CRYPTOSPORIDIOSIS IN MALNOURISHED CHILDREN AT KENYATTA NATIONAL HOSPITAL

BY

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D E C L A R A T I O N

I declare that this dissertation is my own original work and has not been published elsewhere, or presented for a degree in any other University.

Signed: .....

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We certify that this dissertation has been submitted to the University of Nairobi with our approval as University Supervisors.

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Dedication

To

My Parents

ABBREVIATIONS

KNH	-	Kenyatta National Hospital
PEW	-	Paediatric Emergency Ward
HIV	-	Human Immunodeficiency Virus
PEM	-	Protein Energy Malnutrition
VRC	-	Virology Research Centre
CMR	-	Centre for Microbiology Research
KEMRI	-	Kenya Medical Research Institute
ELISA	-	Enzyme Linked Immunosorbent Assay
OR	-	Odds Ratio
CI	-	Confidence Interval

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SUMMARY

Cryptosporidiosis in malnourished children at the Paediatric Emergency Ward (PEW) of Kenyatta National Hospital was studied. This was done over a three months period, September to December 1989. Two hundred and eighty five patients were studied, 24 of whom were HIV positive. Their age range was 14 days - 60 months, with a mean range of 17 months. The male to female ratio was 1:1.1.

Among the 256 patients negative for HIV, 46 had Cryptosporidium oocyts in stool giving a prevalence of 18%. These were 22 males and 24 females giving a M:F ratio of 1:1.1. Their age range was 8 to 37 months with a mean age of 18.2 months and a median of 16 months. The highest prevalence occurred in patients with marasmic-kwashiorkor (21.7%) followed by underweight, kwashiorkor and marasmus with 20.3%, 18.4% and 10.5% respectively.

Ninety nine (38.7%) of the 256 patients had diarrhoea. Eighteen (18%) of the ninety nine were positive for Cryptosporidium as were 28 (18%) of the 157 without diarrhoea.

Fever (Temp. ≥ 37.5°C) was present in 93 out of the 256 forming 36.7%. Among these with fever, 17.2% were Cryptosporidial positive compared to 18.4% Cryptosporidial positive with no fever.

Out of 256 patients, 30 had measles, 90% of which occurred within 6 weeks prior to the study. Nine of these were Cryptosporidial positive giving a prevalence of 30% compared to 16.4% in the non-measles cases.

HIV results were available for 285 patients. Of these, 24 were HIV positive giving a prevalence of 8.4% among the malnourished children. Two (8.3%) out of 24 HIV positive patients had Cryptosporidium oocyts in stool.

The report of this study shows the Cryptosporidium is highly prevalent in Kenyan malnourished children. We therefore recommend routine laboratory examination for Cryptosporidium in stool specimens of malnourished children.

INTRODUCTION

Cryptosporidium is a coccidial protozoa parasite belonging to the same family as Toxoplasma, Sarcocystis, Eimeria, Plasmodium and Babesia. Taxonomic position of Cryptosporidium is shown in appendix I (1).

According to the American Parasitologist E.E. Tyzzer, the first published description of a parasite resembling Cryptosporidium in the gastric epithelium of mice, was by Clark in 1895 (2). In 1907, Tyzzer himself clearly described the organism found on the gastrointestinal mucosa of asymptomatic mice (1,2,3). It was not until 1955 when it was associated with morbidity and mortality by Slavin who reported diarrhoeal illness in turkeys (1,2).

The first case of human cryptosporidiosis was reported in 1976 by None et al (1,3,4). Between then and 1981 the first seven cases were reported five of which were in immunocompromised patients. In 1981 - 1982, 47 additional cases were reported to the Centre for Disease Control, Atlanta, mostly involving patients with the acquired immunodeficiency syndrome (AIDS). The number of reported cases by 1986 was about 500 and since then the number has increased steadily because of development of rapid and convenient screening methods (1,3,4,5).

Prior to its description in human, Cryptosporidium was regarded exclusively as a pathogen of animals. The prevalence of human cryptosporidiosis is not yet well established. Although initially associated with a compromised immunity, it is now clear that persons with normal immunologic functions are affected as well (1,6).

Available evidence shows that human Cryptosporidium infection is a world wide problem (Appendix II). In some areas it has been shown to be a common cause of diarrhoea (1,3).

A report from several studies done in Europe showed that the prevalence of Cryptosporidium infection in humans was between 1 and 23%, the higher figures being from populations with diarrhoea (2). These figures vary from place to place.

Many studies in different parts of the world show that children are more affected by Cryptosporidium than adults (5,9,25,34). Adjel et al in Ghana showed oocysts occurrence to be highest in the age group 0 - 9 years (9).

A number of studies screening for Cryptosporidium specifically in children with diarrhoea have found incidences ranging from 1.4% to 22% (Appendix III).

It has been suggested that the prevalence of Cryptosporidium is higher in developing countries (1,2,3,6). Neils Hojlyng and others in Liberia in 1984 screened Cryptosporidium in children with diarrhoea and found oocysts in 7.9% (10). Abjel et al (1985) found oocysts in 10.7% of stools from Ghanian patients with diarrhoea, while Addy et al. (1986) also in Ghana found 12.9% of children aged 2 months to 5 years with diarrhoea to have Cryptosporidium (9,11). Simwa et al. in 1985 screened children under five years with diarrhoea in Kiambu and found a prevalence of 3.9% (12).

Cryptosporidium has been implicated as a cause of severe illness in the immunocompromised. Melbye in Rwanda (13) showed Cryptosporidium to be associated with diarrhoea accompanying measles in the acute phase of the diarrhoea at the time of the depression of the cellular immune response; nine (7.1%) out of 127 patients had Cryptosporidium in stool. Six (25%) out of 24 patients who had measles and diarrhoea were positive for Cryptosporidium compared to 2(4.3%) of 48 non-measles patients with diarrhoea. This difference was statistically significant. Macfarlane (1985) in his study of wellnourished and malnourished children in Jamaica showed Cryptosporidium oocysts in 23.7% of stools from malnourished children with diarrhoea compared to 2.2% in diarrhoea stools of the well nourished (14). This difference was highly significant. Sarah Sallon in Israel found a prevalence of 13.5% in Israel children with

malnutrition. In 113 patients who had severe malnutrition 20 (17.6%) had Cryptosporidium in their stools as compared to 10 (9.8%) of the 102 patients with mild malnutrition; this difference was not statistically significant though (15).

