors (92) which can neutralise rotavirus. Not surprisingly, therefore, breast feeding has been shown to modify the severity of rotavirus infection but the level of protection provided is not sufficient to reduce the risk of infection (158,159). During the planning stage of the rotavirus vaccine trial, I was asked by the manufacturers of the RIT vaccine to give artificial formula milk to the children at

the time of vaccination. I refused to do this because of the very considerable danger that such a policy might help to promote artificial feeding in a community which has traditionally relied almost exclusively on breast feeding. Mothers in Bakau feed their children on demand and usually gave them breast milk immediately after a vaccination to provide comfort. I decided to allow the mothers to continue this practice as it would have been pointless to try to modify this very well established behaviour pattern. Breast milk samples were, however, collected from all the mothers prior to the first vaccination. It would be interesting to measure the rotavirus neutralising capability of these breast milk samples and relate these results to the vaccination response in individual children and also to compare Gambian breast milk with samples from nursing mothers in Finland.

It is difficult to make a confident statement about the antibody response to RIT 4237 in our trial. Declining maternal antibody levels made interpretation of our serological data more difficult than it would have been if children had been seronegative at the time of vaccination. In the presence of a background of declining maternal antibody, it is reasonable to consider any increase in titre to be a vaccine response, a decrease a non-response, and an unchanged titre intermediate. Within the two vaccinated groups the rotavirus neutralising antibody response of individuals showed considerable variation, but following vaccination the mean antibody levels in vaccinees were

significantly higher than in the controls. A higher proportion of vaccinees showed an increase in neutralising rotavirus antibody titre following three vaccinations than did controls but less than half the children in our study showed any evidence at all of producing serum neutralising antibody in response to vaccination and only a quarter increased their titres by two dilutions or more. It is, therefore, difficult to compare the vaccine response in The Gambia with previous trials although it is perhaps not unreasonable to argue that vaccine take was poor.

Another indication that vaccine take was poor was our inability to detect any evidence of post-vaccination rotavirus excretion. Stools were collected daily from a subgroup of children for a week after vaccination. In addition, many children had stools examined in the post-vaccination period because they developed a coincidental episode of diarrhoea (diarrhoea was not more commonly detected in the post-vaccination period). None of these samples had rotavirus present in sufficient quantities to be detected by ELISA. Nor was there any evidence that the vaccine virus was circulating in the community as none the viruses which we were able to electropherotype showed the bovine vaccine pattern of migration.

Since the rotavirus vaccine was given orally some of the vaccine virus would, undoubtedly, have made its way out in the stool. Our failure to detect vaccine virus in the stool probably means that replication of the virus in the gut was such that concentrations of the vaccine virus remained

fairly low. A similar finding has been reported with the oral polio vaccine in the tropics in as much as even when the vaccine does take only small amounts of virus are excreted in the stool. In the case of oral polio the low concentrations of virus in the stool are associated with a poor immunological response to the vaccine so it might be reasonable to postulate the same for rotavirus. It should, however, be pointed out that there was no apparent post-vaccination excretion of the RIT 4237 vaccine virus during the Finnish studies either so it is not possible to argue that the apparent absence of vaccine virus in the Gambian stools provides evidence of poorer vaccine take, at least compared with the Finnish studies. However, it is likely that there was poor replication of the vaccine virus in the gut as reflected in the poor serological responses observed in our study.

In our study the absolute level of post-vaccination neutralising rotavirus antibody did not correlate with clinical protection. This suggests that the neutralising antibody measured in our study was not in itself protective. It is not at present clear which part of the immune response is the most protective but most investigators seem to be agreed that local gut immunity appears to have the greatest protective role (88,89,90). Consequently serum antibody is not always a reliable indicator of protection. Recent studies have, however, shown the value of measuring neutralising antibody against each of the four main serotypes known to infect man. Neutralising

antibody measured in this way does seem to correlate with protection against subsequent infection with a rotavirus of the same serotype and, to a lesser extent, heterotypic virus infection (68). In our study we measured the neutralising capability of serum samples against the bovine vaccine virus. This measure has provided a reasonable index of the antibody response to vaccination but, perhaps not surprisingly, it did not correlate with clinical protection.

A further area for speculation is the possible effect that the high frequency of enteric viruses and other pathogens in The Gambia may have had on the immune response to rotavirus vaccination. It should be remembered that the background against which any virological work in the tropics has to be done is that of a very considerable load of natural infection with, mostly, enteroviruses. As their name suggest, the enteroviruses (polio viruses, coxsackie A viruses, coxsackie B viruses and echoviruses) inhabit the intestinal tract and poor hygiene aids their dispersal (161). As a consequence enteroviruses are frequently encountered in the intestines of children in the tropics. When a vaccine virus is introduced into the gut at the time of oral vaccination there is at least a theoretical possibility that viral interference between the two viruses will take place.

This problem came to prominence when it was discovered that the measured serological response to oral polio vaccination was much poorer in warm than in temperate countries (162,163). Factors implicated as the cause of this poor

response include breast feeding, inhibition by circulating enteroviruses and a gastrointestinal or salivary inhibitor.

Although polio virus can be inhibited by breast milk (164) this is thought not to be the major suppressive factor as seroconversion rates have been shown to be similar in breast-fed Ugandan infants and those receiving artificial feeding (160). In addition, Indian investigators could not improve conversion rates by withholding breast feeding for varying periods before and after immunisation (165).

Although circulating enteroviruses have been suggested as the cause of the poor response to oral polio in the tropics, reports on the importance of enterovirus infection are conflicting. Several studies have reported that infants carrying enteroviruses at the time of vaccination have a lower antibody response than controls (160), but such a correlation was not found in studies carried out in Nigeria (166) and India (167). Thus it seems possible that some form of local gastrointestinal inhibitor may be the cause of the poor serological response to oral poliovirus obtained in the tropics.

The whole issue of virus interference is, however, highly complex and still a matter of great debate. Interference phenomenon are important in the context of this study for the following reasons. It is important to demonstrate that

the simultaneous administration of the two oral vaccines did not have any detrimental effect on each other. If naturally circulating enteroviruses do have a detrimental effect on the immune response to the oral polio vaccine it is at least possible that the deliberate introduction of the rotavirus vaccine virus would add to this effect. It is also possible that naturally circulating enteroviruses inhibited the immune response to the rotavirus vaccine RIT 4237. This is certainly the explanation favoured by the investigators in Rwanda (151) who conducted a smaller but equally unsuccessful trial of RIT 4237 but some of our results seem to contradict this conclusion.

In the Gambian trial rotavirus vaccine was administered with oral polio vaccine in group A and with killed polio in group C. There was, however, no evidence that the concurrent administration of oral polio vaccine and RIT 4237 had any adverse effect on the rotavirus vaccine response. It is this fact which casts doubt on the theory that the poor response to RIT 4237 was due to interference from naturally circulating enteroviruses. If the mean post-vaccination antibody levels and the rotavirus vaccine response rates were almost identical for children who received oral polio (an enterovirus) and those who received placebo at the time of rotavirus vaccination it is hard to see how naturally circulating enteroviruses could have had a detrimental effect. It is, none the less, clearly possible that the same sort of factors which inhibit the response to oral polio in the tropics were having a similarly negative effect on the rotavirus vaccine. The identity of such a factor or factors

is presently open to speculation.

There was some evidence that the rotavirus vaccine interfered with the immune response to the oral polio vaccine. Antibody levels to polio type 1 and 3 were lower in infants who received concurrent rotavirus vaccine than in controls. During the course of the trial polio antibody levels were analysed elsewhere in batches. When, after 3 batches of sera had been analysed, a statistically significant difference between the two groups was obtained the trial was stopped prematurely. Subsequent analysis of further batches of sera did not show such a marked difference between groups although the trend for lower type 1 and type 3 antibodies in those who had received rotavirus vaccine remained. Interference might be increased if a new less attenuated rotavirus vaccine virus was used and the issue of mutual interference between these two vaccines will require careful consideration in future rotavirus vaccine trials. All vaccine trial participants received a booster dose of oral or intramuscular polio vaccine after the trial had been concluded.

Whatever effect the rotavirus vaccine had on the response to oral polio vaccination the effect was small compared with that caused by whatever factors are operating in all tropical environments to compromise the response to oral polio immunisation. The results from the control group confirm those from other studies that oral polio vaccine frequently fails to result in adequate seroconversion.

Interestingly, the response to killed intramuscular polio in group C was, on the whole, better than that of either oral polio group (A or B). From a serological viewpoint, three doses of I.M. polio vaccine were better overall than three oral doses. This trend was reversed for polio 2 but both vaccines gave good responses and low failure rates for polio 2, so I considered this to be relatively unimportant. Since higher failure rates were observed in polio types 1 and 3 after three doses of oral vaccine, these types would be the logical focus of attention in considering the value of booster doses. In both cases, the I.M. vaccine performed better as a means of boosting irrespective of the initial polio vaccine regime. It should be emphasised, however, that serological response is only one of many factors which should be considered in selecting a vaccine for widespread use, particularly in developing countries.

These results add weight to the view that, despite the undoubted success of oral polio vaccination programmes throughout the world, there are nonetheless several reasons why the relative merits of oral and intramuscular vaccination should be reviewed (168,169). This issue is particularly relevant in The Gambia where there has been a major outbreak of paralytic polio since our study was completed despite a high level of vaccination coverage. Between April and November of 1986, 237 cases of paralytic polio occurred resulting in 19 deaths. Although none of the children involved in our studies developed polio many cases occurred in children who had been fully vaccinated at centres where the integrity of the cold chain could be

guaranteed. The epidemic was investigated by epidemiologists from the Centers for Disease Control in Atlanta. I supplied them with the serological data we had collected and, following a detailed investigation, they concluded in their report to the Gambian Government that "the most likely explanation for the Gambian epidemic was that, despite good vaccination coverage, a proportion of children did not respond to the vaccination and were, therefore, susceptible to paralysis".

