CRYPTOSPORIDIAL DIARRHOEA IN LUSAKA CHILDREN

A cross sectional survey to determine the frequency of isolation of cryptosporidium Spp in Stool Specimen of Children in three low cost areas of Lusaka chosen for their major type of water supply and to assess the risk factors associated with the disease.

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This study is dedicated to the memory of my late father Lowe R.D Malama.

This dissertation is submitted in partial fulfilment of th requirements of the award of the master of Public Health degre University of Zambia December 1995

Signed:....................

Supervisor

DECLARATION

I hereby declare that the work presented in this study for the degree of master of public health has not been presented either wholly or in part for any other degree.

Signed:............................

Candidate

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APPROVAL

THIS DISSERTATION OF MBIKO F.M. NCHITO

has been approved as fulfulling the requirement for the award of the degree of Master of Public Health by the University of Zambia.

Signature	Date
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ABSTRACT

A health centre based survey carried out in November and December 1995 to determine the prevalence of cryptosporidium in under-five children presenting with diarrhoea in three areas of Lusaka chosen for the type of water supply and to assess the risk factors associated with the disease. Reponse rate = 61%Ccryptosporidium was isolated 33 times out of 156 specimen = 21% Cryptosporidial diarrhoea was found to be associated with location X = 17.94, P = 0.0001, source of water, Borehole x = 13.2 P = 0.0001. Always having water was associated with the disease O.R = 2.37 C.I (1.0, 5.66) X = 4.73 P = 0.03,

Age sex, number of persons in household keeping animals, boiling water, economic status, nutritional status and the type of latrine were not associated with the disease. Cryptosporidium would appear to be an important cause of diarrhoea in children under five years old and transmission through water is probably the most important route.

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1. INTRODUCTION

Cryptosporidium is a coccidian parasite that is found worldwide and is a major cause of diarrhoea in both imunocompetent and immunosuppressed person, (1,10). Human infection was first described in 1976 when the parasite produced a self-limiting diarrhoeal illness in an immunocompetent child. Since then it has been widely recognised as an important pathogen in immunosuppressed individuals, particularly those with HIV (2,3). Cryptosporidium is an obligate parasite of the gastro-intestinal epithelial cells. It develops just under the host's epithelial membrane, in parasitophorous vacuoles at the surface of, rather than inside cells; an intracellular yet extracytoplasmic position. In this way and its smaller size it is different from other coccidia (2).

Of the four species recognised, cryptosporidium parvum (10) according to Current (4) is the major species responsible for clinical illness in humans and animals. Tzipori et al (5) demonstrated how that oocysts of cryptosporidium isolated from calves with diarrhoea infected seven other species of animals with or without diarrhoea. Because it is an obligate parasite, it can replicate only within its host.

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The life cycle of Cryptosporidium parvum begins with ingestion of the oocyst which releases four sporozoites after excystation. This stage initiates infection within the epithelial cells of the gastrointestinal tract. The Sporozite differentiates into the trophozoite, which undergoes asexual multiplication to form type 1 meronts and then merozoites, which may infect new host cells. Merozoites from type II meronts produce microgametocytes and macrogametocytes which undergo sexual reproduction to form the oocyst and transmission of the disease is via this enviromentally robust oocyst excreted in the faeces of the infected host (1). The oocysts can remain viable for up to two months, outside the body of the host, and are not destroyed by the level of the chlorination adequate for water treatment (1,6). There have been outbreaks of cryptosporidiosis associated with treated water from which the oocysts have been isolated, (6,23). These have also been recoved from animal stool indicating that animals may serve as a reservoir for cryptosporidium (7). From the animals the transmission can be directly to humans (8,10), or indirectly through water that has been contaminated with faeces of an infected animal as would happen in a rural area (9,18) with unprotected water sources. Piped city water can also be contaminated if infected animals drink from the river or dam, the source of the piped water. (9)

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Of the possible routes of infection, water would appear perhaps to be the most important as cryptosporidiosis has been shown to be seasonal, the peak incidence coinciding with the rains. (19,22,24). However in the Haitian Study (28) no seasonal predilection was observed.

The zoonotic transmission route is also important and a number of animals have been implicated. Infected calves are particularly important in this respect (26). Oocysts have also been recovered from pigs, goats, fowls, dogs and some non domesticated animals. Infact cryptosporidiosis was first known as a disease of animals (1,3).

Person to person spread does not seem to be very important. A study in Brazil (7) demonstrated frequent familial clustering suggesting a person to person spread but the possibility of exposure to a common source of infection was not eliminated (33). A study in Bangladesh (24) also showed a rather low frequency of intrafamilial transmission.

In developed countries cryptosporidiosis is a disease of both adults and children (10) but in developing nations it would appear to be mainly a disease of childhood. (10,11,17,20).

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The clinical presentation varies from asymptomatic carriage to severe life-threatening watery diarrhoea in the immunosuppresed patient. In the immunocompetent individual the infection has an incubation period of 1-7 days followed by fever, copious watery diarrhoea abdominal cramps, nausea and vomiting. (3,8,11) Sypmptoms may resolve in 48 hours but may continue for 2 - 3 weeks and rarely for months (3). However some patients may have diarrhoea for three to four weeks and shed oocysts for a further three weeks or more. In about half the patients diarrhoea is not continous but occurs as alternating bouts of diarrhoea and constipation (21)

This disease in children who might already be malnourished would further debilitate them and hence render them susceptible to still other infectious which would reduce their nutritional status which in turn would leave them open to other infections. In cryptosporidial diarrhoea fat, carbohydrate and vitamin B12 malabsorption as well as reversible lactose intolerance have been observed. (2)

The relationship between nutrition and cryptosporidiosis is not very clear.

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Theoretically children with poor nutritional status are at a higher risk of the disease than those whose nutritional status is good.

Does poor nutrition status predispose to the disease or does the disease lead to malnutrition? Oocysts have been detected in malnourished children. Garcia velarde E.et al (27) found that Cryptosporidium was present with increased frequency in malnurished children with diarrhoea.

In the Rwandese Study (29) in 4 out of 20 children cryptosporidiosis was associated with severe malnutrition.

Although self-limited, the illness is often severe enough to warrant therapeutic intervention. Apart from exacerbating the illness, poor nutritional status could be a risk factor.

2. PROBLEM

Cryptosporidium would appear to be endemic in Lusaka with records from the University Teaching Hospital Parasitology laboratory showing the pathogen isolated on average in 42 stools out of the total stool specimen of 5162.

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Cryptosporidium for the same period 1992, 1993 and 1994 contributed 42-1330 of the parasite isolated = 3.16%. This of course does not reflect the true incidence of cryptosporidiosis in Lusaka, because hospital data can not be generalised to the rest of the community.

As at present little has been done to assess the incidence or prevalance of crytoporidium in Lusaka in under five children with diarrhoea. A survey carried out in the University Teaching Hospital by Khumalo-Ngwenya in 1993 (unpublished) showed an isolation of 6.3% in Lusaka children with diarrhoea. Compounds in the south east of Lusaka had the highest incidence of cryptosporidiosis. This survey also showed a seasonality in the disease which peaked at the beginning of the rainy season (33).