It is worthy to note that the protozoa parasitises the epithelial surfaces of patients with severe T-cell defects (1,6). Children with PEM have impaired immunity and characteristically present with an increased incidence and severity of infection. In PEM the following defects in the immune system have been described; impaired cell mediated immunity, humoral immunity, inflammatory response, complement system, intracellular killing and reduced interferon production (22). Available evidence shows that children with severe kwashiorkor have the lowest percentage of Rosette-forming T-cells. Moderate kwashiorkor cases have slightly higher levels while mild kwashiorkor have levels between moderate and controls (22). It has also been shown that children with kwashiorkor have a lower percentage of T-cells than the marasmic children and that both have a lower percentage than the well nourished children (22).

In the Macfarlane study in Jamaica 23.7% of the malnourished children with diarrhoea had Cryptosporidium isolated from their stools while 19.5% of the malnourished without diarrhoea had stools positive for Cryptosporidium.

But only 2.2% of well nourished with diarrhoea were positive for Cryptosporidium (14). This showed that malnourished children are particularly predisposed to infection with this organism. He also found that the malnourished children had a more protracted gastroentrities with dehydration, fever and vomiting.

In rural Kenya about 40% of children under 5 years of age suffer from PEM, 2-5% being of severe category (18). Observations from Paediatric Emergency Ward at KNH (1987) show that malnutrition accounts for 7% of admissions and one should note that most of mild to moderate cases are treated elsewhere as outpatient (29). Mortality at PEW is 5.2%, 15% of which is accounted for by PEM (29).

Wasunna et al in 1983 studied children with diarrhoea in PEW and found no pathogens in stools of 37.3% of patients. In this study the malnourished children were excluded (19). Oburra et al in 1986 in a similar study in the same place found no pathogens in 55% of stools; this study comprised both malnourished and well nourished children (20). The difference probably suggests that there are other pathogenic organisms in diarrhoea which could be commoner in the malnourished children. Since malnutrition is a prominent cause of morbidity and mortality in our set up, the search for other diarrhoeal pathogens should be advocated in order to broaden our knowledge on this problem and subsequently manage it better.

RATIONALE

Data on Cryptosporidial infection is especially scanty in developing world. This protozoa has been reported to cause severe diarrhoea disease in immuno-suppressed patients e.g. malnutrition. Malnutrition is a prominent cause of morbidity (7% of all admissions) and mortality (15% of all deaths in PEW) in our set up. The prevalence of Cryptosporidium in malnourished children in KNH, Kenya, and many other countries of the world is not known. In order to broaden our knowledge on this organism, the author was prompted to carry out a prevalence study in the malnourished children age 0 - 5 years at the PEW of Kenyatta National Hospital, Nairobi, Kenya.

STUDY OBJECTIVE

To determine the prevalence of Cryptosporidium in malnourished children at Kenyatta National Hospital, Kenya.

HYPOTHESIS:

The proportion of malnourished children in which Cryptosporidium is isolated is 20%; this was infered from prevalence studies in developing world where incidences of 4 - 20% are described coupled with Macfarlane's and Sarah Sallon's findings of higher prevalences in the malnourished (14,15,37).

STUDY DESIGN:

This was a cross-sectional descriptive study carried out in an attempt to describe the prevalance of Cryptosporidium in the malnourished children.

SAMPLE SIZE:

With an estimated prevalence of cryptosporidiosis of 20%, the sample size required to give a 95% confidence interval of width 5% is:

$$N = \frac{P(100 - P) \quad (Z_1 - \frac{\alpha}{2})^2}{d^2}$$

Where P is the estimated prevalence $Z_1 - \frac{\alpha}{2}$ is the standing normal deviate for 0.05.

d is the width of the confidence interval.

$$N = \frac{20(80) \times 3.842}{5^2}$$

$$= \frac{1600 \times 3.842}{25}$$

$$= 246 \simeq 250 \text{ patients.}$$

STUDY FACTOR:

Malnourished children.

1. STUDY AREA

The study was carried out at the Paediatric Emergency Ward of Kenyatta National Hospital. This is the acute admission ward for the department of paediatrics.

2. STUDY POPULATION AND CASE SELECTION:

Malnourished patients 5 years and below identified using the Wellcome classification (23,31) formed the study population. The investigator visited PEW twice a day and all the new admissions of the particular day were weighed unclothed using the "Toledo Scale Model 1361 Sentinel" spring balance which weighs to 0.5 Kg accuracy. For those identified to be malnourished using the Wellcome classification, the investigator explained the rationale, benefits and risks of the study to the parent or guardian; subsequently a verbal consent was obtained.

The patient's history of illness was taken, physical examination done and details filled in the study proforma (Appendix IV).

After the interview, 2 - 3 ml of blood was taken from a peripheral vein under aseptic precautions and put in a plain bottle. It was forwarded within 1 hour to Virology Research Centre (VRC), KEMRI, for HIV screening.

Western blot test was done for those patients with an ELISA positive test. The same blood specimen was used for the western blot. HIV positive patients were taken as those positive on both ELISA and western blot.

Stools were taken and sent to Centre for Microbiology Research (CMR), KEMRI, within 1 hour of collection. Two slides were made from each specimen and both were subjected to Kinyoun acid-fast stain (Appendix V) for detection of Cryptosporidium. This was done by the investigator who subsequently examined the slides together with a technician from CMR. Whether one or both slides showed Cryptosporidium oocysts that patient was considered as having Cryptosporidiosis.

2.1 Inclusion criteria

(a) Presence of protein energy malnutrition as per Wellcome classification (23,24) with or without diarrhoea. Diarrhoea was defined as passage of 3 or more, watery or loose stools in 24 hours (19,20,30,32).

