SOME ASPECTS OF THE EPIDEMIOLOGY OF INTESTINAL PROTOZOAN INFECTIONS IN KWAZULU-NATAL, SOUTH AFRICA

By

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ABSTRACT

This study was carried out to investigate different aspects of the epidemiology of the common intestinal protozoan infections in children in KwaZulu-Natal. The main aspects studied were to:

i. monitor changes in the prevalence and intensity of the common intestinal protozoons in children after receiving antihelmintic drugs,
ii. determine whether environmental and/or socio-economic factors are important in the prevalence of the common intestinal protozoons,
iii. determine the occurrence of *Cryptosporidium parvum* in stool samples of paediatric patients with gastroenteritis and in healthy school children.

Intestinal protozoan species often co-occur with intestinal nematodes in infected children and it is important to determine the effect that anti-nematode treatment has on concomitant protozoan infections. The study included the analysis of stool samples by the Formol-Ether Concentration Technique, examination of the obtained sediments for intestinal helminths and protozoan species, and treatment of nematode-infected individuals. Four surveys were carried out to determine the prevalence and intensity of intestinal protozoons in school children before and after anthelmintic chemotherapy, and the χ^2 test was used to determine significant changes. Prevalences and intensities of helminths were determined for significant changes after treatment using the Student's *t*-test. Additional pre- and post-treatment prevalence data were obtained from four schools in Health Region A of KwaZulu-Natal. The prevalence of *Ascaris lumbricoides* decreased significantly from 75.2% to 9.7% after the first treatment, that of *Trichuris trichiura* decreased from 77.7% to 62.1%, and hookworm infections (presumably *Necator americanus*) dropped from12.7% to 1.0% and remained very low for the rest of the study. Very few individuals were infected with protozoan species and for statistical analysis, these were combined. The prevalence of protozoan infections increased from 33.0% to 50.3% after the first treatment and from 35% to 42% after the second treatment. The prevalence of *A. lumbricoides* decreased significantly again after the second treatment while there was no significant decrease in the prevalence of *T. trichiura*.

The intensities (number of eggs/gram of feaces) of the three nematodes also decreased significantly after the two treatments. It is recommended that children who have been treated for nematode infections should also be examined for protozoan infections, and these should also be treated accordingly.

A retrospective analysis of protozoon prevalence data from different surveys in KwaZulu-Natal was done in order to determine the importance of environmental and/or socio-economic factors in the distribution of intestinal protozoons. These data were plotted on the map of KwaZulu-Natal using Geographic Information System (GIS). Univariate analysis was carried out to determine significant correlations between the prevalences of protozoan species and selected variables. The significant correlations obtained were moderate and no strong correlations were obtained. Univariate stepwise regression analysis was performed to determine the factors that combine best in facilitating the transmission of protozoan species and significant associations were obtained between the prevalence of protozoon species and a combination of environmental and socio-economic factors. In most cases, the association between prevalence and mid-summer temperature and rainfall were the most significant. This is an indication of increased summer transmission. Altitude was significantly correlated only with the prevalence of *Endolimax nana*. The fact that moderate correlations were obtained between prevalence of intestinal protozoons and climatic and socio-economic factors indicates that these factors are important in the distribution of the common intestinal protozoons. However, lack of strong correlations suggests that in addition to climatic and socio-economic factors, there are other factors that have an effect on the distribution of intestinal protozoan species. In the multivariate analysis where the variables were simultaneously considered, the presence of electricity was the only factor that was significantly associated with the variation seen in the prevalence of intestinal protozoons in the different study locations.

Cryptosporidium parvum is a parasitic protozoon that is associated with severe fatal diarrhoea in children and immunocompromised individuals. Oocysts of this parasite were found in 18.2% of stool samples collected from children (aged 6 to 48 months) who were admitted in the paediatric wards at King Edward VIII Hospital, Durban. The

stool samples were firstly concentrated using the Formal-ether method and the obtained sediment was mixed with the Sheather's Sucrose solution and examined microscopically. No oocysts were found in stool samples collected from older primary school children. Although the diarrhoea in these children might have also been due to other causes, the results obtained further show the importance of *C. parvum* as a cause of diarrhoea in children below the age of five years. Knowledge of the epidemiology of *C. parvum* is crucial in the control of this parasite as there is currently no effective treatment. More intensive surveys are needed in determining the epidemiology of this pathogen in the South African population.