The Department of Medical and Health in Banjul decided to respond to the problem by employing the tactic, initially tried in India (170), of increasing the number of doses of oral polio vaccine used. Since the end of 1986 Gambian children have receive five doses of the oral vaccine during the first year of life.

Rotavirus vaccination did not decrease the severity of subsequent rotavirus infections in The Gambia. Our analysis was confined to the 85% of all detected rotavirus cases who were seen and examined by a study clinician. The remaining 15% may have suffered milder attacks but this cannot be assumed as mothers had been taught how to administer water, sugar and salt solution and sometimes treated their own children at home despite our request to see the child during the acute phase of each episode. Of the cases seen by a clinician the majority (88%) suffered a severe attack, as defined by the W.H.O. guidelines, and all of the detected episodes lasted more than 24 hours. There was, however, no

significant difference in the proportion of severe cases in the vaccinated and non vaccinated groups. It is possible that the children in our study had, in general, more severe episodes of diarrhoea than those in the Finnish studies, perhaps because they were exposed to a higher infecting dose of virus.

We are not able to say from the available data whether the strain of infecting virus contributed to the poor vaccine performance but this is clearly an important issue which needs to be addressed in the future. The rotaviruses responsible for the Gambian outbreak were predominantly of a short electropherotype. Experience to date suggests that rotaviruses with short electropherotypes belong to subgroup I serotype 2. The predominant virus in Finland has been of subgroup II serotype 1, although the second study did demonstrated some evidence of protection against natural infection with a subgroup I serotype 2 virus (127).

I am, therefore, unable to provide a single adequate explanation for the failure of the rotavirus vaccine in The Gambia. The poor level of protection provided by RIT 4237 in this study is probably best explained by a combination of factors including the relatively severe spectrum of rotavirus infection, the younger age of administration associated with high levels of pre-vaccination antibody and local factors which may have decreased the vaccine take.

The possibility that naturally circulating viruses, or some other as yet undefined factor, interfered with the rotavirus

vaccine is clearly an important but complex issue which requires further investigation. However, from a practical community health perspective it is clearly much more important to find a vaccine which will protect children in this environment even if we do not understand why our previous effort failed.

An effective and inexpensive rotavirus vaccine would make an important contribution to the control of severe, acute diarrhoeal disease in The Gambia. A less attenuated vaccine may now be required but future trials of further rotavirus vaccines are certainly justified.

CHAPTER VII

FACTORS AFFECTING VACCINE COMPLIANCE

INTRODUCTION.

If disease due to rotavirus is ever to be fully prevented in The Gambia by vaccination, then an effective new vaccine will have to be administered through an efficient vaccine delivery system. By African standards The Gambia is relatively small which facilitates the delivery of health care. It has a well developed national Maternal and Child Health (M.C.H.) programme of which its Expanded Programme for Immunization (E.P.I.) is an important part. Consequently, the country's population has a high rate of access to basic health services including immunisation. Vaccination coverage was measured in 1984 when it was found that 48% of children were fully immunised (171). Despite the success of the E.P.I. programme in The Gambia, coverage has not been uniform and the country still experiences serious measles outbreaks and in 1986 suffered a major outbreak of paralytic poliomyelitis.

Experience in other developing countries suggests that poor vaccination compliance is usually the result of poor availability or delivery of vaccines combined with problems of belief, attitude and perception in the families of potential vaccine recipients (172,173,174). In the towns of The Gambia, vaccine availability and delivery is not a major obstacle to effective vaccination but a significant minority of children still remain unvaccinated or poorly vaccinated.

It seemed possible that these poorly vaccinated children might share common features with respect to family background or socioeconomic status and if these could be identified the task of targeting "at risk" children would be facilitated. This project was devised at the beginning of the rotavirus vaccine trial at which time we anticipated that the vaccine would be successful and that knowledge of factors influencing vaccine compliance would be important to any programme of implementation.

We collected vaccination compliance data retrospectively for 251 children living in Bakau. As expected, the general level of vaccine compliance was high, but a significant minority of children (10%) had received less that half the expected number of vaccinations. These children were compared with a larger group of children who were fully vaccinated.

Data on 29 socioeconomic and attitudinal variables were collected by means of a pre-tested questionnaire (Appendix II) which was administered to the children's mothers by one of the investigators who remained blind to the child's vaccination status.

A proportion of the variables showed significant correlation with vaccine compliance. These results are shown in Table XXIX. All other variables listed in Appendix II showed no significant correlation.

Table XXIX

Variables showing a significant association with poor vaccination compliance together with some of the more important negative results.

Factor	Good Compliance (N=42)	Poor Compliance (N=23)	Significance
Completed primary school(mothers)	10	0	$X^2 = 4.77$ $p < 0.05$
Completed primary school(fathers)	14	2	$X^2 = 3.62$ $p < 0.10$
Mothers literate	8	1	$X^2 = 1.6$ n.s.
Fathers literate	14	3	$X^2 = 2.20$ n.s.
Monthly income (hundreds of dalasis)	mean = 3.21 s.d. = 2.02	mean = 3.00 s.d. = 1.28	t = 0.45 n.s.
Number of assets *	mean = 2.21 s.d. = 1.09	mean = 1.39 s.d. = 1.03	t = 2.96 $p < 0.01$
Number of rooms	mean = 2.71 s.d. = 1.15	mean = 2.61 s.d. = 0.99	t = 0.35 n.s.
Mother employed	26	15	$X^2 = 0.05$ n.s.
Mother self employed	18/26	15/15	$X^2 = 3.94$ $p < 0.05$

Live-born children	mean = 3.55 s.d. = 1.98	mean = 6.17 s.d. = 3.0	t = 4.23 p < 0.001
Had a stillbirth	2	6	X^2 = 4.44 p < 0.05
Living children	mean = 3.12 s.d. = 1.74	mean = 5.44 s.d. = 2.50	t = 4.39 p < 0.001
Children had died	13	12	X^2 = 2.00 n.s.
Complaints about health centre	9	8	X^2 = 0.77 n.s.
Number of visits to health centre	mean = 14.78 s.d. = 5.79	mean = 5.57 s.d. = 3.49	t = 6.96 p < 0.001
Number of weights recorded in 1st year.	mean = 10.50 s.d. = 2.96	mean = 4.22 s.d. = 1.88	t = 9.17 p < 0.001
Would attend for weighing or vaccination only	36	2	X^2 = 24.1 p < 0.001
Knows at least one vaccine-preventable disease	26	7	X^2 = 4.70 p < 0.05
Believes in "scientific" cause of disease	26	2	X^2 = 15.06 p < 0.001

Note.

* Assets were 1.Sheep 2. Goats 3. Cattle 4. Land 5. Motorcycle 6. Bicycle 7.Radio/Cassette 8. Refrigerator.

Access to Immunisation Clinics.

There was no significant difference in the distance mothers of well and poorly vaccinated children had to walk to the Health Centres.

Education and Socioeconomic Status of Parents.

Both parents of poorly vaccinated children had significantly less formal education than parents of well vaccinated children. Mothers and fathers spent less years at school and a smaller proportion of mothers completed primary education while a smaller proportion of fathers finished secondary school (table XXIX). There was also a trend towards poorer literacy in the parents of poorly vaccinated children but this did not reach statistical significance.

There were no differences in family income or housing but families of well vaccinated children had more assets and consumer goods. Paternal occupations were essentially similar, but a significantly larger proportion of the mothers of poorly vaccinated children were self-employed petty traders.

Attitudes of Mothers to Disease and its Prevention.

Mothers of poorly vaccinated children were less aware of the diseases against which immunisation was possible (table XXIX). Mothers were also asked for their opinion with respect to the causes of disease. More than one reply was

accepted and the replies grouped according to whether mothers favoured more scientific reasons like contamination of food, dirtyness, nutrition, feeding practices, and poor child care, or alternatively more superstitious or fatalistic answers like evil spirits and fate. Mothers of poorly vaccinated children favoured the less scientific and more fatalistic answers (table XXIX).

Mothers were asked under what circumstances they took their children to the Health Centre. There was no significant difference in the proportion of mothers who replied "when the child is sick" but a larger proportion of the mothers with well vaccinated children replied that they also took the child for weighing and for vaccination even when the child was well. This aspect was also reflected in the fact that the total number of health centre visits during the first year of life, and the number of weights recorded, was higher in the well vaccinated group (table XXIX).

Mothers of poorly vaccinated children had significantly more children and had experienced more stillbirths. There were, however, no significant differences in the reported number of deaths occurring among liveborn children.

Analysis by Multiple Logistic Regression.

Since some of the factors found to be associated with poor vaccination compliance were highly correlated a multiple logistic regression was carried out in order to examine the effects of risk factors in combination.

The independent variables considered were those showing statistical significance on univariate analysis, except that "number of visits to the health centre" and "number of weights recorded" were omitted as they were so closely related to vaccination compliance. The variable representing attitude "would attend for vaccination, or weighing, even if the child was not sick" was the most important of the remaining variables in discriminating between the two groups, but an argument of circularity could be invoked here, and so the data were analysed both with and without this variable.

Without "would attend for vaccination or weighing, even if the child was not sick", the model which provided the best fit to the data incorporated "number of children born alive", "belief in scientific cause of disease" and "number of assets". The addition of other variables to this model did not significantly improve its fit.

Of the models which included "would attend for vaccination or weighing, even if not sick", the one which proved the best fit contained "number of children born alive" and "number of assets", but not "belief in scientific cause of disease".

Comparison of Vaccination Compliance in 1984 and 1985.