(b) Age: 0 to 60 months.

2.2 Exclusion criteria

(a) Drug therapy of any kind in the preceding one week (enquired from parent or guardian by author).

(b) Patients with chronic illnesses such as tuberculosis, renal disease, congenital heart disease or mental retardation.

3. ETHICAL CONSIDERATIONS

Stool for examination was collected in plastic containers (polypots). Where stools were very watery, sterile swabs were used to get the specimen from the rectum.

Two to three millilitres of venous blood was withdrawn from a peripheral vein, after cleaning the area with methylated spirit, and put in a plain bottle for HIV screening.

Data on Cryptosporidiosis in KNH, Kenya, is not available and it is hoped that the results of this study will benefit in patient management country-wide.

4. DATA ANALYSTS

The data was summarised into frequency tables. Percentages were calculated and cross-tabulations done for comparison of various factors. Chi-square statistics and Fisher's Exact Test were used for statistical analysis. Point estimates and 95% confidence intervals were calculated.

RESULTS

The study was carried out between September to December 1989. Two hundred and eighty five patients were recruited. Five patients did not give a stool specimen and were subsequently not described in Cryptosporidium analysis. Of the 280 patients whose data was complete, 24 were HIV positive. Detailed analysis for the 256 negative for HIV are presented and the HIV positive cases are described.

The age range of the 285 patients was ½ - 60 months with a mean age of 17 months and M:F ratio of 1:1.1. The same holds for the sub-groups of 280 patients with complete data and the 256 HIV negative patients.

Among the 256, 122 (47.7%) were males and 134 (52.3%) were females. Their age distribution is shown in Table I.

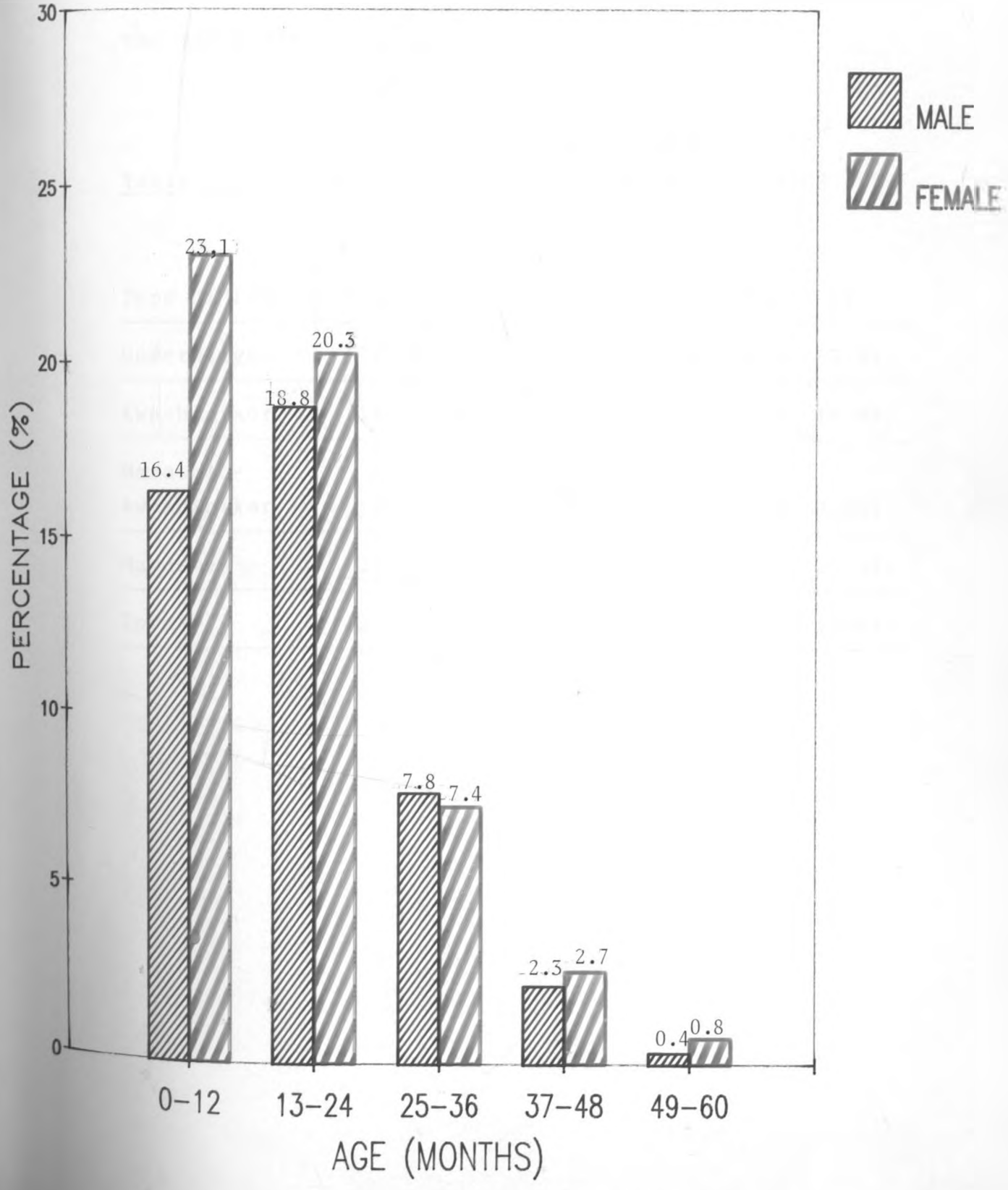
Table I: AGE DISTRIBUTION OF PATIENTS

AGE (MONTHS)	FREQUENCY	%	CUMM. %
0 - 6	42	16.4	16.4
7 - 12	59	23.0	39.4
13 - 24	100	39.1	78.5
25 - 36	39	15.2	93.7
37 - 48	13	5.1	98.8
49 - 60	3	1.2	100
TOTAL	256	100.0	

78.5% of these patients were 2 years and below, confirming that protein energy malnutrition is found in the younger child.