PREFACE

The work described in this thesis was carried out in the School of Life and Environmental Biology, University of Natal, Durban, from February 1999 to December 2000, under the supervision of Professor Chris C. Appleton.

The study represents original work by the author and has not been submitted in any form to any tertiary institution. Where use has been made of the work of others, it has been duly acknowledged in the text.

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M.E. Khabisi

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CHAPTER 1

INTRODUCTION

Diarrhoeic diseases in children are one of the major medical problems in developing tropical countries, with one third of the beds in paediatric wards being occupied by children with diarrhoea (Villod *et al.*, 1979). This casts a heavy burden on the health budgets of these countries. In South Africa, about 43 000 people die from diarrhoeal diseases every year and the annual health care costs incurred due to diarrhoea in this country amount to at least R3.0 billion (Pegram *et al.*, 1998). Diarrhoea in children influences the general health through decreasing intestinal absorption due to accelerated intestinal transit, increasing loss of water and important minerals, and growth retardation (Villod *et al.*, 1979).

Worldwide, parasitic protozoan infections account for about 10% of the gastroenterology cases in children (Ebrahim, 1977) and transmission is mainly person to person and through ingestion of food and water contaminated with cysts and oocysts, while flies play an important role in long distance transmission (Ebrahim, 1977). A survey done in 1985 showed that intestinal infections, together with perinatal and respiratory diseases accounted for 82.5% of infant mortality (number of deaths in children under one year of age/1000 live births) in South Africa, and these infections were found to be the most common causes of infant deaths in this country (Kustner, 1989). More recent data indicate that 2.87% of all deaths in South Africa are caused by water-related intestinal infections (Bourne and Coetzee, 1997) and these are the major cause of death in the 0-5 year age-group where they account for about 20% of all deaths in South Africa.

Pathogenic human intestinal protozoons include *Giardia intestinalis*, *Entamoeba histolytica*, *Balantidium coli*, *Dietamoeba fragilis*, *Blastocystis hominis*, *Isospora belli*, *Cryptosporidum parvum*, microsporidia and cyclosporidia (Fitzgerald and Clark, 1988; Tzipori and Griffiths, 1998) and infection with any of these protozoons may result in diarrhoea. Less than two decades ago, microsporidia and cyclosporidia were completely

unknown while *Cryptospridium* was not considered as an important cause of enteric disease in people (Tzipori and Griffiths, 1998). The importance of diseases caused by these protozoons in humans has only recently become known with the presence of the Human Immunodeficiency Virus (HIV).

Diarrhoea is also a common complication in symptomatic HIV infected individuals (Brandonisio *et al.*, 1999), and as in paediatric diarrhoea, this may have multiple aetiologies but opportunistic intestinal protozoan infections account for most of the disease (Chaisson *et al.*, 1998; Kotler and Orenstein, 1998; Fontanet *et al.*, 2000). These infections have a significant impact on survival of these patients as they further weaken the immune system that is already engaged in the fight against HIV.

The digestive tract provides an ideal habitat for protozoons as it is rich in nutrients and bacteria on which protozoan species can feed, and surfaces on the tract provide suitable attachment sites for these organisms.

Giardia intestinalis interferes with the absorption of nutrients by attaching to the mucosa while *E. histolytica* and *B. coli* are invasive and therefore cause tissue injury. Pathogenesis depends on the host's age, nutritional and immune status, genetic disorders and socio-economic factors (Elson-Dew and Freedman, 1952; Yardley and Bayless, 1967; Ebrahim, 1977; Meyer and Radulescu, 1979; Webster, 1980). Other factors that play an important role in the pathogenesis of infection are infecting dose (Yardley and Bayless, 1967) and interaction with metozoan parasites (Powell *et al.*, 1961; Christensen *et al.*, 1987).