Table XXX shows the results of compliance within the rotavirus vaccine cohort (all born in 1985), while table XXXI shows the retrospective data we collected in Bakau for

Table XXX

Vaccination compliance during 1985 in Bakau (during the rotavirus vaccine trial) for the rotavirus vaccine cohort. Children were withdrawn form the study when they outmigrated and these figures only relate to vaccinations which were carried out during 1985, hence the declining numbers eligible for vaccination.

Vac.	Number eligible	Came within one month	Came after one month	Did not attend
1st	338 (100%)	332 (98%)	6 (2%)	0
2nd	294 (100%)	293 (100%)	1 (0%)	0
3rd	250 (100%)	248 (99%)	2 (1%)	0
Measles	106 (100%)	101 (95%)	5 (5%)	0

Table XXIX

Vaccination compliance during 1984 in Bakau (outwith the rotavirus vaccine trial) in a group of 153 Bakau children still resident in 1985 (information was not available for children who had out-migrated). The information was collected retrospectively.

Vac.	Number attended	Came within one month	Came after one month	Did not attend
1st	151 (99%)	104 (68%)	47 (31%)	2 (1%)
2nd	143 (93%)	101 (66%)	42 (27%)	10 (7%)
3rd	128 (84%)	90 (59%)	38 (25%)	25 (16%)
Measles	103 (67%)	63 (41%)	40 (26%)	50 (33%)

children born in the first half of 1984. The two groups were not exactly comparable since I knew a great deal more about the compliance of the vaccine trial cohort and the two sets of data were collected in different ways.

Despite these differences, it is possible to make a comparison between the two groups for vaccination status within one month of dates on which vaccination should have been given. Such a comparison shows that vaccination compliance was significantly higher during the rotavirus vaccine trial than during the previous year (Mantel-Haenszel test allowing for differences between vaccination 1 to 3, $X^2_{M-H} = 33$, 1d.f., $p < 0.001$). However, the fall-off rate between the three vaccinations was not significantly different when a similar comparison is made with allowance for the pre- and post-trial periods ($X^2_{M-H} = 5.8$, 2 d.f.) The anthropometry and measles vaccine call made at 39 weeks in the rotavirus vaccination trial also shows a significantly higher completion rate ($X^2 = 77$, 1 d.f., $p < 0.001$)

DISCUSSION.

In this survey mothers of poorly vaccinated children showed a number of differences from those of well vaccinated children. There is little doubt that a number of these variables were correlated with each other and it was difficult to disentangle which variables are true risk factors even with the help of multiple logistic regressional analysis. Other surveys examinining aspects of socioeconomic

status in the Gambia have encountered similar problems (109). We did, however, find that the mothers of poorly vaccinated children had more children, had fewer assets, were less well educated, had less knowledge of the diseases their children should be vaccinated against and had a more superstitious view of disease causation.

These mothers were also less orientated to the health centre as a source of preventive, as opposed to simply curative, services. By contrast, the mothers of poorly vaccinated children were less inclined to bring their children to the Health Centre for weighing and vaccination but tended to confine their visits to times of acute illness.

It is possible that the heavier workload involved in looking after a larger number of children gave these mother less time to visit the health centre. The intense pressure of work and family cares of many women in developing countries influences their ability to use available health services. A similar explanation may underlie the fact that more of the mothers of poorly vaccinated children worked as self-employed petty traders. These women had to spend a great deal of time selling their wares at the local markets, which may have decreased their opportunities for visiting the Health Centre.

No difference was found in family income although the families of well vaccinated children had more assets and consumer items. This is surprising as wealth might have been

expected to correlate to a more marked degree with education. It has, however, been pointed out in previous studies of Bakau families that the occupation of the father, housing, and possession of consumer items are poor indices of true family wealth, but they have been used in this study in the absence of more suitable indicators.

Studies from other countries have shown that poor vaccine compliance is often associated with the difficulties experienced by mothers in obtaining vaccinations for their child allied to problems of attitude, perception and belief (172,173,174). In our study area, vaccination clinics were run efficiently on a weekly basis and all mothers had easy access to them. None of the mothers interviewed believed that vaccination could cause any harm to their child and there was no difference between the groups with respect to complaints about, or difficulties with, the Health Centre staff.

In this study I have identified a number of characteristics common to mothers and families of poorly vaccinated children. One of the more interesting findings to emerge from this study was the relationship between poor vaccination compliance and poor education. These poorly educated women also had a poorer knowledge of disease and vaccines. In the light of this, it would seem logical to employ health education techniques to compensate for these disadvantages. It has, however, also been demonstrated that these mothers pay fewer visits to the Health Centre which is the traditional site of most formal health education

activity. For this reason it may be necessary for the health workers to take the health education messages to the homes. Fortunately these is a cadre of health workers in The Gambia who are ideally suited to confronting this problem. In Bakau, and throughout much of The Gambia, Community Health Nurses (C.H.N.s) form part of the primary health care team. Their main function is health education and health promotion but there is some doubt about how effectively they are fulfilling this role.

Despite the methodological problems involved in comparing vaccination compliance for children born in 1985 with retrospective data collected for children born in 1984, vaccination compliance in Bakau was measureably improved during the rotavirus vaccine trial. There were, undoubtedly, many reason for this but my strong impression was that the major factor which improved compliance was the paying of reminder visits to the child's compound on the day before vaccinations were due. Similar visits could be paid by C.H.N.s, not only to remind the mothers about vaccination but also for health educational purposes. It is clearly impractical for this type of visit to be paid to all children but it may be possible for C.H.N.s to target "at risk" children who have poor vaccination records, thereby encouraging the mothers to bring their children. From the evidence of this study, self-employed mothers with large families and a low standard of education need special help if their children are to benefit from preventive health measures.

Other methods of health education could also be usefully employed to increase awareness of the importance of vaccinations. These include radio messages and posters which are already used extensively in The Gambia to promote other health activities.

CHAPTER VIII

FINAL DISCUSSION AND CONCLUSIONS

The World Bank estimates that even if optimistic rates of economic growth are achieved in the developing world, there will still be 600 million people trapped in a state of absolute poverty in the year 2,000. This, the Bank defines as a condition of life so characterised by malnutrition, illiteracy, disease, high infant mortality, and low life expectancy as to be beneath any reasonable definition of human decency. The number of people in the world who are not in a state of absolute poverty, but none the less suffer from preventable disease and a low standard of living, is much larger. The existence of such an enormous reservoir of human disease and suffering is a challenge not just to the medical profession, but to the world at large. Medical personnel, on their own, can do very little to change the lot of the most disadvantaged people in the world but health care and promotion is a vital ingredient in any comprehensive development programme.

The World Health Organisation has responded to this challenge with its "Health for all by the year 2,000" programme. This programme has emphasised the need to take medical care out of the hospitals and into the community, where low cost treatments can be delivered by village health workers. As this programme has gathered momentum, so too have a number of problems and questions become evident. What are the most cost effective interventions? How can the

consumer's desire for good treatment of diseases be married to the obvious need for disease prevention? Can efficient, sustainable primary health care programmes be maintained without continuing international financial support and expertise?

Those of us who are involved in tropical medicine research need to be sensitive to these issues. It is very easy for the research agenda to be unconsciously set by academics working in the rich industrialised countries. From such a context basic scientific issues seem more important and it is easy to depreciate the value of operational research and investigations into social and environmental issues. On the other hand, doctors working in the developing world are acutely aware of the urgency of the need. Both sets of values are clearly required but in practice a compromise has to be arrived at. The research I carried out in The Gambia was one such compromise.

The development of oral rehydration solutions has revolutionised the treatment of diarrhoea but, despite this, several million children die every year in the Third World from a condition which is usually little more than a mild inconvenience in Europe or North America. The basic problem is that a mother still needs to mix the water, sugar and salt solution in the correct quantities and give enough of it to her sick child. This requires knowledge of how to mix the solution and the necessary discernment to start treatment in time. For these reasons effective management of

diarrhoea will continue to be a problem, although oral rehydration therapy is a vitally important development which is presently saving many lives throughout the world.

These difficulties, encountered with even simple treatment measures, make vaccination an even more attractive proposition in the developing world. Vaccination to prevent any form of diarrhoea would also have the added benefit that it would prevent the weight loss which is known to be associated with repeated diarrhoeal episodes.

It was within this context that the rotavirus vaccine RIT 4237 was developed. The case for a rotavirus vaccine was effectively argued soon after this virus was discovered, but it would be wrong to overestimate the potential benefits. From the evidence of the work carried out in Bakau, complete elimination of rotavirus disease would only reduce the total diarrhoea burden on children less than 2 years old by less than 10%. This figure reflects the extraordinarily high frequency of diarrhoeal episodes in young Gambian children but, looked at from another perspective, it is equally true to say that rotavirus vaccination could prevent a severe and potentially life threatening illness in one third to one half of all infants. Rotavirus vaccination would also have a disproportionate impact on hospital admissions with gastroenteritis as rotavirus has been shown to cause a relatively severe illness.

I have no data to prove that more severe episodes of diarrhoea are associated with greater weight loss but such a

hypothesis has a strong theoretical basis. Approximately half of all rotavirus cases experienced obvious weight loss in the post infection period. This issue clearly requires further study.

Another issue, arising from our observations, which requires further investigation is the relationship between climate and rotavirus infection. The very strict epidemic nature of rotavirus in The Gambia, occurring as it does at exactly the same time each year, and the highly predictable change in season which precedes the epidemic by a matter of weeks, point to an association between climate and rotavirus survival in the environment. Experiments relating atmospheric humidity and temperature to rotavirus survival on fomites would be an obvious approach to this problem.