The age and sex distribution is as shown in Figure I. In the infants, there were more females 59 (58.4%) than males 42 (41.6%) compared to the equal distribution in the older children. However this showed no statistical significance with P value of 0.87.

FIGURE 1
AGE AND SEX DISTRIBUTION OF PATIENTS.



Among these patients, 138 (53.9%) were underweight, 38 (14.8%) had kwashiorkor, 23 (9%) had marasmic - kwashiorkor and 57 (22.3%) had marasmus as shown in Table II. The sex distribution was the same in each of the malnutrition categories.

Table II: SEX DISTRIBUTION BY NUTRITIONAL STATUS

	S E X		
Type of PEM	Males (%)	Females (%)	Total (%)
Underweight	72 (59%)	66 (49.3%)	138 (53.9%)
Kwashiorkor	18(14.8%)	20 (14.9%)	38 (14.8%)
Marasmic-kwashiorkor	10 (8.2%)	13 (9.7%)	23 (9.0%)
Marasmus	22 (18.0%)	35 (26.1%)	57 (22.3%)
Total	122 (100%)	134 (100%)	256 (100%)

Table III: CRYPTOSPORIDIUM OCCURRENCE BY AGE

AGE (Months)	CRYP +ve	TOTALS	% +ve	P. Value*
0 - 6	0	42	%	
7 - 12	16	59	27.1%	0.0004
13 - 24	19	100	19%	0.002
25 - 36	9	39	23%	0.002
37 - 48	2	13	15.3%	0.07
49 - 60	0	3	0%	0.9999
Total	46	256	18%	

* By Fisher's exact test.

Table III shows that Cryptosporidium was found in 46 patients giving a prevalence of 18% and that Cryptosporidium occurred between 7 months to 4 years. The peak age group was 7 - 12 months. The mean age was 18.2 months and a median of 16 months. No patient 6 months and below had Cryptosporidium in stool.

The occurrence of the infections in the age groups 7 - 12 months, 13 - 24 months, and 25 - 36 months were highly significant, using age group 0 - 60 months as the reference point. For the age group 49-60 months there was no statistical difference in the occurrence of infection, a factor that is probably due to the small sample size in this age group.

Table IV: DISTRIBUTION OF CRYPTOSPORIDIUM INFECTION BY SEX

Sex	Cryptosporidium		Total
	+ve	-ve	
Male	22 (47.8%)	100 (47.6%)	122
Female	24 (52.2%)	110 (52.4%)	134
Total	46	210	256

X^2 = 0.019

P = 0.89

OR = 1.008

95% C1 0.5 - 2.001

Table IV shows that Cryptosporidium has no sex predilection.

Table V: CRYPTOSPORIDIAL INFECTION AMONG THE TYPES OF MALNUTRITION.

Type of malnutrition	Number of Patients Cryp +ve	Cryp -ve	% +ve	P Value+
Underweight	28	110	20.3	
Kwashiorkor	7	31	18.4	0.98
Marasmic-kwashiorkor	5	18	21.7	0.91
Marasmus	6	51	10.5	0.15
*Severe malnutrition	18	100	18	0.38

+ By X^2 test.

* Severe malnutrition = kwashiorkor, marasmic-kwashiorkor and marasmus.

Cryptosporidium was most prevalent in the marasmic-kwashiorkor patients (21.7%), followed by underweight, kwashiorkor and marasmic patients having 20.3%, 18.4% and 10.5% respectively. When these groups were subjected to statistical test taking underweight as the baseline, there was no statistical difference. This holds true when each group is tested individually or when they are all combined. All P values were greater than 0.1.

Table VI: SEX DISTRIBUTION OF CRYPTOSPORIDIUM IN MALNOURISHED CHILDREN.

Type of Malnut.	S E X					
	Males			Females		
	Total	Cryp +ve	% +ve	Total	Cryp +ve	% +ve
Underweight	72	15	20.8%	66	13	19.7
Kwashiorkor	18	5	27.8%	20	2	10%
Marasmic-Kwashiorkor	10	1	10%	13	4	30.8%
Marasmus	22	1	4.5%	35	5	14.3%
Total	122	22	18%	134	24	17.9%

Cryptosporidium occurrence in the underweights seem to be uniformily distributed among the sexes, with 15 (20.8%) out of 72 males positive compared to 13 (19.7%) out of 66 females. Larger proportions (30.8% and 14.3%) are found in the females than in the males among the marasmic-kwashiorkor and marasmic children, but among the kwashiorkor patients comparatively more males than females were involved. The figures in these groups are however too small to make any conclusions.

Table VII: PREVALENCE OF CRYPTOSPORIDIUM AMONG PATIENTS WITH AND WITHOUT DIARRHOEA

Cryptosporidium	Diarrhoea +	Diarrhoea -	Total
+	18(18%)	28(18%)	46
-	81	129	210
Total	99	157	256

X^2 = 0.009

P = 0.92

OR = 1.02

95% C1 0.51 - 2.10

This shows that 99 (38.7%) out of 256 patients had diarrhoea. Eighteen of these patients were Cryptosporidium positive.

As is shown in Table VII above, there was equal prevalence (18%) of Cryptosporidial infection among those presenting with diarrhoea and with no diarrhoea (P value 0.92).

Table VIII: DURATION OF DIARRHOEA IN THE PATIENTS STUDIED.

Duration (days)	Cryptosporidium +ve	Cryptosporidium -ve	Total
⩽ 7	11(15%)	63	74
>7	7(28%)	18	25
Total	18	81	99

X^2 = 1.37

P = 0.24

OR = 0.45

95% C1 0.13 - 1.51

Table VIII shows that majority 74(74.7%) of the diarrhoeal cases presented with short duration (⩽ 7/7) diarrhoea. Cryptosporidium was present in 11 (15%) of those with short duration diarrhoea compared to 7 (28%) of patients with long duration diarrhoea. This difference was not statistically significant with P = 0.24, OR = 0.45, and 95% C1 of 0.13 to 1.51.