1.1 Motivations and objectives of the study

A lot of research in KwaZulu-Natal has focused on determining the prevalence of intestinal nematodes and their effect on the general health of children. High prevalences and intensities of the geohelminths, *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworm, *Necator americanus*, have been found in school children living on the coastal plain of KwaZulu-Natal (Schutte *et al.*, 1981; Maurihungirire, 1993; Taylor *et al.*, 1995; Appleton and Gouws, 1996; Mabaso, 1998; Appleton *et al.*, 1999). These infections have been associated with morbidity such as impaired physical and mental development in school children in this province and elsewhere (Cole and Parkin, 1977; Nokes *et al.*, 1991) and this often results in poor academic performance and absenteeism from school. Because of the high infection rates in school children, the Department of Health in KwaZulu-Natal has set up a school-based Parasite Control Programme (presently the only one in the country) which mainly aims at reducing the transmission rate of these nematodes in school children in this province. Intestinal nematode infections however, commonly co-occur with intestinal protozoan infections and therefore knowledge of interactions between these organisms, and the effect that de-worming has on protozoan infections is essential for efficient control. An increase in the prevalence of *G. intestinalis* after anti-nematode treatment was noted in northern Bangladesh (Rousham, 1994) and southern KwaZulu-Natal (Taylor *et al.*, 1995).

Knowledge of the distribution of intestinal protozoan species, especially the pathogenic ones, with regard to the topography and climate of KwaZulu-Natal is also important for optimal cost-effectiveness of parasite control programmes (Appleton and Gouws, 1996). Unlike intestinal nematodes, intestinal protozoons do not require development in the outside environment and can therefore be directly transmitted from person to person i.e (hand-to-mouth). Socio-economic factors would therefore play a more important role than environmental factors in the transmission of protozoan infections. This hypothesis on the distribution of intestinal protozoan infections depends more on socio-economic factors tested by analysis of epidemiological data collected from different studies in the province of KwaZulu-Natal.

The pathogenesis of *Cryptosporidium* in animals has long been established, but it's role as a cause of human disease was only recently discovered (Meisel *et al.*, 1976; Nime *et al.*, 1976). In healthy individuals, *C. parvum* infection is usually asymptomatic or might cause a self-limiting diarrhoea, but it causes a fatal watery diarrhoea in immunocompromised individuals, especially those with HIV infection (Fripp and Bothma, 1987; Fripp *et al.*, 1991). Although the occurrence of this parasite in South Africa was discovered at King Edward VIII Hospital, Durban (Smith, 1985), it is important to constantly determine its prevalence in this province where HIV/AIDS infections keep escalating (Kustner *et al.*, 1998). *Cryptosporidium parvum* infections were determined in healthy school children and in paediatric patients admitted to King Edward VIII Hospital in Durban, South Africa.

The objectives of this study were to expand knowledge on the protozoan parasites of man in KwaZulu-Natal in the following three important areas by:

- monitoring the changes in both prevalence and intensity of protozoan infections in school children after anthelmintic chemotherapy
- determining whether or not environmental and/or socio-economic factors determine the distribution of intestinal parasites in KwaZulu-Natal
- determining the prevalence of *Cryptosporidium parvum* in healthy school children and in paediatric patients

The first two have an impact on the Parasite Control Programme referred to earlier and the third is an important consequence of the current HIV/AIDS pandemic.

CHAPTER 2

DIVERSITY OF INTESTINAL PROTOZOONS IN SOUTHERN AFRICA

2.1 Human intestinal protozoons

Intestinal parasitic infections are among the most common human infections throughout the developing world and are particularly common in the poorest communities. Although they are not classified as contagious and rarely attain epidemic status, they can be harmful to their hosts by influencing basic physiological processes and facilitating secondary infections. Their co-existence with different micro-organisms may elicit conditions much more severe than when either agent is present alone (Kuntz and Myers, 1972).