A great deal remains to be discovered about the epidemiology of different rotavirus strains. Our studies have not added greatly to this knowledge, but The Gambia is now rare among developing countries in that rotavirus epidemiology has been studied for four consecutive years and data have been collected on the R.N.A. electropherotypes circulating in the community during each of these years. A limited amount of data is also available concerning the subgroup and serotype of rotaviruses circulating in Bakau during this period. If these observations are continued it should be possible to define strain-specific characteristics.

My failure to identify specific risk factors for rotavirus

infection does not in any way reduce the need for improvements in housing, sanitation water supply etc., but it does underline the fact that improvements in health will come about only when a wide range of improvements are made in living standards and that it is relatively futile to search for individual environmental interventions.

There is also a need to discover a great deal more about the microbiological environment of children in The Gambia. In particular, efforts should be made to determine the aetiology of diarrhoea and further define the effect of enteroviruses on polio and other oral vaccines. Such work would require individuals with a high level of expertise in various aspects of laboratory science and so, given the present availability of funds and the medical priorities in The Gambia, it seems unlikely that such work will be carried out in the near future. Whether it is right to pursue vaccination trials while such knowledge is lacking is open to debate but my own feeling is that research in the tropics has to be the art of the possible and the need is so great that any vaccine with a reasonalbe chance of success should at least be assessed.

The relatively poor performance of the rotavirus vaccine RIT 4237 in The Gambia was a major disappointment but a variety of other candidate vaccines are nearing the stage of field trials and it is possible that a new vaccine will be more successful. From the evidence of the trial of RIT 4237, any new vaccine will need to be more immunogenic and it is possible, but as yet unproven, that it will have to be

polyvalent, containing each of the major serotypes.

The age of administration is now an important issue. In The Gambia maternal antibody levels to rotavirus in young children were high and may have had a detrimental effect on the response to vaccination with RIT 4237. Whether a more immunogenic vaccine could overcome the effect of maternal antibody remains to be seen, but it would be a major disadvantage if rotavirus vaccination had to be delayed until the second half of the first year of life.

Vaccination as early as possible in life is not only important because the disease affects all infants, but also because the compliance to vaccination is higher for vaccines given during the first six months of life when the child is brought for other vaccinations and weighing. Although we were able to improve the overall vaccine compliance of the children in our study, compared with historical controls born the previous year, the relative fall-off in vaccination compliance for D.P.T. and polio (administered at two, three, and four months) to measles and yellow fever (administered at nine months) was not improved, which shows that whatever the overall level of compliance, there is a clear advantage if a vaccine can be given before six months of age and at the same time as D.P.T. and polio.

Rotavirus vaccination in The Gambia would only be justified if an effective vaccine could be developed which was relatively inexpensive. The cost of RIT 4237 was not known.

The market price of any future vaccine would obviously depend on a large number of factors. As was argued earlier in the text, administration of rotavirus and oral polio vaccines together would reduce the delivery cost of the new vaccine, but we were not able to exclude a negative effect on oral polio vaccination by the rotavirus vaccine. Any new vaccine would have to be assessed carefully with respect to this potential interaction.

In conclusion, it can be said that there are several ways of reducing morbidity and mortality due to diarrhoeal disease in general, and rotavirus infection in particular, in The Gambia. These interventions are effective rotavirus vaccination, a general rise in living standards and effective treatment of acute cases when they arise. It is to be hoped that the long-term goal of a rise in living standards will eventually come to The Gambia and that it will not be long before an effective and inexpensive rotavirus vaccine is available.

REFERENCES

1. Puffer R R, Serrano C V. Patterns of Mortality in Childhood. Scientific Publication No. 262. Pan American Health Organisation, Washington D.C. 1973.

2. Chen L C, Rahaman M, Sarder A M. Epidemiology and Cause of Death Among Children in Rural Areas of Bangladesh. International Journal of Epidemiology 1980; 9 : 25 - 33.

3. Walsh J A, Warren K C. Selective Primary Health Care: An Interim Strategy for Disease Control in Developing Countries. New England Journal of Medicine 1979; 103 : 967 - 974.

4. Snyder J D, Merson H/M. The Magnitude of the Global Problem of Acute Diarrhoeal Disease: A Review of Active Surveillance Data. Bulletin of the World Health Organization 1982; 60 : 605 - 613.

5. Mata L J. The Children of Santa Maria Cauque. M.I.T. Press 1978.

6. Rohde J E, Northrup R E. Taking Science Where the Diarrhoea Is. In: Acute Diarrhoeal Diseases in Childhood. CIBA Foundation Symposium 42. Elsevier/Exerpta Medica, Amsterdam 1978.

7. Gordon J E, Guzman M A, Ascoli W, Scrimshaw M S. Acute Diarrhoeal Disease In Less Developed Countries. Bulletin of The World Health Organisation 1964; 31 : 9 - 20.

8. Pickering L K, Evans D J, Munoz O, Dupont H L, Coello-Ramirez P, Vollet J J, Conklin R H, Olarte J, Kohl S. Prospective Study of Enteropathogens in Children in Huston and Mexico. The Journal of Pediatrics 1978; 93 : 383 - 388.

9. Chen L C. Interactions of Diarrhoea and Malnutrition. In: Chen L C, Scrimshaw N S, eds. Diarrhoea and Malnutrition: Interactions, Mechanisms, and Interventions. New York: Plenum, 1983; 3 - 23.

10. Rowland M G, Cole T J, Whitehead R G. A Quantitive Study into the Role of Infection in Determining Nutritional Status in Gambian Village Children. British Journal of Nutrition 1977; 37 : 441 - 450.

11. Mata L J, Kromal R A, Urrutia J J, Garcia B. Effects of Infection on Food Intake and The Nutritional State. American Journal of Clinical Nutrition 1977; 30 : 1215 - 1227.

12. Martorell R, Yarbough C, Yarbough S, Klein R E. The Impact of Ordinary Illness on the Dietary Intakes of Malnourished Children. American Journal of Clinical Nutrition 1980; 33 : 345 -350.

13. Baqui R, Zuberi S J, Khan M A. Significance of E. coli and Rotavirus in Infantile Diarrhoea. Journal of The Pakistani Medical Association 1985; 35 : 307 -308.

14. Black R E, Merson M H, Huq I, Alim A R M A, Yunus M D. Incidence and Severity of Rotavirus and E. coli Diarrhoea in Rural Bangladesh. Lancet 1981; i : 141 - 143.

15. Gordon I, Ingraham H S, Kerns R F. Transmission of Epidemic Gastroenteritis to Human Volunteers by Oral Administration of Faecal Filtrates. Journal of Experimental Medicine 1947; 80 : 409 - 415.

16. Bishop R F, Davidson G P, Holmes I H, Ruck B J. Virus Particles in Epithelial Cells of Duodenal Mucosa from Children with Acute Gastroenteritis. Lancet 1973; ii : 1281 - 283.

17. Kapikian A Z, Wyatt R G, Greenberg H B, Kalica A R, Hyun W K, Brandt C D, Rodriguez W J, Parrott R H, Chanock R M. Approaches to Immunisation of Infants and Young Children Against Gastroenteritis Due to Rotaviruses. Review of Infectious Diseases 1980; 2 : 459 - 469.

18. Bishop R F. Rotavirus in Perspective - A Personal View. Australian Paediatric Journal 1984; 20 : 9 - 12.

19. Steinhoff M C. Rotavirus: The First Five Years. The Journal of Pediatrics 1980; 4 : 611 - 622.

20. Soenarto Y, Sebodo T, Ridho R, Alrasjid H, Rohde J E, Bugg H C, Barnes G L, Bishop R F. Acute Diarhoea and Rotavirus Infection in Newborn Babies and Children in Yogyakarta, Indonesia. Journal of Clinical Microbiology 1981; 14 : 123 -129.

21. Black R E, Merson M H, Rahman A S M M, Yanus M, Alim A R, Huq I, Yolken R H, Curlin G T. A Two Year Study of Bacterial, Viral, and Parasitic Agents Associated with Diarrhoea in Rural Bangladesh. Journal of Infectious Diseases 1980; 142 : 660 -664.

22. Mata L, Simhon A, Padilla R, Gamboa M, Vargas G, Hernandez F, Mohs E, Lizano C. Diarrhoea Associated With Rotavirus, Enterotoxigenic Escherichia coli, Campylobacter, and Other Agents in Costa Rican Children. American Journal of Tropical Medicine and Hygiene 1983; 32 : 146 -153.

23. Stoll B J, Glass R I, Huq M I, Khan M V, Holt J E, Banu H. Surveillance of Patients Attending a Diarrhoeal Disease Hospital in Bangladesh. British Medical Journal 1982; 285 : 1185 - 1188.

24. Linares A C, Moncao H C, Gabbay Y B, de Araujo V L C, Serriya C, Loureiro E C B. Acute Diarrhoea Associated with Rotavirus Among Children Living in Belem, Brazil. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1983; 77 : 384 - 390.

25. George M C, Wachmuth I K, Meunier D M V, Nebout N, Didier F, Siopathis M R, George A J. Parasitic, Bacterial, and Viral Enteric Pathogens Associated with Diarrhoea in the Central African Republic. Journal of Clinical Microbiology 1984; 19 : 571 - 575.

26. Davidson G P, Bishop R F, Townley R R W, Holmes I H, Ruck B J. Importance of a New Virus in Acute Sporadic Enteritis in Children. Lancet 1975; i : 242 - 246.

27. Veskari T, Maki M, Sarkkinen H K, Arstila P P, Halonen P E. Rotavirus, Adenovirus, and Non-Viral Enteropathogens in Diarrhoea. Archives of the Diseases of Childhood 1981; 56 : 264 - 270.

28. Person B L, Thoren A, Tufvessen B, Walder M. Diarrhoea in Swedish Infants. Acta Paediatrica Scandavica 1982; 71 : 909 - 913.