Table IX: PREVALENCE OF DIARRHOEA IN THE DIFFERENT TYPE OF MALNUTRITION IN CRYPTOSPORIDIUM POSITIVE PATIENTS

Type of Malnutrition	Diarrhoea		Total
	+ve	-ve	
Underweight	8(28.6%)	20	28
Kwashiorkor	4(57.1%)	3	7
Marasmic-kwashiorkor	3(60.0%)	2	5
Marasmus	3(50.0%)	3	6
Total	18	28	46

Diarrhoea occurred among all classes of malnutrition with the frequencies as shown in Table IX.

Diarrhoea was prominent among the marasmic-kwashiorkor (60.0%), kwashiorkor (57.1%) and marasmus (50.0%) as compared to underweight (28.6%) patients; however the numbers are too small to make a definite conclusion within separate PEM entities.

Table X: PREVALENCE OF DIARRHOEA BY DEGREE OF MALNUTRITION.

Type of Malnutrition	Diarrhoea		Total
	+ve	-ve	
*Severe malnutrition	10(55.6%)	8	18
Underweight (mild malnutrition)	8(44.4%)	20	28
Total	18	28	46

X^2 = 2.31

P = 0.13

OR = 3.13

95% C1 0.77 - 13.16

*Severe malnutrition = kwashiorkor, marasmic-kwashiorkor and marasmus.

Table X above shows that diarrhoea occurred with the same frequency among the patients with severe malnutrition as it did among the patients who were underweight.

Table XI: PREVALENCE OF DIARRHOEA IN CRYPTOSPORIDIUM POSITIVE MALNOURISHED CHILDREN.

Type of Malnutrition	Duration of diarrhoea (Days)		Total
	>7	⩽7	
Severe Malnutrition	6(66.7%)	4(33.3%)	8
Underweight	2(25%)	6(75%)	10
Total	8(44.4%)	10(55.6%)	18

P = 0.16 by Fisher's exact test.

Short duration diarrhoea occurred in 55.6% of patients compared to 44.4% with long duration diarrhoea. Long duration diarrhoea was more common among severe forms of malnutrition (66.7%) compared to 25% in the underweight patients. This difference was not statistically significant with a P = 0.16.

Table XII: ASSOCIATION BETWEEN CRYPTOSPORIDIAL INFECTION AND FREQUENCY OF STOOLS IN 24 HOURS.

Number of stools in 24 hours	Diarrhoea		Total
	Cryp +ve	Cryp -ve	
>5	9(50%)	31(38.3%)	40
3 - 5	9(50%)	50(61.7%)	59
Total	18	81	99

X^2 = 0.42

P = 0.51

OR = 1.61

95% C1 0.52 - 5.06

Table XII shows that 50% of those with Cryptosporidial infection had more than 5 diarrhoeal stools per day compared to 38.3% amongst those without Cryptosporidium. This difference was not statistically significant, P = 0.51, OR = 1.61 and 95% C1 between 0.52 and 5.06.

Table XIII: FEVER AMONG CRYPTOSPORIDIUM POSITIVE CHILDREN.

Fever	Cryptosporidium		Total
	+ve	-ve	
≥ 37.5°C	16(17.2%)	77(82.8%)	93
< 37.5°C	30(18.4%)	133(81.6%)	163
Total	46	210	256

X^2 = 0.005

P = 0.94

OR = 0.92

95% C1 0.45 - 1.89

Since there is no standardised definition of fever, the author took temperature ≥ 37.5°C as fever.

Table XIII therefore shows that 93(36.3%) patients had fever. Sixteen (17.2%) of these patients had Cryptosporidial infection while among those without fever, 30(18.4%) had Cryptosporidial infection. There was no statistical difference between the two groups (P = 0.94, OR = 0.92 and 95% C1 of 0.45 to 1.89).

MEASLES CASES:

Out of the 256 patients, 30 had measles, 27(90%) of whom had suffered measles within 6 weeks before recruitment into the study. Nine out of the 30 were Cryptosporidium positive giving a prevalence of 30% among measles patients compared to 16.4% in the non-measles patients. This information is shown on Table XIV.

Table XIV: CRYPTOSPORIDIOSIS AMONG POST-MEASLES PATIENTS

Measles	Cryptosporidium		Total
	+ve	-ve	
Yes	9(30%)	21	30
No	37(16.4%)	189	226
Total	46	210	256

X^2 = 2.48

P = 0.12

OR = 2.19

95% C1 0.85 - 5.53

When the two groups were subjected to statistical test, there was no statistical difference (P = 0.12, OR = 2.19 and 95% C1 between 0.85 and 5.53).

Table XV: TYPE OF MALNUTRITION IN POST-MEASLES PATIENTS

Type of Malnutrition	Number of Patients Cryp +ve	Cryp -ve	Total
Underweight	4 (25%)	12	16
Kwashiorkor	3 (42.9%)	4	7
Marasmic-kwashiorkor	0 (0%)	1	1
Marasmus	2 (33.3%)	4	6
Total	9	21	30

Only one patient had marasmic-kwashiorkor. Among the other classes of malnutrition, kwashiorkor patients had the highest prevalence (42.9%) of Cryptosporidiosis followed by marasmus and underweight with 33.3% and 25% respectively, but the numbers are too small for any definite conclusion.

Table XVI shows the same information where underweight children were compared with those having severe forms of malnutrition.

Table XVI: PREVALENCE OF CRYPTOSPORIDIUM IN POST-MEASLES CHILDREN.

Degree of Malnutrition	Number of Patients		
	Cryp +ve	Cryp -ve	Total
Severe Malnutrition	5	9	14
Underweight	4	12	16
Total	9	21	30

P = 0.40 by Fisher's exact test.

There was no statistical significance in the occurrence of Cryptosporidium among underweight and the severe forms of malnutrition.

Table XVII: PREVALENCE OF CRYPTOSPORIDIUM AMONG MALNOURISHED POST-MEASLES PATIENTS WITH DIARRHOEA

Diarrhoea	Cryptosporidium		Total
	+ve	-ve	
+ve	4(22.2%)	14	18
-ve	5(41.7%)	7	12
Total	9	21	30

P = 0.26 by Fisher's exact test.