The intestinal protozoons (protists) commonly found in people in KwaZulu-Natal and other parts of Southern Africa, are shown in Table 2.1. These include the pathogenic *E. histolytica*, *G. intestinalis*, *B. coli* and *Isospora* spp. Although all the other protozoons listed are considered non-pathogenic (i.e. they are commensals) and are often present in light infections in healthy individuals, their importance lies in the fact that they are an indication that oral-faecal transmission has occurred and if present, one has to be on the look-out for the possible presence of the pathogenic species (WHO, 1998). Protozoan cysts and oocysts are excreted in the faeces of infected individuals. Due to poor hygiene and lack of sanitation, the cysts and oocysts are transmitted by the faecal-oral route via contaminated food and water. Both pathogenic and commensal protozoons can be life-threatening in immunocompromised patients, particularly those with AIDS (Mendez *et al.*, 1994).

Table 2. 1. Prevalences (%) of intestinal protozoons found in Man at different localities in KwaZulu-Natal and other parts of Southern Africa

	Locality *						
	A	B	C	D	E	F	G
Protozoon							
Amoebae							
Entamoeba coli	34.9	-	40.2	60.5	40.0	52.8	53.2
Entamoeba histolytica/E.dispar	5.3	6.0	2.2	4.3	11.0	1.4	14.5
Entamoeba hartmanni	-	9.8	-	4.3	5.0	5.9	6.7
Iodamoeba butschlii	7.6	7.7	5.8	4.3	9.0	2.2	1.7
Endolimax nana	1.1	-	6.6	7.0	32.0	18.1	3.7
Flagellates							
Giardia intestinalis	2.5	13.0	8.0	3.4	5.0	4.5	4.1
Chilomastix mesnili	0.3	9.8	-	4.2	14.0	11.8	6.1
Trichomonas hominis	-	-	-	-	6.0	-	-
Ciliates							
Balantidium coli	0.1	-	-	-	-	-	-
Coccidia							
Isospora hominis	0.3	-	-	1.0	2.0	-	-

* Localities - indicates that the parasite was not found

A - Durban (Elson-Dew and Freedman, 1952)

B - Southern KwaZulu-Natal (Taylor *et al.*, 1995)

C - foothills of the Drakensberg Mountains (Appleton and Gouws, 1996)

D - Northern KwaZulu-Natal (Schutte *et al.*, 1981)

E - Durban (Powell *et al.*, 1961)

F - QwaQwa (Mosala, 1995)

G - Lesotho (Kravitz *et al.*, 1993)

2.1.1 Flagellates (Phylum Sarcomastigophora)

All the above-mentioned protozoons, with the exception of *T. hominis*, have both the trophozoite and cystic stages in their life-cycle. *Trichomonas hominis*, which is sometimes referred to as *Pentatrichomonas hominis* because of the presence of five flagella, only have the trophozoite stage. *Trichomonas hominis* is usually found in the colon or caecum where it feeds on carbohydrates in food and may be found in diarrhoeic stool, although its presence in stool is just an indication of an upset in the host's carbohydrate metabolism (Fripp, 1995). Another intestinal protozoon that does not have a cyst stage in its life-cycle is *Dientamoeba fragilis*. Because of the absence of a cyst stage and the fact that it disappears rapidly after stool passage, it is rarely found in stools (Juckett, 1996). *Dientamoeba fragilis* was initially classified as an amoeba but is now considered to be a flagellate (Fripp, 1995). It is normally found in association with *Enterobius vermicularis* (pinworm), leading to the speculation that it is transmitted on the eggs of the pinworm (Sawangjaroen *et al.*, 1993; Fripp, 1995). The protozoon was previously thought of as a commensal but is now considered as a potential pathogen, particularly in children 5-10 years old (Smyth, 1994; Grendon *et al.*, 1995), and may cause nausea, diarrhoea, weight loss and abdominal discomfort (Juckett, 1996).