Khumalo-Ngwenya et al (11) also looked at the different intestinal tract parasites in HIV patients in Lusaka. The isolation rate for cryptosporidium was 4.2%.

There was need to asses the proportion of Cryptosporidium caused diarrhoea as this will serve as a baseline for other studies, undertaken in the same field that is still relatively unknown in Zambia.

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This study will also help us identify the risk factors associated with the disease in order to put preventive measures in place. As at present there is no recognised cure for cryptosporidiosis though spiramycin, clindamycin and erythromycin have shown to be of some benefit.(3). It is also probable that in diarrhoea of unknown aetiology <u>cryptosporidium</u> could be the causative agent especially as this pathogen is not routinely looked for in hospitals in Zambia apart from U.T.H and perhaps a few other major hospitals.

CHAPTER 2

3. LITERATURE REVIEW

There have been a number of studies done on HIV related cryptosporidiosis as it is an important infection in this category of people, (1,2,3,10).

However studies have also shown cryptosporidiosis to be an important cause of diarrhoea in immunocompetent individuals and especially in children. (12,14,15). The children most affected would appear to be especially those 2 years and below (14,15,19,27) Other risk factors that have been identified apart from age are contact with animals (18,26). The Guinea-Bissau study identified pigs and dogs (22), other studies have implicated dogs, pigs, donkeys and goats as reservoir of infection. Infected calves are particularly important as these have been shown to shed as many as 10 oocysts per grain of faeces (9,26). The child's sex has also been put forward as a risk factor for cryptosporidiosis with male children at a higher risk than females (22).

Khumalo-Ngwenya et al(11) in their study found cryptosporidium only in diarrhoea patients with HIV - 1 infection and non in the control group who were HIV negative. This would seem to imply immunity in adults who had probably been exposed to the infection in childhood. This finding would also indicate that the infection is probably endemic in Lusaka.

The population prevalence rates are estimated between 1 - 5% in developed and up to 10% in developing countries. In Africa the prevalence rates among children of 5 - 10% have been reported from Nigeria, Rwanda and Liberia, (10). Sinwa et al (14) found this peaked at 7 - 18 months. They also found no difference in the proportion of households with animals.

A study in Khartoum, Sudan found 16/100 children with cryptosporidium diarrhoea and 7/16 had had contact with animals (12). In Ethiopian children with acute diarrhoea there was a 9% isolation rate and 6/9 children were below 12 months of age (15). It is believed that this infection is rare in breast fed infants (2) but a study in Malawi showed no difference between those breast fed and those who are not (16).

10/....

The study in Haiti showed that breast feeding was protective only for those infants who were breastfed exclusively (28).

A Gambian study (19) found an overall prevalence of 9% in children with diarrhoea. Cryptosporidiosis in the Gambia showed a well defined peak in association with rains and high relative humidity. Molbak et al(21) also found that parasite was mainly present at the beginning of the rainy season.

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CHAPTER 3

OBJECTIVES

1. General Objective

The aim of this study was to assess the frequency of isolation of *cryptosporidium* oocysts in children under five years of age, presenting with diarrhoea in three areas of Lusaka irrespective of their immune status and to assess the risk factors associated with the disease.

2. Specific objectives

A. To assess the frequency of isolation of *cryptosporidium* oocysts in under five children in three low cost areas in Lusaka according to the water supply

(i) Chawama Compound - boreholes and quarry supply line

(ii) George Compound - main reticulation system + boreholes

(iii) Chipata Compound - main reticulation system

B. To see if there is an association between the factors enumarated below and cryptosporidiosis

(i) age

(ii) source of water

(iii) unboiled drinking water

(iv) socio economic status

(v) nutritional status of child

(vi) number of persons in household

(vii) keeping of animals

Definition of terms

(i) Nutritional Status

Using the Gomez classification as in the under five cards

a. below the 25th percetile = underweight

b. between the 25th and 75th pecetible = normal

c. above the 75th percetile = overweight

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(ii) Socio-economic status

a. below K25,000.00 group one (1)

b. K25,000 to K49,000.00 group two (2)

c. K50,000 to K69,000.00 group three (3)

d. K70,000 to K99,000.00 group four (4)

e. K100,000.00 and above group five (5)

(iii) Diarrhoea:- as defined by mother (normally more than 2 loose or watery stools in 24 hours)

(iv) Animals including any domesticated animal kept in the house or in the vicinity of the house.

(v) Number of persons in the households. This was taken to be the total number of people resident in the house at the time of the interview.

(vi) Source of water

a. Main reticulation system - piped water

b. Borehole

c. Protected well

d. Unprotected well

14/....

(vii) Age

a.	0 - 6 months	group 1
b.	7 - 12 months	group 2
c.	13 - 18 months	group 3
d.	19 - 24 months	group 4
e.	Above 24 months	group 5

CHAPTER 4

METHODOLOGY

1. Location

The study was conducted in three areas of Lusaka chosen for their major source of water. Lusaka Water and Sewerage Company is responsible for the supply of water to Lusaka residents. Because it is difficult to get information on privately owned water sources, it is assumed that the Water and Sewerage Company provides 100% of the water supply in Lusaka. This water comprises 110,000m3 from the Kafue river and 50,000m3 from boreholes and other sources around the city per day.

The total of 160,000m3 per day is more than sufficient for the needs of the city but the water actually available for use in Lusaka is much less than this due to losses which amount to about 40%. (Nyirenda Edwin, Personal communication).

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Chawama lies to the South-eastern side of the city and has a population of 48,000 (1990 census). it is served by one health centre which also cater for the surrounding compounds of John Howard, Cook, Misisi etc. Chawama like other shanty townships is a high density area characterised by poor standard of living substandard housing, poor sanitation and inadequate water supply. The area has no sewer system and the most common latrine is the pit type and there is about one for every 4 - 5 houses. Garbage collection is non existent such that the residents live in perpetual unhygienic surroundings. Children and animals are left free in this enviornment. Chawama is supplied largely by two boreholes and partly by the quarry supply line. It is not connected to the main reticulation system. The water supply is erratic, so that for certain hours in the day or sometimes even for days water is not available. George and Chipata compounds on the other hand are both supplied by water from the Kafue river.

But like Chawama, the water supply is not consistent forcing the residents to look for alternative sources. Both George and Chipata lie to the north of the city towards the north-west and north-east respectively.

The housing and sanitation in these two areas is similar to Chawama. There is no evidence to suggest that there is a difference in the overal economic status of the three areas.

Most residents of George until a few months ago, depended on two boreholes maintained by the Lusaka Water and Sewarage Company (L.W.S.C) and the main reticulation from the Matero reservoirs (but the latter is a very poor source of water). The water from these two sources is not sufficient to meet the water needs of the residents, so some have resorted to drawing water from shallow wells. About four months ago, two new boreholes were sunk by a multi national Non-governmental Organisation. These supply water to only a segment of the compound. Chipata is also connected to the main reticulation system and water is drawn from the Matero reseviors. This however as noted before, is an inadequate source of water.

Both Chipata and George are each served by a health centre. The population of these compounds is 42,000 and 40,000 respectively.