Among the measles patients 18 (60%) out of 30 had diarrhoea; thus majority of measles patients presented with diarrhoea. The prevalence of Cryptosporidium in the diarrhoeal cases was 22.2% (4 out of 18) compared to 41.7% in the non-diarrhoeal cases. There was no statistical difference between the two groups with a P value of 0.26.

HIV POSITIVE CASES:

Among the 285 patients, 24 were HIV positive giving a prevalence of 8.4% among the malnourished children. These comprised of 13 males and 11 females. Their mean age was 14.2 months while in the HIV negative patients it was 17 months.

Two out of the 24 HIV positive patients had Cryptosporidium oocysts in the stool. This gives a prevalence of 8.3% which is lower than in the HIV negative patients (18%). This information is depicted in Table XVIII.

Table XVIII PREVALENCE OF CRYPTOSPORIDIUM AMONG MALNOURISHED POSITIVE PATIENTS.

HIV	Cryptosporidium		Total
	+ve	-ve	
+ve	2(8.3%)	22	24
-ve	46(18%)	210	256
Total	48	232	280

P = 0.18 by Fisher's exact test.

There was no statistical significance in Cryptosporidial occurrence among HIV positive and HIV negative patients with a P value of 0.18.

One patient had kwashiorkor (4.2%), 4(16.7%) had marasmic-kwashiorkor, 8(33.3%) had marasmus, while 11(45.8%) were underweight.

Eleven (45.8%) of HIV positive had diarrhoea compared to 38.7% in HIV negative patients.

DISCUSSION

Cryptosporidium was found in 46 (18%) out of 256 malnourished patients who were HIV negative. This is within the range quoted for developing countries of 4 - 20% (37,38). The figure also agrees with figures from several other countries found in malnourished children such as Macfarlane's figure of 19.5% and Sallon's of 13.5% (14,15). It is however much higher than the figures reported for well nourished children. Macfarlane's figures for the well nourished were 2.2% (14) . In Kenya a study by Simwa in 1985 on well nourished children aged 0 - 60 months with diarrhoea in a rural community found a prevalence of any 3.9% (12). Estambale in 1989 looked at stool specimens sent to the laboratory for parasitology examination at KNH,; 4 (10%) out of 40 children's specimens were positive for Cryptosporidium. His sample size was however very small making his figures very unstable and difficult to make conclusive report on.

Cryptosporidium occurred between 8 - 37 months of age. In Macfarlane's study, all his patients with Cryptosporidiosis were 2½ years and below though he did not specify the age range of the patients he studied. Juan in Chile (28) studied children 0 - 13 years and all the positive cases were less than 4 years old.

The peak age group of Cryptosporidium is variable in different studies. Simwa found the peak age group of 7 - 12 months of age (12) as was found in this study. Neils in Liberia studied children 0 - 60 months and found a peak age group of 6 - 12 months old (10) while Addy in Ghana (11) studied children aged 2 - 60 months and found that Cryptosporidiosis was most common in the youngest age group (2 - 12 months), decreasing with increasing age. In Haiti, however, Pape found a peak age group of 18 - 24 months though he studied children under 2 years (39). Similarly in Chile, Juan found Cryptosporidiosis to peak during 2nd year of life (28). All these studies show that Cryptosporidiosis is a disease of the younger child.

No patient 7 months and below had Cryptosporidium in this study. This is the group likely to be on breast milk only. Pape in Haiti (39) found children who were exclusively on breast feeds to have the lowest incidence of cryptosporidiosis. He suggested that exclusive breastfeeding appeared to protect against the development of cryptosporidiosis although some cases did occur in wholly breast fed older infants. John Wolfson in Boston (5) similarly found no cases under one year of age and suggested this may reflect on breast feeding or other unexplained factors. In other studies those patients below 6 months seem to have a lower occurrence of Cryptosporidium, for example in the

Haiti study, Pape found that below 6 months the incidence was half as common compared to those over 6 months of age (39).

The M:F ratio of the Cryptosporidium positive cases was 1:1.1 in this study which is the same for the whole study group. There was no statistical difference between males and females (P=0.89). This agrees with series which indicate no sex difference (1, 2, 7).

Cryptosporidium was most prevalent in the marasmic-kwashiorkor (21.7%), followed by underweight, kwashiorkor and marasmus having 20.3%, 18.4% and 10.5% respectively. No other studies have categorised their patients in a similar manner for comparison. The high prevalence among the underweight cases cannot be explained in this study since one expects them to have a better immunological status compared to other categories of PEM (22).

Ninety nine (38.7%) out of 256 patients had diarrhoea. Eighteen (18%) of these patients were Cryptosporidium positive. Cryptosporidium has been reported to be a common cause of diarrhoea (1 - 4, 6 - 8). However this study shows that the prevalence of diarrhoea in those infected with Cryptosporidium is similar to that in those without Cryptosporidium, suggesting that Cryptosporidium may not be a major cause of diarrhoea in the malnourished. One however needs to carry out a prospective study to establish this

causative association.

Seventy four patients (74.7%) of the diarrhoeal cases presented with short duration (≤ 7/7) diarrhoea. Cryptosporidium was present in 11 (15%) of those with short duration diarrhoea compared to 7 (28%) of patients with long duration diarrhoea (> 7/7). Though Cryptosporidium was more associated with prolonged diarrhoea than those with short duration diarrhoea, this difference was not statistically significant (P = 0.16). Failure to show a significant difference may be a factor of small sample size. Several reviews have associated Cryptosporidial infection with prolonged diarrhoea (1,2,6). The finding in this study however agrees with that of Simwa (12) in which he showed no difference between the prevalence of Cryptosporidium in short duration diarrhoea (4.0%) and long duration diarrhoea (3.7%).

Of the Cryptosporidial positive patients with diarrhoea, 9 (50%) had more than 5 diarrhoeal stools per day compared to 31 (38.3%) among those without Cryptosporidiosis. This difference was not statistically significant (P = 0.51) suggesting that Cryptosporidium may not be associated with severe diarrhoea in the malnourished. A review by Gracia (33) indicated that Cryptosporidium is associated with 5 - 10 stools per day, and other studies have reported Cryptosporidium to be associated with profuse, watery, frequent diarrhoea with some reports giving as many as 71 stools per day in adults (2,4).