There is no universally accepted name for the *Giardia* species found in Man, with *G. lamblia, G. intestinalis* and *G. duodenalis* being specific names used by different authors (Ackers, 1980; Lymbery and Tibayrenc, 1994). However, according to Tibayrenc (1994), and Mayrhofer and Andrews (1994), *G. duodenalis* is a complex of species that parasitize vertebrates while *G. intestinalis* particularly infects man. The name *G. intestinalis* is therefore used in this thesis. *Giardia* parasitizes the small intestine of man where it feeds on mucous secretions, although it is not invasive (it does not penetrate the mucosa), it causes mechanical damage by adherence to the intestinal wall. The clinical manifestations of infection range from asymptomatic to severe diarrhoeal conditions. Intestinal damage due to *G. intestinalis* results in fat and vitamin malabsorption, rapid weight loss and growth retardation in children, particularly those under the age of three (Cole and Parkin, 1977; Rowland and McCollum, 1977; Farthing *et al.*, 1986; Esrey *et al.*, 1989). Giardiasis is the most common parasitic protozoal

infection, with prevalences ranging from 2-5% in the developed world and 20-30% in the developing world (Farthing, 1994). It is most common in children below the age of 10 years and outbreaks usually occur in children living in overcrowded areas (Fripp, 1995). *Giardia* outbreaks have been reported in day-care centres, residential institutions, schools, and in people who travel to giardiasis endemic areas (Ebrahim, 1977; Farthing, 1994). *Giardia* infection is however, rare in breast-feeding infants due to immunity from the mother's milk (Gillin *et al.*, 1983; Farthing *et al.*, 1986; Savioli, 1994). Sexual transmission of *Giardia* between male homosexuals with and without HIV infection has been reported (Farthing, 1994), and *Giardia* spp. indistinguishable from *G. intestinalis* at the light microscope level have been isolated from beavers and calves, and therefore Erlandsen (1994) has proposed that giardiasis is a zoonosis.

Giardia cysts are highly resistant as they can withstand extreme environmental conditions, and as a result have been found in a variety of places. They survive well in untreated water and can resist chlorine levels that are effective against bacteria, leading to water-related diarrhoeal outbreaks throughout the world (Goldin *et al.*, 1990; Hilner and Kfir, 1992; Steiner *et al.*, 1997). Wallis (1994) has reported cases of waterborne giardiasis in the United States, Canada, Scotland, Sweden, Australia and New Zealand. Human and animal faeces are the main source of *Giardia* cysts in water bodies and faecal contamination of water bodies usually occurs when the water is used both as a source of raw water and a receiving body for treated and untreated sewage effluent (Wallis, 1994).

Giardia cysts have been isolated in selected South African drinking waters by various researchers (Hilner and Kfir, 1992; Kfir *et al.*, 1995; Gericke *et al*, 1996; Jarmey-Swan, 1999). Jarmey-Swan (1999) also detected *Giardia* cysts in treated wastewater effluents and sludge from different sites of the Darvill wastewater works in Pietermaritzburg. She suggests that the treated effluents discharged into rivers, and the sludge disposed of onto land, should always be monitored for protozoan parasites as this could result in the contamination of drinking water sources and grazing land. In a recent study, du Preez and Gericke (1999) isolated *Giardia* cysts from 17% of samples from surface and ground water which serves rural and urban informal settlement communities in the

Northern and Northwest provinces. High concentrations of cysts (of up to 2000 cysts/100 ℓ) were found in these water-bodies.

The amount of water used for personal and domestic use is also a risk factor in protozoan infections. During a study in rural areas of Lesotho, Esrey *et al.* (1989) observed that it is the small amount of water used for personal and domestic hygiene rather than the quality of drinking water that encourages the transmission of *G. intestinalis*. As *G. intestinalis* infections are directly passed from person to person, the use of large amounts of water for personal hygiene is as important as the elimination of human and animal faeces from the environment.

2.1.2 Ciliates (Phylum Ciliophora)

Balantidium coli is the only ciliate and the largest protozoon to parasitize man, where it lives in the colon and appendix of the host. Infection with *B. coli* can lead to severe and fatal diarrhoeal conditions due to invasion of the mucosa and penetration to the submucosa where the parasite forms ulcerations. In severe cases, the ulcers may involve the whole bowel wall and result in frequent bloody and mucoid stools (WHO, 1998). *Balantidium coli* is generally associated with pigs and infections have been reported from pig handlers (Currie, 1990; Pinheiro and de Lima, 1990; Appleton *et al.*, 1995; Juckett, 1996). Balantidiasis has also been reported in AIDS patients (Clyti *et al.*, 1998).