2. Experimental design of research

This was a health centre based cross sectional survey with passive case finding to assess the proportion of children with cryptosporidial diarrhoea. The study also sought to establish whether or not associations exist between cryptosporidiosis and possible risk factors like age, source of water, unboiled drinking water, social economic status, nutritional status of child, number of persons in households and keeping of animals.

A structured interview was used to collect the data (see appendix).

Specimens from the health centres were collected everyday beginning from the second week in November only a few specimens were collected before the rains as the study was delayed.

3. Inclusion Criteria

The subjects were enrolled individually, children presenting with diarrhoea (subjective definition by the mother) five years and below both male and female who were residents in their respective areas.

4. Procedure

After the protocol was approved by the research and ethics committee, the study begun in November. Permission to conduct the study in the urban health centres was granted by the director of public Health in Lusaka.

In as far as it was possible, written consent by the parent was requested before the child was enrolled into the study. (see appendix 2).

Upon being enrolled a stool specimen was collected from each child into a clean specimen container. All mothers with children who had diarrhoea and were at the clinic at the time were asked to participate in the study but only those who were willing to give a stool specimen were enrolled.

Ideally the stool specimen was collected the same day the interview was done, but most parents went away with the containers and brought the specimen the following day.

Entry into the study was entirely voluntary and no force or coercion was used but to encourage participation the importance of the study was stressed to the mothers.

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The parents of the children were all interviewed at the health centre.

After collection, the stool specimens were taken to the University Teaching hospital Parasitology laboratory for processing.

5. <u>Laboratory Procedure</u>

Thin smears one per specimen were made, air dried and fixed using methanol. They were then stained using the modified Ziehl - Neelsen technique (16), though some (21,23,24) have labelled this method slow and insensitive and inferior to Safranin-methylene blue and phenol auramine. But others have shown it to be of comparable sensitivity to other methods (8,19). A cryptosporidial antibody test is available but it would not be very helpful in this kind of study where what is being measured is the amount of disease and not just exposure.

Smears after fixing in methanol for a minute or so were air-dried and stained with cold carbol fuchsin for 10 minutes.

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The smears were then rinsed in running tap water, decolourized with 1% acid alcohol for about 1 minute, washed in water and counter stained with 1% malachite green for 1 minute before being rinsed again in water, air dried and examined with an oil immersion objective.

Consistency and the presence of elements like blood and mucus were noted for each stool. Parasitologic examination for enteric parasites was done by light microscopy of a direct saline preparation. This was repeated after formol-ether concentration of the stool. The results were sent back to the respective health centres.

Each stool smear after staining was read under 0.1 immersion lens (x100) by two people independent of each other. At least 20 minutes was spent on each slide before it was declared negative. Cryptosporidium oocysts were identified as pink round bodies against a green background measuring 5 in diameter. One oocyst properly identified would be sufficient to declare a stool smear positive. One hundred and fifty four smears were made and read in the five week period beginning 2nd week of November to the end of the 2nd week of December. Approximately 6 slides were read per day. 6 x 20 = 2 hours a day for slide reading.

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For the remainder of the stool specimen

(i) a wet preparation was made

(ii) formal either concentration was done.

6. Sample Size Calculations

Since the prevalence of cryptosporidiosis is not known we assumed it was not more than 20%

With a prevalence of about 20% and allowing a margin of error of 5% at 90% confidence intervals the sample size required was 173 Allowing for 80% response rate the adequate sample size was calculated to be 215

Ideally 80 patients were to be recounted from each of the three areas bringing the total sample size to 240 (32).

Total population of the three areas = 130,000 Number of children under 5 years (percentage of children under 5 taken to be 5% of total population) = 6 500.

Number of diarrhoea episodes per child per year in Africa = 5 (31)

23/...

Total number of diarrhoea episodes per year in three areas = 5 x 6 500 = 32,500.

Research was carried out over a period of 6 weeks

$$\frac{6 \times 32\ 500}{52} = 3750$$

According to the Lusaka Urban Public health department 1994 records these were on average 300 episodes of diarrhoea for the last quarter, of the year per 6 weeks per clinic.