29. Brandt C D, Kim H W, Rodriguez W, Paediatric Viral Gastroenteritis During Eight Years of Study. Journal of Clinical Microbiology 1983; 18 : 71 - 78.

30. Hjelt K, Krasilnikoff P A, Grauballe P C. Incidence of Hospitalisation and Outpatient Clinical Visits Caused by Rotavirus and Non-Rotavirus Acute Gastroenteritis. Danish Medical Bulletin 1984; 31 : 249 - 251.

31. Ellis M E, Watson B, Mandal B K, Dunbar E M, Craske J, Curry A, Roberts J, Lemox J. Micro-Organisms in Gastroenteritis. Archives of the Diseases of Childhood 1984; 59 : 848 - 855.

32. Mata L. Acute Diarrhoea: Its Nutritional Consequences in Children. ed. Belanti J A. Nestle Vevey Raven Press, New York 1983; 85 - 97.

33. Greenwood B M. Serum Survey of Gambian Children in a Rural Gambian Environment. Personal Communication.

34. Gust I D, Pringle R C, Barnes G L, Davidson G P, Bishop R F. Complement-Fixing Antibody Response to Rotavirus Infection. Journal of Clinical Microbiology 1977; 5 : 125 - 130.

35. Blacklow N R, Echeverria P, Smith D H. Serological Studies with Reovirus-like Agent. Infectious Immunology 1976; 13 : 1563 - 1566.

36. Black R E, Greenberg H B, Kapikian A Z, Brown K H, Becker S. Acquisition of Serum Antibodies to Norwalk Virus and Rotavirus and Relation to Diarrhoea in a Longitudinal Study of Young Children in Rural Bangladesh. Journal of Infectious Diseases 1982; 145 : 483 - 489.

37. Echevarria P, Burke D S, Blacklow N, Cukor G, Charoenkul G, Yanggratoke S. Age-specific Prevalence of Antibody to Rotavirus, Escherichia coli Heat Labile Enterotoxin, Norwalk Virus and Hepatitis A Virus in a Rural Community in Thailand. Journal of Clinical Microbiology 1983; 17 : 923 - 925.

38. Wenman W M, Hinde O, Feltham S, Gurwith M. Rotavirus Infection In Adults: Results of a Prospective Family Study. New England Journal of Medicine. 1979; 301 : 303 - 306.

39. Grimwood K, Abbot G D, Fergusson D M, Jennings L C, Allan J M. Spread of Rotavirus Within Families: A Community Based Study. British Medical Journal 1983; 287 : 568 - 575.

40. Gurwith M, Wenman W, Hinde D, Feltham S, Greenberg H. A Prospective study of Rotavirus Infection in Infants and Young Children. Journal of Infectious Diseases 1981; 144 : 218 - 224.

41. Black R E, Brown K H, Becker S, Alim A R M A, Huq I. Longitudinal Studies of Infectious Diseases and Physical Growth of Children in Rural Bangladesh. II Incidence of Diarrhoea and Association with Known Pathogens. American Journal of Epidemiology 1982; 115: 315 - 322.

42. Mata L, Simhon A, Urrutia J J, Kronmal R A, Fernandez R, Garcia B. Epidemiology of Rotavirus in a Cohort of 45 Guatemalian Mayan Indian Children Observed from Birth to the Age of 3 Years. Journal of Infectious Diseases 1983; 148 : 452 - 460.

43. Guerrant R L, Kirchoff L V, Shields D S, Nations M K, Leslie J. Prospective Study of Diarrhoeal Illness in Northeastern Brazil: Patterns of disease, Nutritional Impact, Etiologies, and Risk Factors. Journal of Infectious Diseases 1983; 148 : 986 - 997.

44. Rowland M G M, Goh S G J, Williams K, Campbell D, Beards G M, Sanders R C, Flewett T W. Epidemiological Aspects of Rotavirus Infection in Young Gambian Children. Annals of Tropical Paediatrics 1985; 5 : 23 - 28.

45. Monto A S, Koopman J S. The Tecumseh Study, XI. Occurence of Acute Enteric Disease in the Community. American Journal of Epidemiology 1980; 112 : 323 - 334.

46. Spencer H C, Wells J G, Gary G W, Sondy J, Puhr N D, Feldman R A. Diarrhoea in a Non-Hospitalised Rural Salvadorian Population. American Journal of Tropical Medicine 1980; 29 : 246 - 253.

47. Monto A S, Koopman J S, Longini I, Isaccson R E. The Tecumseh Study XII. Enteric Agents in The Community. Journal of Infectious Diseases 1983; 148 : 284 - 291.

48. Davidson G P, Barnes G L. Structural and Functional Abnormalities of the Small Intestine in Infants and Young Children with Rotavirus Enteritis. Acta Paediatrica Scandinavica 1979; 68 : 181 - 186.

49. Cukor G, Blacklow N R. Human Viral Gastroenteritis. Microbiological Review 1984; 48 : 157 - 179.

50. Wyn-Jones A P, Lillington A W, Alzaka A. An Investigation into the Possible Role of the Family Unit in the Transmission of Rotavirus Infection in Childen. Public Health, London 1978; 92 : 291 - 293.

51. Holmes I H. Rotaviruses. In: The Reoviridae , Jolik W K. ed, Plenum, New York 1983; 359 - 423.

52. Brandt C D, Kim H W, Rodriguez W, Arrobio J O, Jeffries B C, Parrott R H. Rotavirus Gastroenteritis and The Weather. Journal of Clinical Microbiology 1982; 16 : 478 - 482.

53. Sattar S A, Lloyd-Evans N, Springthorpe V S, Nair R C. Institutional Outbreaks of Rotavirus Diarrhoea : Potential Role of Fomites and Environmental Surfaces as Vehicles for Virus Transmission. Journal of Hygiene 1986; 96 : 277 - 289.

54. Cameron D J S, Bishop R F, Davidson G P, Townley R R W, Holmes I H, Ruck B J. New Virus Associated With Diarrhoea in Neonates. The Medical Journal of Australia 1976; 1 : 85 - 87.

55. Totterdell B M, Chrystie I L, Banatvala J E. Rotavirus Infection in a Maternity Unit. Archives of the Diseases of Childhood 1976; 51 : 924 - 928.

56. Bishop R F, Cameron D J S, Veenestra A A, Barnes G L. Diarrhoea and Rotavirus Infection Asociated with Differing Regemens for Postnatal Care of Newborn Babies. Journal of Clinical Microbiology 1979; 9 : 525 - 529.

57. Chrystie I L, Totterdell B M, Banatvala J E. Asymptomatic Endemic Infection in the Newborn. Lancet 1978; i : 1176 - 1178.

58. Flewett T H. Rotavirus in the Home and Hospital Nursery. British Medical Journal 1983; 287 : 568.

59. Van Renterghem L, Borre P, Tilleman J. Rotavirus and Other Viruses in the Stools of Premature Babies. Journal of Medical Virology 1980; 5 : 137 - 142.

60. Rocchi G, Vella S, Resta S, Cochi S, Donelli G, Tanqucci F. Outbreak of Gastroenteritis among Premature Infants. British Medical Journal 1981; 283 : 886.

61. Bryden A S, Thouless M E, Hall C J, Flewett T H, Warton B A, Mathew P M, Craig I. Rotavirus Infection in a Special Baby Care Unit. Journal of Infection 1982; 4 : 43 - 48.

62. Crewe E, Murphy A M. Further Studies in Neonatal Rotavirus Infection. Medical Journal of Australia 1980; 1 : 61 - 63.

63. Rodriguez W J, Kim H W, Brandt C D, Gardner K M, Parrott R H. Use of Electrophoresis of RNA from Human Rotaviruses to Establish the Identity of Strains Involved in Outbreaks in a Tertiary Care Nursery. Journal of Infectious Diseases 1983; 148 34 - 40.

64. Dearlove J, Latham P, Dearlove B, Pearl K, Thomson A, Lewis G. Clinical Range of Neonatal Rotavirus Gastroenteritis. British Medical Journal 1983; 286 : 1473 - 1475.

65. Walther F J, Bruggeman C, Daniels-Bosman M S M. Rotavirus Infection in High Risk Neonates. Journal of Hospital Infection 1984; 5 : 438 - 443.

66. Perez-Schael I, Daoud G, White L, Urbina G, Daoud N, Peres M, Florez J. Rotavirus Shedding in Newborn Children. Journal of Medical Virology 1984; 14 : 127 - 135.

67. Bishop R F, Barnes G L, Cipriani E, Lund J S. Clinical Immunity After Neonatal Rotavirus Infection: A Prospective Longitudinal Study in Young Children. New England Journal of Medicine 1983; 309 : 72 - 76.

68. Chiba S, Yokoyama T, Nakata S, Morita Y, Urasawa T, Taniguchi K, Urasawa S, Nakao T. Protective Effect of Naturally acquired Homotypic and Heterotypic Rotavirus Antibodies. Lancet 1986; ii : 417 - 421.

69. Tao H, Gaungmu C, Chengan W, Henli Y, Zhaoying F, Tungzin C, Zinyi C, Weiwe Y, Zuejian C, Shuasen D, Xiaoquang L, Weicheng C. Waterbourne Outbreak of Rotavirus Diarrhoea in Adults in China Caused by a Novel Rotavirus. Lancet 1984; i : 1139 - 1142.

70. Echeverria P, Blacklow N R, Cukor G, Vibulbandhitkit S, Changchawalit S, Boonthal P. Rotavirus as a Cause of Severe Gastroenteritis in Adults. Journal of Clinical Microbiology 1983; 18 : 663 - 667.