Literature associates Cryptosporidium with low grade fever (Temp. < 39°C) (1,2,6,7). In this study 16 (17.2%) out of the 93 patients who had fever (Temp. ≥ 37.5%) had Cryptosporidium compared to 30 (18.4%) out of 163 who had no fever. No studies in malnourished children have shown the pattern of temperature in Cryptosporidial cases. However patients in this study had intercurrent illnesses other than Cryptosporidiosis and so fever here cannot be explained on any one disease entity.

Patients with measles have immunological derangements, worse during the first six weeks after an attack but can continue upto 1 year (35, 36). Out of the 256 patients, 30 patients had measles attack of whom 27 (90%) had the attack 6 weeks prior to the study. Nine out of the 30 were Cryptosporidium positive giving a prevalence of 30% among measles patients compared to 16.4% in the non-measles patients. These figures are higher but consistent with those of Melbye (13) in which 7 (16.3%) out of 43 patients with measles had Cryptosporidium in stool compared to 2(2.3%) out of 84 non-measles cases. The high prevalence of Cryptosporidium in measles patients in this study is because the two factors of measles and malnutrition are possibly additive as far as immunosuppression is concerned (22,35,36). However the prevalence of Cryptosporidiosis (30%) among measles cases in this study was not statistically different from that in the non-measles patients (16.4%).

Among the 24 patients who were HIV positive, 2 had Cryptosporidium oocysts in stool. This gave a prevalence of 8.3% which is lower than in the HIV negative patients (18%). Though HIV positive patients also have depressed T lymphocytes, this finding disputes literature which suggests that Cryptosporidium is mainly found in HIV patients (1,3,4).

DRAWBACKS AND COMMENTS:

1. Drug exclusion in this study was from history only and therefore may be considered inaccurate.

2. Most mothers would know a number of illnesses like measles. Therefore, for every patient the author enquired the history and date of onset of measles. This was used as a basis for calculating the duration between measles attack and date of recruitment into the study. A few patients were having measles and the date of onset of the skin rash was taken to calculate the duration of illness. Thus most of the cases I analysed as "measles cases" were actually post measles patients.

3. The results of this study show no statistical significance between Cryptosporidial occurrence and diarrhoea, measles and HIV positive patients as shown by some workers. This could be due to the fact that the staining method used has high specificity (33) and low sensitivity (40).

CONCLUSIONS

1. The prevalence of Cryptosporidium in the malnourished children is 18%.

2. Cryptosporidium in the malnourished children is more prevalent in the young child between ½ - 3 years but rare before 6 months.

3. There is an equal sex distribution of Cryptosporidial infection.

4. The prevalence of Cryptosporidium infection among malnourished children presenting with diarrhoea and among those presenting with no diarrhoea is the same.

5. The prevalance of Cryptosporidium is higher in malnourished post-measles children compared to non-measles cases.

6. HIV positive malnourished patients seem to have a lower prevalence of Cryptosporidium than malnourished HIV negative patients.

7. There is no evidence that Cryptosporidium is associated with chronic diarrhoea.

RECOMMENDATIONS

1. Routine laboratory studies to identify this parasite in stools of malnourished children should be considered.

2. Case controlled studies to find the prevalence of the organism in well nourished and compare with the malnourished should be carried out.

3. Studies to identify all the possible pathogens in diarrhoea should be done in order to see clearly what role Cryptosporidium plays as a cause of diarrhoea.

4. A prospective study to establish the causal relationship between Cryptosporidium and diarrhoea should be carried out.

5. A study to compare the malnourished with diarrhoea and well nourished with diarrhoea should be considered to find out the morbidity pattern(s) in the two groups.

6. Possibly drug trials should be done in order to come up with treatment regime(s) for Cryptosporidial infection.

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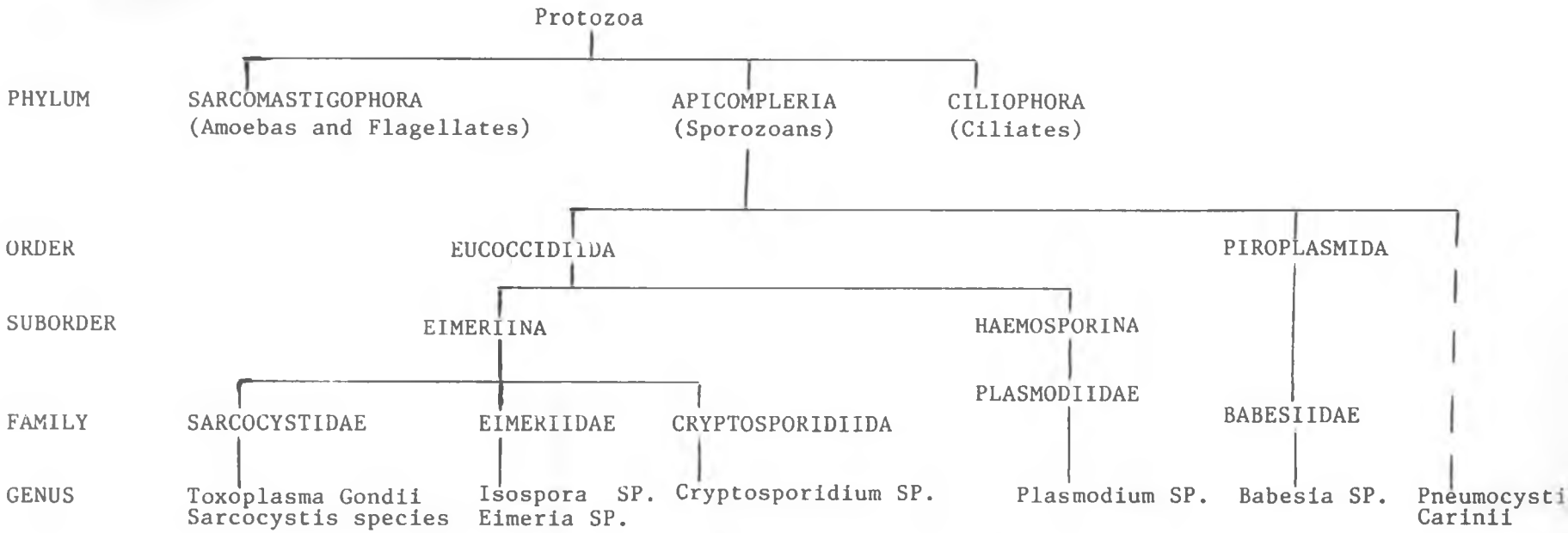
Appendix I:

Taxonomic Position of Cryptosporidium



Appendix II

Epidemiologic Surveys of Cryptosporidial Infection.