Our desired sample size = 215

As a (percentage of the total diarrhoea episodes for the six week

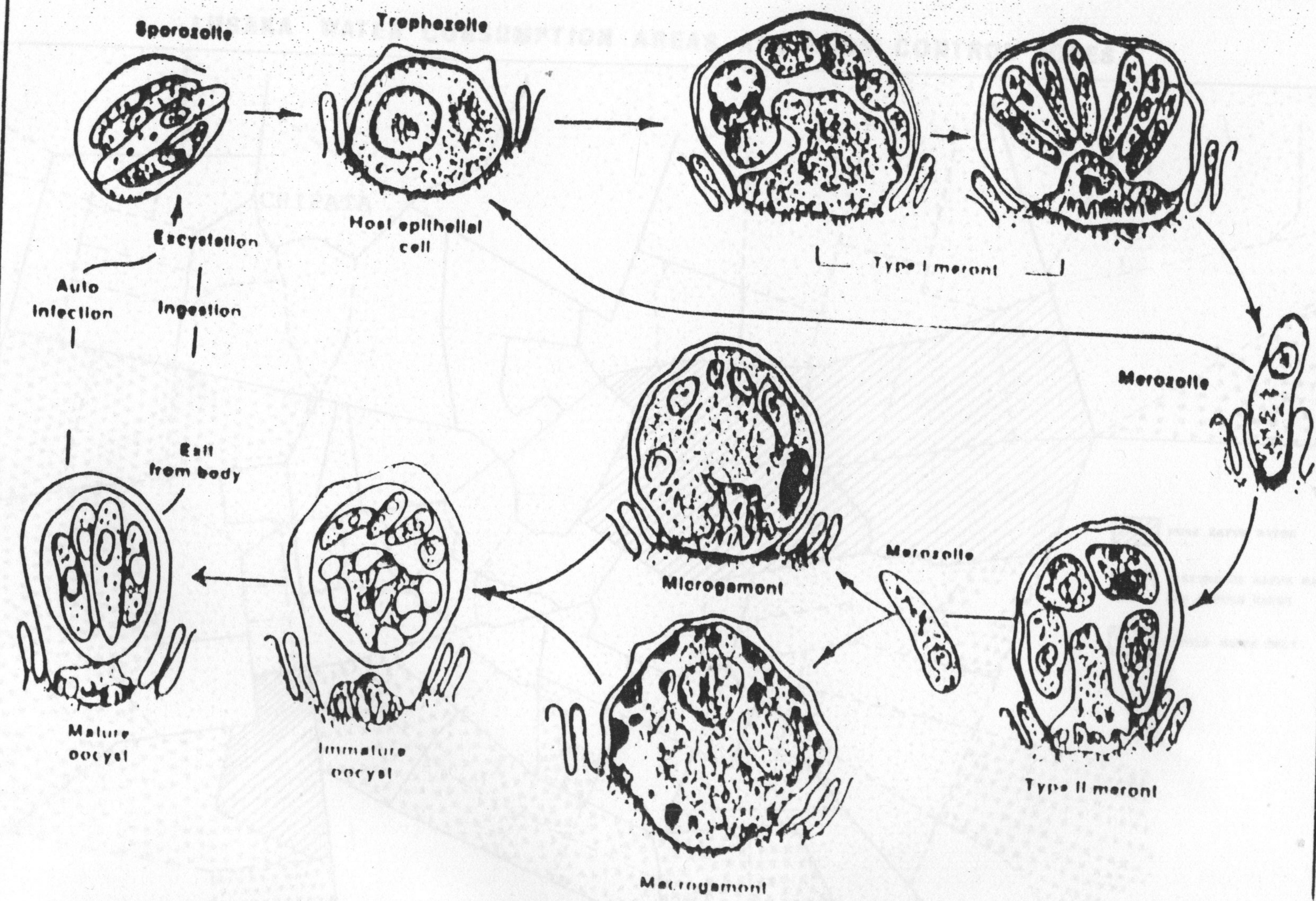
$$\frac{215}{3750} = 5.7\%$$

Actual respondents as percentage of the total diarrhoea episodes

$$\frac{154}{3750} = 4.1\%$$

The data collected was analysed using EPi-6

In Chawama 95 patients were interviewed over the study period but only 38 submitted stool specimen. In Chipata 98 patients were interviewed and 67 submitted stool specimen. Response rates of 40% and 68% respectively. In George compound out of those interviewed 49 brought the specimen indicating a response rate of 80 %