71. Kim H W, Brandt C D, Kapikian A Z, Wyatt R G, Arrobio J O, Rodriguez W J, Chanock R M, Parrott R H. Human Reovirus - Like Infection: Occurence in Adult Contacts of Paediatric Patients With Gastroenteritis. Journal of the American Medical Association 1977; 238 : 404 - 407.

72. Estes M K, Graham D Y, Dimitrov D H. The Molecular Epidemiology of Rotavirus Gastroenteritis. Progress in Medical Virology (Karger-Basel) 1984; 29 : 1 - 22.

73. Rodger S M, Bishop R F, Birch C, McLean B, Holmes I H. Molecular Epidemiology of Human Rotaviruses in Melbourne, Australia, from 1973 to 1979 as Determined by Electrophoresis of Genome Ribonucleic Acid. Journal of Clinical Microbiology 1981; 13 : 272 - 278.

74. Follett E A C, Sanders R C, Beards G M, Hundley F, Desselberger U. Molecular Epidemiology of Human Rotavirus. Journal of Hygiene, Cambridge 1984; 92 : 209 - 222.

75. Albert M J, Suenarto Y, Bishop R F. Epidemiology of Rotavirus Diarrhoea in Yogyakarta, Indonesia as Revealed by Electrophoresis of Genome RNA. Journal of Clinical Microbiology 1982; 16 : 731 - 733.

76. Dimitrov D H, Graham D Y, Lopez J, Muchinik G, Velasco G, Stenback W A, Ellis M K. RNA Electropherotypes of Human Rotaviruses from North and South America. Bulletin of the World Health Organisation 1984; 62 : 321 - 329.

77. Nicholas J C, Pothier P, Cohen J, Lourenco M H, Thompson R, Guimbaud P, Chenon A, Dauvergue M, Bricout F. Survey of Human Rotavirus Propagation as Studied by Electrophoresis of Genomic RNA. Journal of Infectious Diseases 1984; 149 : 688 - 693.

78. Konno T, Sato T, Suzuki H, Kitaoka S, Katsushima N, Sakamoto M, Yasaki N, Ishida N. Changing RNA Patterns in Rotavirus of Human Origin: Demonstration of a Single Dominant Pattern at the Start of the Epidemic and Various Patterns Thereafter. Journal of Infectious Diseases 1984; 149 : 683 - 687.

79. Dolan K T, Twist L M, Norton-Slight P, Forrier C, Bell L M, Plotkin S A, Clark H F. Epidemiology of Rotavirus Electropherotypes Determined by a Simplified Diagnostic Technique with RNA Analysis. Journal of Clinical Microbiology 1985; 21 : 753 - 758.

80. Sanders R C. Molecular Epidemiology of Human Rotavirus Infections. European Journal of Epidemiology 1985; 1 : 19 - 32.

81. Anon. Nomenclature of Human Rotaviruses: Designation of Subgroups and Serotypes. Bulletin of the World Health Organisation 1984; 62 : 501 -503.

82. Wyatt R G, James H D, Pittman A L, Hoshino Y, Greenberg H B, Kalica A R, Flores J, Kapikian A Z. Direct Isolation in Cell Culture of Human Rotaviruses and Their Characterisation into Four Serotypes. Journal of Clinical Microbiology 1983; 18 : 310 - 317.

83. Hoshino Y, Wyatt R G, Flores J, Midthun K, Kapikian A Z. Serotypic Characterisation of Rotaviruses Derived from Asymptomatic Human Neonatal Infection. Journal of Clinical Microbiology 1985; 21 : 425 429.

84. Lambert J P, Marissens D, Marbehant P, Zissis G. Prevalence of Subgroup 1,2, and 3 Rotaviruses in Belgian Children Suffering from Acute Diarrhoea. Journal of Medical Virology 1983; 11 : 31 - 38.

85. Flewett T H, Thouless M E, Pilfold J N, Bryden A S, Candeias J A S. More Serotypes of Human Rotavirus. Lancet 1978; ii : 632.

86. Zissis G, Lambert J P. Different Serotypes of Human Rotaviruses. Lancet 1978; i : 38 - 39.

87. Beards G M, Campbell A D, Cottrell N R, Peiris J S M, Rees N, Sanders R C, Shirley J A, Wood H C, Flewett T H. Enzyme-linked Immunoabsorbant Assay based on Polyclonal Antibodies for Rotavirus Detection. Journal of Clinical Microbiology 1984; 19 : 248 - 254.

88. Riepenhoff-Talty M, Duffy L, Offer E, Suzuki H, Ogra P L. Pathogenic Mechanisms and Immunity to Rotavirus Infection. In: Development of Vaccines and Drugs Against Diarrhoea. Editors, Holmgren J, Linberg A, Mollby R. Studentlitteratur 1985; 171 - 184.

89. Riepenhoff-Talty M, Bogger-Goren P, Li P, Carmody H J, Ogra P L. Development of Serum and Intestinal Antibody Response to Rotavirus after Naturally Acquired Rotavirus Infection In Man. Journal of Medical Virology. 1981; 8 : 215 - 222.

90. Davidson G P, Hogg R, Kirubakaran C. Serum and Intestinal Immune Response to Rotavirus Enteritis in Children. Infection and Immunity 1983; 40 : 447 -482.

91. Totterdell B M, Chrystie I L, Banatvala J E. Rotavirus Infection in a Materity Unit. Archives of the Diseases of Childhood 1976; 51 : 924 - 928.

92. Thouless M E, Bryden A S, Flewett T H. Rotavirus Neutralisation by Human Milk. British Medical Journal 1977; II : 1390.

93. Totterdell B M, Chrystie I L, Banatvala J E. Cord blood and Breast - Milk antibodies in Neonatal Rotavirus Infection. British Medical Joural 1980; 828 - 831.

94. Snodgrass D R, Wells P W. Rotavirus Infection in Lambs: Studies on Passive Protection. Archives of Virology 1976; 52 : 201 - 205.

95. Saif L J, Redman D R, Smith K L, Theil K W. Passive Immunity to Bovine Rotavirus in Newborn Calves Fed Colostrum Supplements From Immuninised or Nonimmunised Cows. Infection and Immunity 1983; 41 : 1118 - 1131.

96. Ebina T, Sato A, Umezu K, Ishida N, Ohyama S, Ohizumi A, Aikawa K, Katagiri S, Katsushima N, Imai A, Kitaoka S, Suzuki H, Konno T. Prevention of Rotavirus Infection by Cow Colostrum Containing Antibody against Human Rotavirus. Lancet 1983; ii : 1029 - 1030.

97. Snodgrass D R, Madeley C R, Wells P E, Angus K W. Human Rotavirus In Lambs: Infection and Passive Protection. Infection and Immunology 1977; 16 : 268 - 270.

98. Snodgrass D R, Wells P W. Passive Immunity to Rotavirus Infection. Journal of the American Vetinary Association 1978; 173 : 565 - 569.

99. Ryder R W, Singh N, Reeves W C, Kapikian A Z, Greenberg H B, Sack R B. Evidence of Immunity Induced by Naturally Acquired Rotavirus and Norwalk Virus Infection on Two Remote Panamerican Islands. Journal of Infectious Diseases 1985; 151 : 99 - 105.

100. Riepenhoff-Talty M, Ping L C, Carmody P J, Barrett H J, Ogra P L. Age-Dependent Rotavirus-Enterocyte Interactions. Proceedings of the Society for Experimental Biology and Medicine 1982; 170 : 146 - 154.

101. Rowland M G M. Epidemiology of Childhood Diarrhoea in The Gambia. In Chen L C, Scrimshaw N S, eds. Diarrhoea and Malnutrition: Interactions, Mechanisms and Interventions. New York: Plenum Publishing Corporation; 1982; 87 - 98.

102. Goh S G J, Lloyd-Evans N, Williams K, Rowland M G M. The Aetiology of Diarrhoea in the Community in Young Gambian Children. Journal of Diarrhoea Disease Research 1985; 3 : 7 -13.

103. Rowland M G M, Goh Rowland S G J, Dunn D T. The Relation Between Weaning Practices and Patterns of Morbidity from Diarrhoea: An Urban Gambian Case Study. In: Walker-Smith J A, McNeish A S, eds. Diarrhoea and Malnutrition in Childhood. London Butterworths, 1986: 7 - 13.

104. Annual Report of the Royal Victoria Hospital, Banjul 1985; Medical and Health Department, Banjul.

105. Greenwood B M. Morbidity and Mortality Data From Farafeni Study Area. Personal Communication.

106. Rowland M G M, McCollum J P K. Malnutrition and Gastroenteritis in The Gambia. Transactions of the Royal Society of Tropical Medicine and Hygiene 1977; 71 : 199 - 203.

107. Rowland M G M. Growth Faltering in Diarrhoea. In: Nutritional Implications of Diarrhoea in Young Children. Proceedings of the XIII International Congress of Nutrition, Brighton, U.K. John Libbey, 1986: (In Press).

108. Rowland M G M, Goh S G J, Cole T J. The Impact of Infection on the Growth of Children from 0 to 2 Years in an Urban West African Community. In Press.

109. Pickering H. Social and Environmental Factors Associated with Diarrhoea and Growth In Young Children: Child Health in Urban Africa. Social Science in Medicine 1985; 21 : 121 - 127.

110. Rowland M G M, Leung T S M, Marshall W C. Rotavirus Infection in Young Gambian Village Children. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1980; 74 : 663 - 665.

111. Vesikari T, Isolauri E, Delem A, D'Hondt E, Andre F E, Zissis G. Protection of Infants against Rotavirus Diarrhoea by RIT 4237 Attenuated Bovine Rotavirus Strain Vaccine. Lancet 1984; i : 977 - 981.