Year	Location of Study	No. of Persons examined	Positive %
1983	Australia	884	4.1
	Finland	154	9.1
	United Kingdom	500	1.4
1984	Costa Rica	278	4.3
	Canada	1317	1.1
	Denmark	800	2.0
	Peru	111	8.1
	Rwanda	293	7.8
	Rwanda	72	11.1
	United Kingdom	867	5.0
	United Kingdom	1967	1.4
1985	Haiti	824	16.7
	Brazil	117	7.7
	U.S. (Massachussetts)	1290	2.6
	Finland	4545	2.6
	Madrid	91	1.0
	Venezuela	120	9.2
	United Kingdom	213	3.2
	Mexico	57	32.0
	Bangladesh	578	4.3
	France	90	2.1
	India	687	11.1
	Canada (British Colombia)	7300	0.6
1986	Ghana	474	12.9
	U.S. Oregon	1710	0.5
	Liberia	374	8.4

Appendix III:

Cryptosporidial occurrence in children with diarrhoea.

	Geographic Location	Age(MO)	Incidence	References
1.	New Zealand		22%	2
2.	India	0-36	13.1%	2,27
3.	Venezuela	0-24	10.8%	2,26
4.	Ghana	2-60	12.9%	9,11
5.	Rwanda		10.4%	13
6.	Australia		9.6%	2
7.	Liberia	6-60	7.9%	10
8.	Chile	0-156	6.4%	2,28
9.	Nigeria	2-60	5.3%	25
10.	Costa Rica		4.3%	2
11.	Kenya	0-60	3.9%	12
12.	Thailand		3.2%	2
13.	London		3.2%	2
14.	Liverpool		1.4%	2

Appendix IV:

STUDY PROFORMA

STUDY NUMBER: _____

A. HISTORY:

1. DATE OF INTERVIEW _____

2. NAME _____

3. IP NO. ☐

4. SEX ☐

5. AGE (MONTHS) ☐

6. DATE OF ONSET ILLNESS ☐

7. DATE OF ONSET DIARRHOEA ☐

8. CHARACTERISTICS OF DIARRHOEA

COLOUR	1. GREENISH 2. YELLOWISH 3. WHITISH	☐
MUCOID	1. YES 2. NO	☐
WATERY	1. YES 2. NO	☐
BLOOD STAINED	1. YES 2. NO	☐

9. NUMBER OF STOOLS PER 24 HOURS

10. ASSOCIATED SYMPTOMS

VOMITING	1. YES 2. NO	☐
FEVER	1. YES 2. NO	☐
DEHYDRATION	1. YES 2. NO	☐
WEIGHT LOSS	1. YES 2. NO	☐
OTHER (specify)		

B. CLINICAL EVALUATION

1. WEIGHT (Kg) _____ ☐

2. HEIGHT (cm) ☐

3. TEMP °C (RECTAL) ☐

4. DEGREE OF MALNUTRITION:

OEDEMA PRESENT	1. YES	2. NO	☐
WASTED	1. YES	2. NO	☐
APATHY	1. YES	2. NO	☐
HAIR CHANGES	1. YES	2. NO	☐
SKIN CHANGES	1. YES	2. NO	☐

5. DEGREE OF DEHYDRATION:

DRY LIPS	1. YES	2. NO	☐
DRY TONGUE	1. YES	2. NO	☐
SUNKEN EYES	1. YES	2. NO	☐
SUNKEN ANTERIOR FONTANELLE	1. YES	2. NO	☐
ABSENCE OF TEARS	1. YES	2. NO	☐
LOSS OF SKIN TURGOR	1. YES	2. NO	☐
THIRSTY	1. YES	2. NO	☐
DROWSY	1. YES	2. NO	☐

CODE:

(1) MILD
(2) MODERATE
(3) SEVERE ☐

6. ANY CONCOMMITTANT ILLNESS (specify type of illness and date on onset)

C. <u>LABORATORY DATA</u>

1. CRYPTOSPORIDIUM PRESENT	1. YES	2. NO	☐
2. HIV TEST POSITIVE	1. YES	2. NO	☐
3. WESTERN BLOT	1. YES	2. NO	☐

Appendix V

Kinyou acid-fast staining method.

1. Prepare a thin smear of fresh or formalin fixed on a slide.
2. Fix with gentle heat at about 60°C
3. Fix with methanol for 30 seconds.
4. Flood with stain for 1 minute
5. Rinse with tap water.
6. Decolourise with acid-alcohol for 2 minutes.
7. Rinse with tap water.
8. Counterstain with malachite green for 2 minutes.
9. Rinse briefly with tap water.
10. Dry slide, mount, and examine under oil immersion.

The oocysts stain red on a pale green background. They are oval, measuring 2 - 6μ in diameter.

Garcia looked at 13 different diagnostic techniques for organism recovery and identification. The quality of Cryptosporidium oocysts morphology was found to be variable with the overall best results being obtained with sheather's sugar flotation concentration, Giemsa, Ziehl-Neelsen acid-fast and Kinyoun acid-fast methods (33). The Kinyoun acid-fast stain was found to be useful

in differentiating oocysts from yeast since the latter are stained green by the counterstain (40). With most of the other methods both oocysts and yeast stain same colour and can only be differentiated by their sizes.