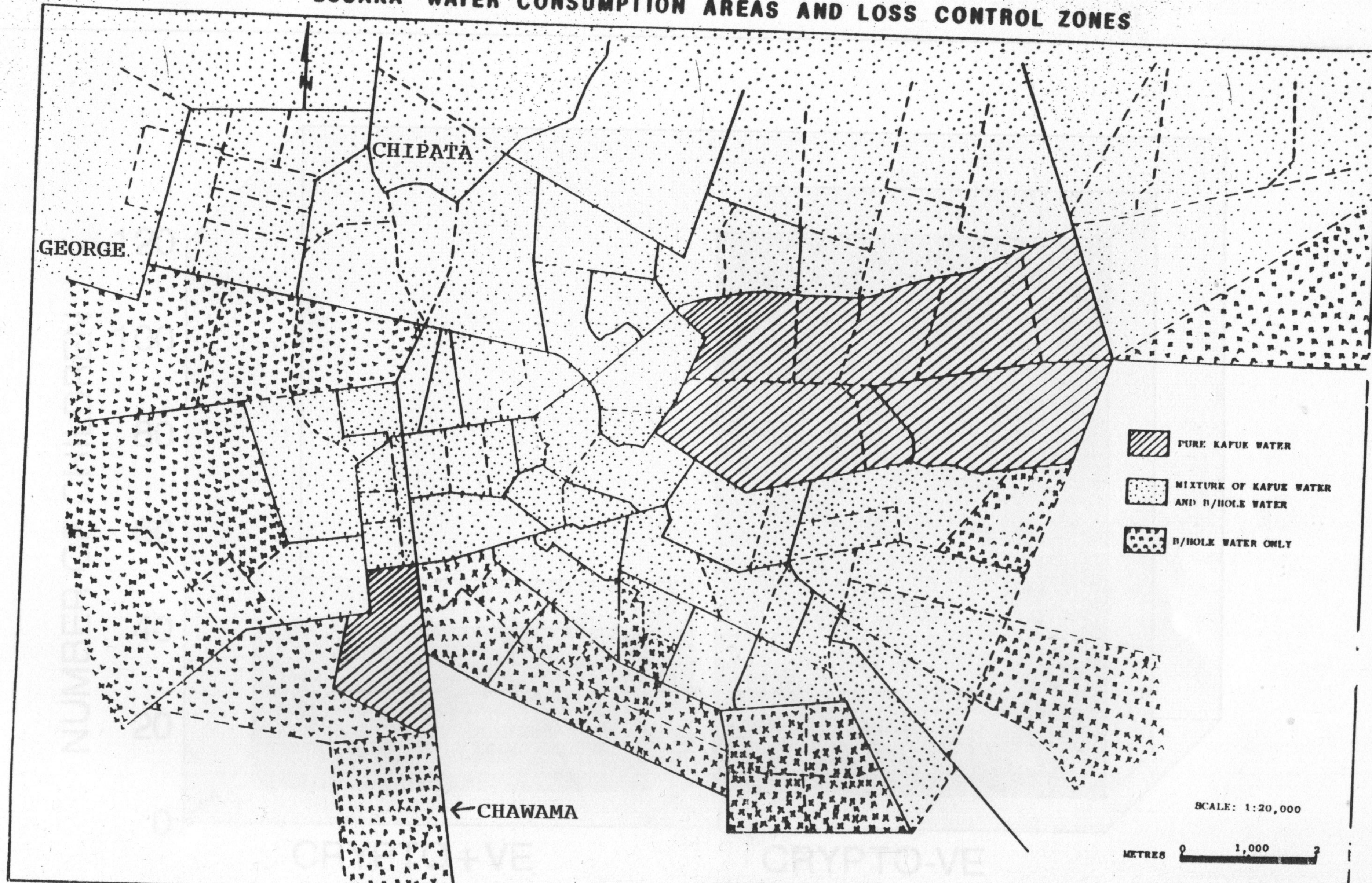


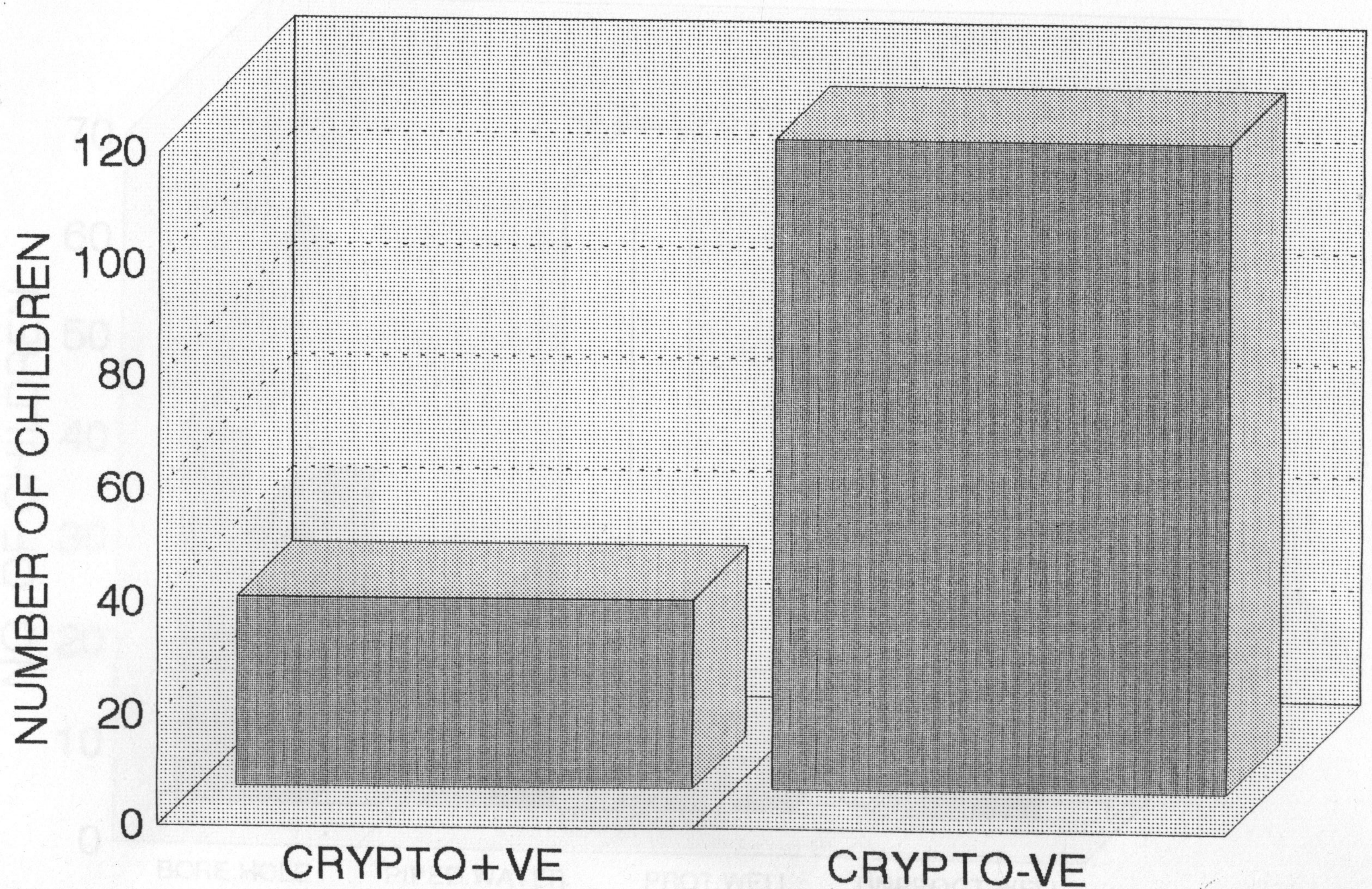
Life cycle of *Cryptosporidium* (*this drawing was originally published by the American Society for Microbiology in* Microbiological Reviews, *50:458 [1986]*)

DRAWING BY R.B. EWING

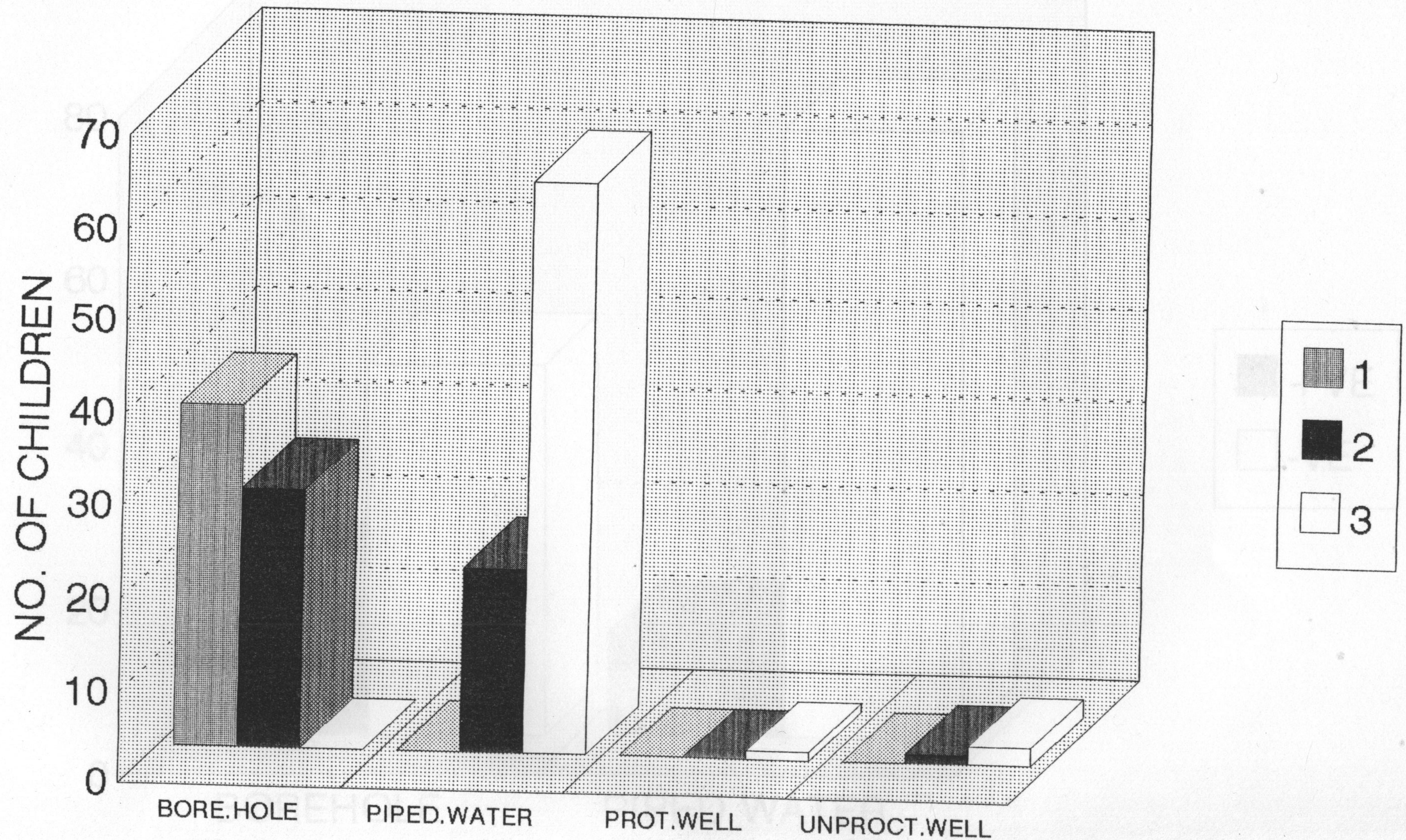
(1)