112. Wyatt R G, Kapikian A Z, Greenberg H B, Kalica A, Flores J, Hoshino Y, Chanock R M, Levine M M. Development of Vaccines Against Rotavirus Disease. Progress in Food and Nutrition Science 1983; 7 : 189 - 192.

113. Kapikian A Z, Wyatt R G, Levine M M, Black R E, Greenberg H B, Flores J, Kalica A R, Hoshino Y, Chanock R M. Studies in Volunteers with Human Rotaviruses. Devel. Biol.Standard. 1983; 53 : 209 - 218.

114. Kapikian A Z, Wyatt R G, Levine M M, Yolken R H, Van Kirk D H, Dolin R, Greenberg H B, Chanock R M. Oral Administration of Human Rotaviruses to Volunteers: Induction of Illness and Correlates of Resistance. Journal of Infectious Diseases 1983; 147 : 95 - 106.

115. Matsuno S. Cold Adaptation of a Human Rotavirus Strain, p. 12. Abstract of Working conference on Rabies, Arboviruses, Including Dengue and Viral Gastroenteritis of Japan-U.S. Cooperative Medical Science Programme Oiso, Japan 1984.

116. Gaul S K, Simpson T F, Woode G N, Fulton R W. Antigenic Relationships Among Some Animal Rotaviruses, Virus Neutralisation and Cross Protection in Piglets. Journal of Clinical Microbiology 1982; 16 : 495 - 503.

117. Wyatt R G, Mebus C A, Yolken R H. Rotavirus Immunity in Gnotobiotic Calves: Heterologous Resistance to Human Virus Induced by Bovine Virus. Science 1979; 203:548 - 550.

118. Kapikian A Z, Midthun K, Hoshino Y. Rhesus Rotavirus: A Candidate Vaccine for Prevention of Human Rotavirus Disease. In: Vaccines 85. Molecular and Chemical Basis of Resistance to Parasitic, Bacterial and Viral Diseases. Eds Lerner R A, Chanock R M, Brown F. 1985; 357 - 367. Cold Spring Harbour Laboratory , Cold Spring Harbour, New York.

119. Greenberg H B, Kalica A R, Wyatt R G, Jones R W, Kapikian A Z, Chanock R M. Rescue of Noncultivatable Human Rotavirus by Gene Reassortment During Mixed Infection with ts Mutants of a Cultivable Bovine Rotavirus. Proceedings of the National Academy of Science of the U.S.A. 1981; 78 : 420 - 424.

120. Midthun K, Greenberg H B, Hoshino Y, Kapikian A Z, Wyatt R G, Chanock R M. Reassortant Rotaviruses as Potential Live Rotavirus Vaccine Candidates. Journal of Viroaogy 1985; 53 : 949 - 954.

121. Richardson M A, Iwamota A, Ikegami A, Namota A, Furuichi Y. Nucleotide Sequence of the Gene Encoding the Serotype - Specific Antigen of Human (Wa) Rotavirus: Comparison with the Homologous Genes from Simian SA-11 and UK Bovine Rotaviruses. Journal of Virology 1984; 51 : 860 - 862.

122. Ellman T C, Hoyne P A, Dyall-Smith M L, Holmes I H, Azad A A. Nucleotide Sequence and Gene Encoding for Serotype-Specific Glycoprotein of U.K. Bovine Rotavirus. Nucleic Acids Research 1983; 11 : 4689 - 4701.

123. Delem A, Lobmann M, Zygaich N. A Bovine Rotavirus Developed as a Candidate Vaccine For Use in Humans. Journal of Biological Standardisations 1984; 12 : 443 - 445

124. Kapikian A Z, Cline W L, Greenberg H B, Wyatt R G, Kalica A R, Banks C E, James H D, Flores J, Chanock R M. Antigenic Characterisation of Human and Animal Rotaviruses by Immune Adherence Haemagglutination Assay (I.A.H.A.):Evidence of Distinctness of I.A.H.A. and Neutralising Antigens. Infectious Immunology 1981; 33 : 549 - 550.

125. Zissis G, Lambert P, Marbehant P, Marissens M, Lobmann M, Charlier P, Delem A, Zygraich N. Protection Studies in Colostrum-Deprived Piglets of a Bovine Rotavirus Vaccine Candidate Using Human Rotavirus Strains for Challenge. Journal of Infectious Diseases 1983; 148 : 1061 - 1067.

126. Vesikari T, Isolauri E, Delem A, D'Hondt E, Andre F E, Zissis G. Immunogenicity and Safety of Live Oral Attenuated Bovine Rotavirus Vaccine Strain RIT 4237 in Adults and Young Children. Lancet 1983; ii: 807 - 811.

127. Veskari T, Isolauri M D, Delem A, D'Hondt E, Andre F E, Beards G M, Flewett T H. Clinical Efficacy of the RIT 4237 Live Attenuated Bovine Rotavirus Vaccine in Infants Vaccinated Before a Rotavirus Epidemic. The Journal of Pediatrics 1985; 2 : 189 - 194.

128. Vesikari T, Ruuska T, Delem A, Andre F E. Oral Rotavirus Vaccination in Breast- and Bottle-Fed Infants Aged 6 to 12 Months. Acta Paediatrica Scandinavica 1986; 75 : 573 - 578.

129. Rowland M G M, Barrel R A E. Ecological Factors in Gastroenteritis. In: SSHB Symposia Proceedings 20. Clegg E J, Garlick J P, eds. Diseases and Urbanization. London: Taylor and Francis 1980; 21 - 35.

130. Gambia Government Population Census 1973. Vol III, General Report. Central Statistics Division, Ministry of Economic Planning and Industrial Development 1976.

131. United Nations Demographic Yearbook. United Nations, Washington 1977.

132. Yamuah M. Personal communication.

133. Gambia Government Primary Health Care Review 1985. General Report. Ministry of Health 1985.

134. Greenwood B M. Farafenni Field Studies. Personal Communication.

135. Mabey D. Outpatient Attendance Statistics. Personal Communication.

136. Gray J M. A History of The Gambia. Cambridge University Press 1940.

137. Byass P. "Computers in Africa - Appropriate Technology?" In Press.

138. Herring A J, Inglis N F, Ojeh C K, Snodgrass D R, Menzies J D. Rapid Diagnosis of Rotavirus Infection by Direct Detection of Viral Nucleic Aacid in Silver-stained Polyacrylamide Gels. Journal of Clinical Microbiology 1982; 16 : 473 - 77.

139. Morrs P O, Erinle E A. Influence of Humidity on Rotavirus Prevalence Among Nigerian Infants and Young Children with Diarrhoea. Journal of Clinical Microbiology 1982; 15 : 212 - 215.

140. Mutanda L N, Kinoti S N, Gemert W, Lichenga E O. Age Distribution and Seasonal Patterns of Rotavirus Infection in Children in Kenya, Journal of Diarrhoeal Disease Research 1984; 2 : 147 - 150.

141. Sitbon M, Lecerf A, Garin Y, Iranoff B. Rotavirus Prevalence and relationship With Climatological factors in Gabon, West Africa. Journal of Medical Virology 1985; 16 : 177 - 182.

142. Moe K, Shirley J A. The Effects of Relative Humidity and Temperature on the Survival of Human Rotaviruses in Faeces. Archives of Virology 1982; 72 : 179 - 186.

143. Champsaur H, Questiaux E, Prevot J, Henry-Amar M, Goldszmidt D, Bourjouane M, Bach C. Rotavirus Carrage, Asymptomatic Infection, and Disease in the First Two Years of Life. I. Virus Shedding. The Journal of Infectious Diseases 1984; 149 : 667 - 674.

144. McKeown T, Lowe C R. An Introduction to Social Medicine 1974. Blackwell, Oxford.

145. Khan M U. The Interruption of Shigellosis by Hand Washing. Transactions of the Royal Society of Tropical Medicine and Hygiene 1982; 72 : 164 - 168.

146. Torum B. Environmental and Educational Interventions Against Diarrhoea in Guatamala. In Malnutrition and Diarrhoea. Eds Chen L C, Scrimshaw N S. 1983 Plenum Press, New York ; 253 - 267.

147. Dugdale A E. Infant Feeding, Growth and Mortality. Australian Medical Journal 1980; 380 - 385.

148. Linderbaum S. The Influence of Maternal Education on Infant and Child Mortality in Bangladesh. I.C.D.D.R.B. Report, 1983.

149. Engleberg N C, Holburt E N, Barrett T J, Gary G W, Trujillo M H, Feldman R H, Hughes J M. Epidemiology of Diarrhoea Due to Rotavirus on an Indian Reservation: Risk Factors in the Home Environment. Journal of Infectious Diseases 1982; 145 : 894 - 898.

150. Samadi A R, Huq M I, Ahmed Q S. Detection of Rotavirus in Handwashings of Attendats of Children with Diarrhoea. British Medical Journal 1983; 286 : 188.

151. De Moy P, Zissis G, Butzler J P, Mutwewingabo A, Andre F E. Failure of Live Attenuated Oral Rotavirus Vaccine. Lancet 1986; ii : 108.

152. Flores J, Perez-Schael I, Gonzalez M, Garcia D, Perez M, Daoud N, Cunto W, Chanock R M, Kapikian A Z. Protection Against Severe Rotavirus Diarrhoea by Rhesus Rotavirus Vaccine in Venezuelan Infants. Lancet 1987; i : 882 - 884.

153. Sack D A, Gioman R H, Kapikian A Z, Aziz K M S. Seroepidemiology of Rotavirus Infection in Rural Bangladesh. Journal of Clinical Microbiology; 11 No.5 : 530 - 532.

154. Yesudoss E S, John T J, Mathan M, Spence L. Prevalence of Rotavirus Antibody in Infants and Children. Indian Journal of Medical Research 1978; 383 - 386.