LUSAKA WATER CONSUMPTION AREAS AND LOSS CONTROL ZONES

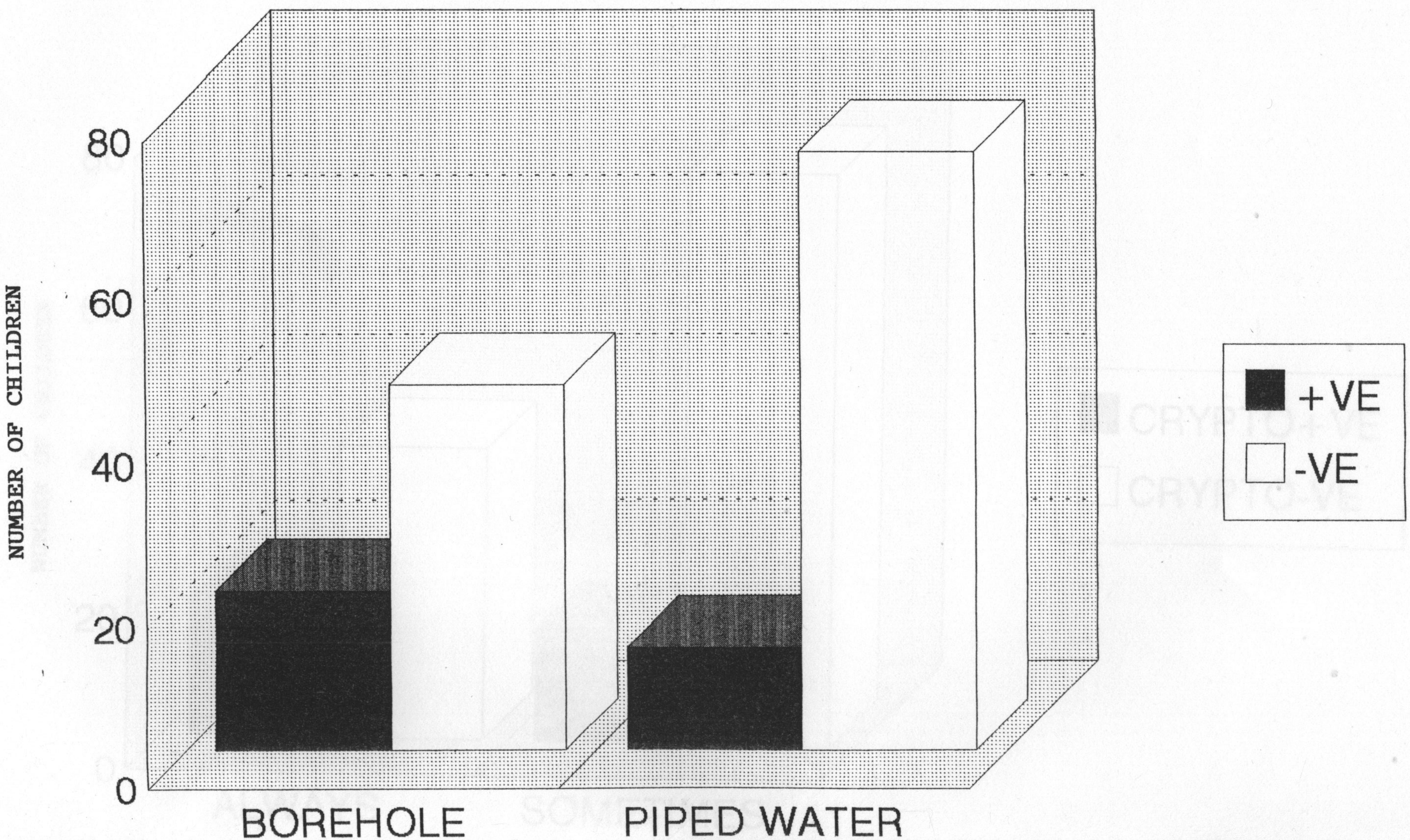



NUMBER OF CHILDREN
120
100
80
60
40
20
0
CRYPTO+VE
CRYPTO-VE

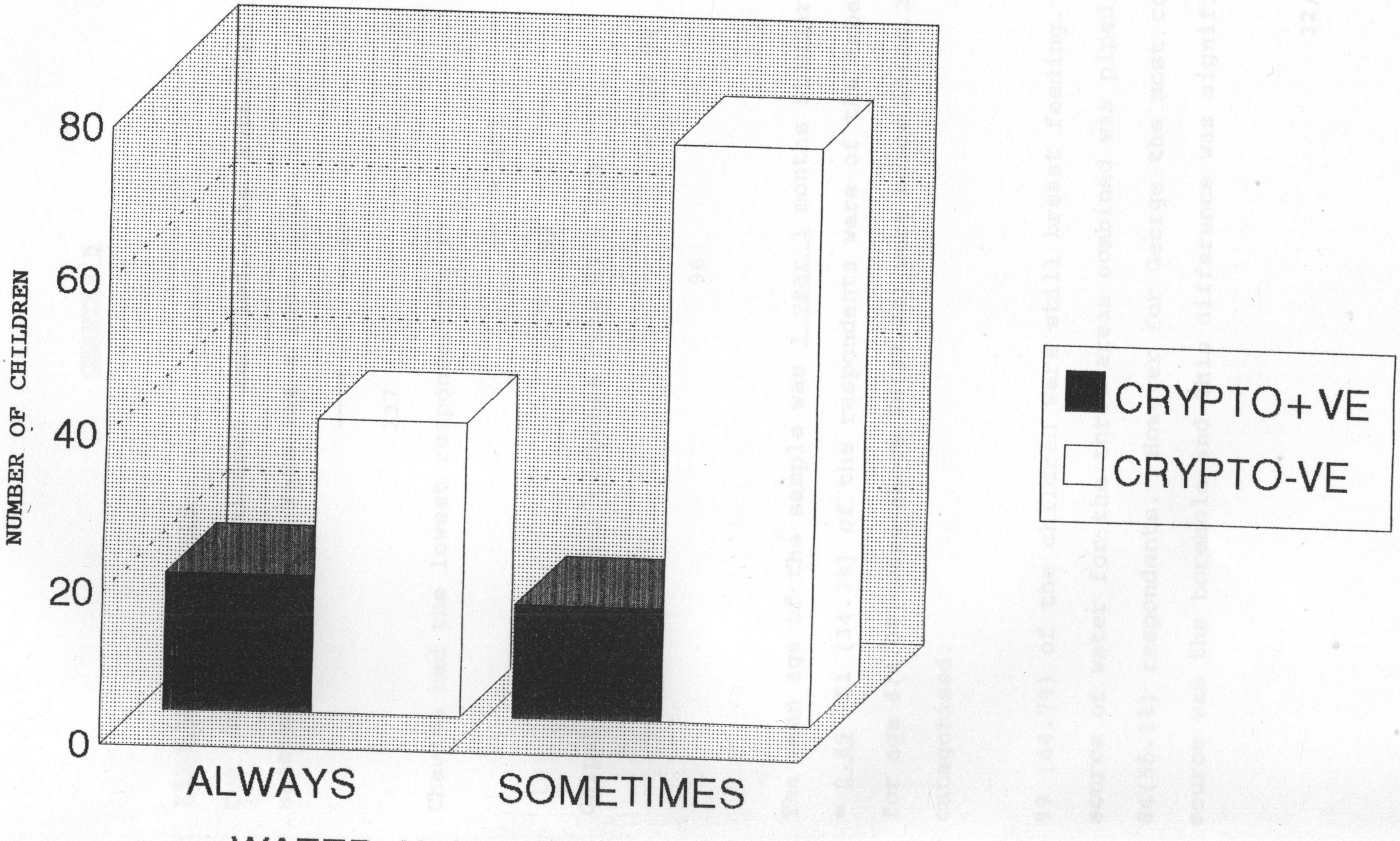
WATER SOURCE AND LOCATION



CRYPTOSPORIDIOSIS AND MAJOR WATER SOURCE



WATER AVAILABILITY AND CRYPTOSPORIDIOSIS



CHAPTER 5

RESULTS AND ANALYSIS

Out of the 237 children enrolled, 154 submitted their stool specimen. Response rate $\frac{154 \times 100}{237} = 65\%$

Chawama had the lowest response rate $\frac{37}{92} = 40\%$

Chipata had a higher response rate $\frac{67 \times 100}{96} = 69.8\%$

The mean age of the sample was 1 year 3 months Standard deviation = 0.89 51 (34.2%) of the respondents were of the expected weight for age 6(4.0%) were above 56(37.6%) were below 36(24.2%) were not categorised.

99 (64.7%) of the children were still breast feeding. The major source of water for the three areas combined was piped water with 84(55.1%) respondents. However for George the most common water source was the borehole and this difference was significant.

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Table 1 showing major source of water in the three different areas

major source	Chawama	George	Chipata	Total
Bore-hole	37	28	0	65
Piped water	0	20	62	84
Protected well	0	0	1	1
Unprotected well	0	1	2	3
	37	49	65	151

(below table
Chi-Square
69.84
P. value 0.000

X = 98.33 P value 0.00

11 (7.3%) respondents boiled their drinking water always, 16 (10.7%) only sometimes and the rest never.

104 (74%) kept animals and the most common ones were Dogs 26% and chickens 18%.

The most common household item owned was the radio 74% followed by the Television 42.4%. The least owned item was the video (1.4%). 51% of the sample population use a communal pit latrine, 69 (45.7%) their own and 5 (3.3%) a water closet.

33/...

Out of the 154 stool smear 33 were positive for cryptosporidium.

Isolation rate = $\frac{33 \times 100}{154} = 21\%$

Table 2 showing distribution of cryptosporidium by residence

Cryptosporidiosis

Residence Code	+ve	-ve	Total
Chawama (001)	5	32	37
George (002)	21	28	49
Chipata (003)	7	60	67
			153

X = 17.94

P value = 0.0001

There was a significant difference in the distribution of cryptosporidial diarrhoea.

34/...

Table 3 Cryptosporidium by sex

	Sex F	M	Total
Crypto +ve	18	16	34
-ve	50	64	114
	68	80	148

Odds ratio 1.44

Confidence intervals 0.62 - 3.55

Chi-Square 0.87 P value 0.4

There is no significant difference in the amount of cryptosporidiosis among male and female children.

Table 4 Cryptosporidiosis and nutritional status

	normal	overweight	underweight	Total
Crypto +ve	18	1	13	34
-ve	32	5	41	112
	50	6	54	146

Chi-Square = 2.26

P value = 0.3

Table 5 Cryptosporidiosis and water availability

		Always	Sometimes	
Crypto	+ve	18	15	33
	-ve	38	75	113
		56	90	140

There was an association between the availability of water and cryptosporidiosis.

Odds ratio = 2.34 (1.00, 5.66)

Chi-Square = 4.37 P value 0.03

Table 6 Availability of water by residence

	Residence			
Available	001	002	003	Total
Always	10	36	12	58
Sometimes	27	13	55	95
	37	49	67	153

36/...

There is a significant difference in the availability of water in the three areas

X = 37.57

P value = 0.00

Table 7 Cryptosporidiosis and major water source

	+ve	-ve	Total
Borehole	20	45	65
Piped water	13	74	87
	33	119	152

Odds ratioı = 2.15 (1.07,6.02)

X = 5.48 P value 0.02

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Table 8 Cryptosporidiosis and keeping animals

		Animals	No animals	Total
Crypto	+ve	21	13	34
	-ve	84	32	116
		105	45	150

Odds ratio = 0.64 (0.25, 1.49)

X = 1.49 P value = 0.2

Table 9 Toilet facilities and Cryptosporidiosis in the three areas

	Crypto +ve	Crypto -ve	Total
Communal pit	21	55	76
Own pit	13	56	69
	54	111	145

Chi-Square	2.49
P value	0.28

There is no oassociation between type of latrine and cryptosporidiosis.

There was no significant difference in the overall economic status of the three areas as shown by possession of household items.

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DISCUSSION

Cryptosporidium has again been shown to be a major cause of diarrhoea in children under five years. The ones at highest risk are those under 2 years of age (14,15,19). In our study only 23 children were above the age of 2 and only one of these had the disease.

The isolation rate for the three areas was 21%. This is much higher than studies in the region have shown. The prevalence rates in similar studies have been 16% in Khartoum (12) and 9% in Ethiopia (15). The majority of the children were below 2 years.

Unlike the Guinea Bissau Study, sex was not a risk factor. Male and female children were at equal risk.

It would appear that there was a very significant difference in the distribution of cryptosporidiosis in the three areas under study X = 17.94 P value 0.0001. Children in George are at a higher risk of cryptosporidiosis than the rest. Chawama and Chipata each had 13.5% and 10% respectively. George on the other hand had an isolation rate of 43%.

The George results would suggest an outbreak because previous records do not show such high prevalence rate (Khumalo-Ngwenya unpublished). George as mentioned above is supplied by three sources (i) main reticulation system from the water reservoirs (ii) two LWSC boreholes and (iii) the newly commissioned boreholes.

A high percentage of the respondents in George indicated their water source as borehole. There is suspicion that the term borehole might actually refer to shallow wells. This is despite the fact that unprotected well was given as an option in the questionaire. This is for two reasons:

(i) A clear misunderstanding of terms. The word borehole is sometimes inappropriately loosely used to mean well. This in the interview would occur if the interviewer did not give all the options beforehand.

(ii) Shallow wells are illegal and the public health authorities do not encourage their use. People using this source would not readily disclose it.

However, there is also the possibility that they were correctly referring to the newly commissioned boreholes as these were functional before the study began.

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If the former is true then cryptosporidiosis in George can be attributed to the contaminated water in the shallow wells. This option is particularly plausible as this year's rains have been heavier than previous years. The surface water run-off would then contaminate the wells.

If however the latter is true then cryptosporidiosis would be due to contaminated ground water. Sampling of water from the different sources for cryptosporidium oocysts would have confirmed this. A study already underway is investigating this, but the results are not yet available.

Newman et all in their study in Brazil demonstrated the presence of cryptosporidium occysts in water cources; 22.2% were positive for the organism. 10.2% of the animal stool specimen were also shown to be positive indicating that animals may serve as a reservoir of cryptosporidium and hence the possibility of contamination of the water in a community.

Our study found no association between cryptosporidiosis and animals but no stool specimen from animals were sampled.

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Persons using borehole water would appear to be at a higher risk than the other sources of water X = 5.48 P = 0.02. Having water always was seemingly associated with cryptospridiosis X = 4.73, P = 0.03, OR = 2.31, ci 91.0 - 5.6), but this was confounded by the fact that residents in George almost always had water, so that it is not the water availability that is the risk factor but rather the quality of the water available; in this case borehole water in George. Keeping animals did not seem to be significant. This finding is similar to that of the Khartuom study where keeping animals was not associated with cryptosporidial diarrhoea. However studies in Brazil and Gambia found strong association between the two. Keeping animals particularly dogs and pigs have been identified as risk factors for the disease. Calves have also been shown to be a very important source of infection for cryptosporidiosis. In this study none of the respondents had had contact with calves.

Cryptosporidiosis would appear to have no significant association with the nutritional status of the child. Underweight children are no more at risk of the disease than those in the expected weight for age bracket. X = 2.26 P = 0.3. In looking at the nutritional status of the children, it is important to note whether or not the malnutrition is current or past. For this, a good anthropometric measure which would detect wasting and stunting is needed.