155. Halsey N A. The Optimal Age for Administering Measles Vaccine in Developing Countries. In: Recent Advances in Immunization. Pan American Health Organization 1983, Publication 451.

156. Vesikari T, Isolauri E, D'Hondt E, Delem E, Andre F E. Increased "Take" Rate of Oral Rotavirus Vaccine in Infants After Milk Feed. Lancet 1984; ii : 700.

157. Yolken R H, Wyatt R G, Mata L, Urrutia J J, Garcia B, Chanock R M, Kapikian A Z. Secretory Antibody Directed Against Rotavirus in Human Milk - Measurement by means of Enzyme-Linked Immunosorbent Assay. The Journal of Paediatrics 1978; 93 : 916 - 921.

158. Weinberg R J, Tipton G, Klish W J, Brown M R. Effect of Breast-Feeding on Morbidity in Rotavirus Gastroenteritis. Pediatrics 1984; 74 : 250 - 253.

159. Berger R, Hadziselimovic F, Just M, Reigel F. Influence of Breast Milk on Nosocomial Rotavirus Infections in Infants. Infection 1984; 12 : 171 - 174.

160. Dömök I, Balayan M S, Fayinka O A, Skrtic N, Soneji A D, Harland P S E G. Factors Affecting the Efficacy of Live Polio Vaccine in Warm Climates. Efficacy of Type 1 Sabin Vaccine Administered Together with Antihuman Gamma Globulin Horse Serum on Breast Fed and Artificially Fed Infants in Uganda. Bulletin of the World Health Organization 1974; 51 : 333 - 347.

161. Metsalaar D, Simpson D I H. Practical Virology for Medical Students and Practitioners in Tropical Countries. Oxford University Press; Oxford 1982: 221 - 252.

162. Metselaar D, McDonald K, Gemert W, van Rens M M, Muller A S. Poliomyelitis: Epidemiology and Prophylaxis. 5. Results of a Two and Three Dose Vaccination Experiment. Bulletin of the World health Organization 1977; 55 : 755 - 759.

163. John T J, Jayabal P. Oral Polio Vaccination of Childen in the Tropics. I. The Poor Seroconversion Rates and the Absence of Viral Interference. American Journal of Epidemiology 1972; 96 : 263 - 269.

164. Plotkin S A, Katz M, Brown R E, Pagana J S. Oral Polio Vaccination in Newborn African Infants. American Journal of Diseases in Children 1966; 111 : 27 - 30.

165. John T J, Deverajan L V, Luther L, Vijayarathnam P. Effects of Breast Feeding on Seroresponse of Infants to Oral Poliovirus Vaccination. Pediatrics 1976; 57 : 47 - 53.

166. Peradze T, Montefiore D, Coker G. Oral Poliovirus Vaccination and Breast Feeding. West African Medical Journal 1968; 17 : 122 - 124.

167. John T J, Sajora C. Oral Polio Vaccination in Childern in the Tropics. III. Intercurrent Enterovirus Infections, Vaccine Virus Take and Antibody Response. American Journal of Epidemiology 1975; 102 : 422 - 428.

168. Anon., Polio Reconsidered (Editorial), Lancet 1984; ii : 1309 - 1310.

169. John T J. Poliomyelitis in India: Prospects and Problems in Control. Review of Infectious Diseases 1984: 6 ; 438 - 441.

170. John T J. Antibody Response of Infants in The Tropics to Five Doses of Oral Polio Vaccine. British Medical Journal 1976; 1 : 812 - 813.

171. Gambia Government Maternal Child Health Service. Internal Statistics: Unpublished.

172. Friede A M, Waternaux C, Guyer B, De Jesus A, Filipp L. An Epidemiological Assessment of Immunisation Programme Participation in the Philippines. International Journal of Epidemiology 1985; 14 : 135 - 141.

173. Belcher D W, Nicholas D D, Ofuso-Amaah S, Wurapa F K. A Mass Immunisation Campaign in Rural Ghana: Factors affecting Participation. Public Health Reports 1978; 93 : 170 - 176.

174. Brown J, Djogdom P, Murphy K, Kesseng G, Heymann D. Identifying the Reasons for Low Vaccination Coverage: A Case Study of Yaounde, Cameroon. In Press.

APPENDIX I

Variables recorded on rotavirus risk factors questionnaire.

Variables concerning the compounds and dwellings.

Compound (lived in by case/control).

No. of buildings in compound ? (counted)

No. of people in compound ? (personal census)

No. of children less than 3 years in compound ?

No. of children less than 2 years ?

No. of European style houses in the compound ?

No. of Cement/corrugate houses in the compound ?

No. of Mud/corrugate houses in the compound ?

No. of Mud/thatch houses in the compound ?

Dwelling (occupied by case/control)

No. of people sharing house ?

No. eating out of common bowl ?

No. under three years ?

No. under two years ?

No. sharing bedroom ?

No. sharing bed ?

No. living in European style house ?

No. living in Cement/corrugate house ?

No. living in Mud/corrugate house ?

No. living in Mud/thatch house ?

Water supply and storage.

Mean distance to standpipe (measured in metres) ?

No. storing water in clay jars ?

No. storing water in metal pots ?

No. storing water in plastic buckets ?

No. storing water in glass bottles ?

No. of people using water ?

No. of children using water ?

No. of times/day water collected ?

No.of times/day containers cleaned ?

Sanitation facilities and behaviour.

No. using same pot ?

No. of children using the ground ?

No. of children using a nappy ?

No. of compounds with a latrine ?

Type of sanitation used by adults in dwelling/compound ?

Hygiene and cleanliness.

No. of times compound swept daily ?

How frequently does mother wash her hands ?

Does mothers prepare fresh food for each meal ?

Does mother prepared fresh food for the child daily ?

Does mothers prepare fresh food for child less than daily ?

Cleanliness/tidyness score allocated by field assistant

on a 0 - 5 scale

Is case/control child allowed to sit on the ground ?

Is case/control child in the habit of eating sand ?

Is the same broom used for inside and outside the house ?

Animal Contact.

Is the case/control child.....

In contact with cows ?

In contact with goats ?

In contact with sheep ?

In contact with pigs ?

In contact with dogs ?

In contact with cats ?

In contact with chickens ?

Family background.

Is the mother Mandinka/Wollof/Fulla/Jolla/other tribal group ?

Is the father Mandinka/Wollof/Fulla/Jolla/other tribal group ?

Is mother married/unmarried/divorced/widowed ?

Is the fathers living in compound ?

Is the mother in paid employment ?

Mean number of sibs ?

No. of stillbirths/abortions experienced by case/control's mother ?

No. of deaths in sibs ?

Parental education and attitude to disease.

Years of maternal education ?

Did mother finishing primary school ?

Did mothers finishing secondary school ?

Years of paternal education ?

Did fathers finishing primary school ?

Did fathers finishing secondary school ?

Mothers were asked an open ended question about the cause of disease and illness in their children. Many answers were given but the catagories were contracted down to simply whether the mother had a superstitious view of disease or not.

Parental income and wealth.

Amount (in Dalasie) spent on food per person per day ?

Estimated cash income per month (mother's estimate) ?

Does mother have a paid maid ?

Does the mother earn a cash salary ?

List consumer items in household (check list provided).

APPENDIX II

Variables recorded on the vaccination compliance questionnaire.

Child's compound.

Distance from health centre ?

Style of house ?

Number of rooms ?

Number of residents ?

Parental education.

Years mother spent at school ?

Years father spent at school ?

Did mother complete primary/secondary/post-secondary education ?

Did father complete primary/secondary/post-secondary education ?

Literacy.

(This section was tested, and not simply reported by the respondent).

Whether mother/father could read 1.English 2.Arabic 3.Local language ?

Whether mother/father could write 1.English 2.Arabic 3.Local language ?

Employment and occupation.

Is mother/father in waged employment ?

Is mother/father self employed ?

What is the nature of mother/father's employment ?

Income.

What is the combined monthly income of the mother and father ?

How many of the following are owed by the mother or the father ?

1.sheep 2.goats 3.cattle 4.land 5.motorcycle 6.bicycle 7.radio/cassette 8.refrigerator.

Family structure.

Ethnic group of mother ?

Ethnic group of father ?

Marital status of mother ? Single parent, divorced, 1st, 2nd or 3rd wife ?

Number of children born alive to the mother ?

Number of stillbirths experienced by mother ?

Number of deaths among children born to the mother ?

Who was the normal care giver for the child ?

Attitudes and knowledge.

Mother's opinion on the causes of childhood disease ?

Mother was asked to name any diseases against which her child should be vaccinated.

Where is the child normally taken when he/she is unwell ?

Under which circumstances does the mother take her child to the health centre ?

a) when the child is sick ?
b) when the child needed vaccination, even if he/she was not unwell at that time ?
c) for weighing, even if the child is not unwell ?

Does the mother believe that vaccination can harm the child in any way ? If yes - in what ways ?

Had the mother experienced any difficulties or problems at the health centre with respect to treatment, vaccinations or with the staff ?

Published papers arising form this work.

Copies of these papers can be found in a wallet in the back cover of this thesis. Where the article is "in press" the final proof version has been substituted.

1. Trial of an Attenuated Bovine Rotavirus Vaccine (RIT 4237) in Gambian Infants.
   Lancet 1987; i : 1342 - 1345.

2. Serological Comparisons of Approaches to Polio Vaccination in Gambian Infants.
   Lancet 1987; i : 800 - 801.

3. Epidemiology of Rotavirus in a Periurban Gambian Community.
   Annals of Tropical Paediatrics 1987; 7 : 6 - 11.

4. Factors Influencing Vaccination Compliance in Peri-urban Gambian Children.
   Journal of Tropical Medicine and Hygiene 1987; In press.