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It would seem obvious that cryptosporidial diarrhoea (where calorie and vitamin mal absorption have been observed) can lead to malnutrition. It might be helpful to compare children with chronic and acute diarrhoea. In this case the sample would have to be stratified into chronic and acute diarrhoea cases. Children with chronic diarrhoea would theoretically be at a high risk of malnutrition than those in the acute category.

In this study breast feeding was not found to be significantly related to cryptosporidiosis as in the study in Malawi (16).

Drinking boiled water did not seem to be protective. This does not suggest that boiling is ineffective against the oocysts but this could be because there were so few people who boiled their water regularly.

The type of latrine used also did not seem to significantly increase the risk for the disease.

The communal pit latrine, by far the most common in this study, would be expected to be a risk factor. That such unhygienic practices would predispose one to infection by cryptosporidium, seems a forgone conclusion, but the study did not show this.

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This finding suggests that the faeco-oral route and therby the person to person spread is not a very important mode of transmission.

Social economic status as measured by income possessions or education would appear to be insignificant. These factors are significant risk factors for most diarrhoeal diseases such that had the study compared children with cryptosporidial diarrhoea and those with illnesses other than diarrhoea, social economic status would most certainly have had significance. (31) page 91

The number of persons in a household also appeared to be insignificant. It was expected that crowding would be identified as a risk factor, however this was not so.

The HIV status of the children in this study was not taken into account because cryptosporidiosis is known to be a significant cause of diarrhoea in both immunosurpressed and immunocompetent individuals. It is a very common infection in children in developing world (10,11,20). For this reason it was not felt necessary to delve into that area. Time and finance also obviously limited what could be done.

It must be noted that the 21% isolation rate cannot be generalised to the rest of the under-five children in Lusaka, since as mentioned above, (i) the three study areas were not selected randomly (ii) they are all in the low income high density group and are not representative of the whole of Lusaka, (iii) the exceptionally high rate in George would perhaps be suggestive of an outbreak, (iv) this was a health centre based survey and not a community one.

The second part of the study was more a comparative one than a case control, the children were not particularly matched. This lends weakness to the study design.

The sampling of the study population is another possible source of bias. The sampling, was with replacement; such that it is possible that the final sample was made up of individuals who are very different from the non respondents and therefore the rest of the community.

One possible way of reducing non respondents would have been to obtain rectal swabs from the children who had difficulties passing stool. The rectal swab method results are comparable to the traditional stool smear (19).

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Although the 21% isolation rate is much higher than anticipated, it is most probable that this could have been even higher had the respondents been asked to submit more than one stool specimen each. Ideally, due to intermittent shedding of the oocysts patients are encouraged to submit about 3 stool specimen in as many days; one stool specimen per day. it is also recommended that at least two thin smears per specimen be made. Two or more persons should read the slides independent of each other spending not less than 20 minutes on each. (S. Sianongo, Personal Communication). In this way non of the positive smears will be missed.

Other parasites isolated from the stools were Giardia Lamblia 3.3%, Ascaris Lumbricoides 2% Blastocystis hominis, Entamoeba histolytica, Chilomastix Mesnili and Retortamonas intestinalis 1% each. Non of the above were significantly associated with cryptosporidium.

CHAPTER 7

CONCLUSION

Cryptosporidium Spp is a common cause of diarrhoea in children, particularly those under 2 years; 21% of the children presenting with diarrhoea in the three clinics had cryptosporidiosis. In this study the infection would appear to be significantly related to (i) Location; those in George were at a higher risk (ii) source of wate; Bore-hole water probably contaminated (iii) Availability of water always. Water borne infection would appear to be the most important transmission route. Boiling drinking water was not protective but this could be because those who boiled their water were few. Age, sex, nutritional status, keeping animals and number of persons in house-hold were found to be non risk factors.

RECOMMDENDATIONS

1. A larger and more comprehensive study should be undertaken to work out the prevalence of cryptosporidiosis in Lusaka children.

2. That the reasons for the high cryptosporidiosis in George be investigated. (This is already being looked into).

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3. Treatment of water should involve filtration to remove <u>cryptosporidium</u> oocysts.

4. <u>Cryptosporidium</u> should be looked for routinely in childhood diarrhoea.

CONSTRAINTS

1. The University of Zambia Time table indicated that research should have began by 1st October 1995. This study did not commence till the end of the month because:
   (a) The Research and ethics Committee did not sit till 19th October

   (b) The Directorate of Human Resources Development (DHRD) the Sponsors of the research did not release the money till after mid-October.

2. Due to the time limitation the sample size was reduced from 308 to 215 reducing the level of confidence from 95% to 90%.

3. Since the study began late it was not possible to collect specimen before the rains as per protocol.

The bulk of the specimen were collected during the rains; there is the possibility that the results were biaised, since cryptosporidiosis has been shown to be seasonal.

4. Sampling with replacement raises the problesm of having a sample that is different from the community from which it is drawn.

5. The response was poor and slow. A pilot study would have brought out some of these problems beforehand. It would also appear that insufficient ground work was done to win the confidence of the mothers. using members of staff to recruit patients seemed to work well as happened in Chipta and George Clincs. Patients also want to benefit from research so that they must be offered something worthwhile in return for their participation in the research.

6. One of the initial areas chosen for the research was later abandoned as it was discovered that the Clinical Officer helping in the collection of specimen was cheating. One specimen would be divided into three specimen bottles bearing different numbers and names. These were all discarded .

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7. Since only one specimen per child was collected, the possibility of having missed some cases of cryptosporidiosis remains.

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APPENDIX 1

QUESTIONNAIRE NUMBER:...

1. Residence...
2. Name of Child...
3. Age:..
4. Sex M.................. F..................
5. Weight now:...
6. Weight before diarrhoea:......................................
7. Nutritional status of child Good.................
   OK:..................
   Bad:.................
8. Is the child still breast-feeding Yes:.................
   No:..................
9. Major water source: Piped water...........
   Borehole..............
   Protected well........
   Unprotected well......
10. Is water available: Always................
    Sometimes.............
11. Alternative source of water: ....
12. Is drinking water boiled? Yes.................
    Sometimes.............
    No..................
13. Socio economic status: High.................
    Medium................
    Low:..................
14. Number of persons in household: ....
15. Animals in the vicinity of the house:.................

16. Household items possessed;

Television	----------------
Video	----------------
Fridge	----------------
Car	----------------
Radio	----------------
Bicycle	----------------

17.	Level of education:	husband	---------------------------
		wife	---------------------------
18.	Income per month:	husband	---------------------------
		wife	---------------------------
19.	Toilet facilities:	Communal	---------------------------
		Own	---------------------------
		Pit	---------------------------
		W.C.	---------------------------

APPENDIX 2

CONSENT FORM

I................................. agree to have my child's/Ward's STOOL specimen taken to be used in the Research. I have voluntarily agreed to have my child enrolled in the study and I am aware that I will not be paid for it. The Research procedure has been explained to me.

SIGNED:..................................

DATE:....................................