2.1.3 Amoebae

Entamoeba histolytica is the main cause of intestinal and extra intestinal amoebiasis (amoebic dysentery) in man. It invades the caecum where it penetrates the mucosa and sub-mucosa, leading to ulcer formation. Severe infections may involve the small intestine and the appendix (Ebrahim, 1977). The onset of the disease is indicated by diarrhoea and mild intestinal discomfort. In mild cases, there is an intermittent diarrhoea with few trophozoites in the stools while severe amoebiasis results in amoebic ulcers in the mucosa and frequent bloody stools containing numerous trophozoites with ingested erythrocytes (Fripp, 1995). Amoebiasis leads to severe abdominal cramps, anorexia,

nausea and vomiting, and can be a life-threatening illness if untreated or mis- diagnosed (Brack, 1987).

The most common complication of amoebiasis is the migration of trophozoites through the portal blood system to the liver, leading to liver abscess. The trophozoites can also migrate to the lungs, brain and genital organs, leading to complications in these organs (Heinz, 1973; Segal *et al.*, 1981). Extra intestinal amoebic abscesses mostly occur in males aged between 20 and 50 years (Brack, 1987). Amoebiasis cases have been reported from different areas in South Africa. Liver and lung abscess due to amoebiasis have been found in patients at Baragwanath Hospital in Johannesburg (Segal *et al.*, 1981) and in children at Tygerberg Hospital in Cape Town (Hendricks, 1994). Heinz (1973) reported three cases of amoebiasis of the female genital tract in Pretoria. One of these patients also had carcinoma of the cervix, and an association was found between amoebiasis and the carcinoma. Table 2.2 is a list of amoebiasis complications reported from King Edward VII hospital, Durban (Hussey, 1998).

Table 2.2. Complications of invasive amoebiasis (1960 to 1982) at King Edward VIII Hospital, Durban (from Hussey, 1998)

Frequent complications	No.	Mortality (%)
Dysentery, peritonitis and amoebic liver abscess	18	100
Dysentery and peritonitis	90	89
Dysentery and amoebic liver abscess	163	50
Dysentery	1176	22
Amoebic liver abscess	273	17
Rare complications		
Pericarditis	7	71
Intussusception	7	57
Amoeboma	5	20
Appendicitis	3	100
Brain abscess	2	100
Colonic stricture	1	-
Volvulus	27	3.7

An explanation of some of the terms as stated by Stanaszek *et al.* (1991) is given below.

Peritonitis - inflammation of the peritoneum (membrane lining the abdominal cavity)

Pericarditis - inflammation of the pericardium (membrane surrounding the heart)

Intusscusception - invagination of one part of the bowel into another

Amoeboma - a tumour that is caused by *E. histolytica* that occurs in the rectum or caecum

Volvulus - twisting of part of the digestive tract, leading to partial or complete obstruction of the affected area

In 1925, Brumpt described another species, *Entamoeba dispar*, which is morphologically identical to *E. histolytica* (Petithory *et al.*, 1994; Clark, 1998; Jackson, 1998). This was however, dismissed by his colleagues as a synonym for *E. histolytica*, and this parasite was then considered to have pathogenic and non-pathogenic forms. It was only with the recent biochemical, immunological and genetic data that the existence of *E. dispar* as a non-pathogenic species was accepted (Petithory *et al.*, 1994; Jackson 1998; Mehlotra, 1998; WHO, 1998). The invasive species is now known as *E. histolytica,* while *E. dispar* is a commensal. However, cysts of the two protozoons are indistinguishable at light microscopy level and can only be distinguished by electron microscopy (WHO, 1998). The name *E. histolytica/dispar* is therefore used in this thesis.

Other pathogenic amoebae include *Naegleria* and *Acanthamoeba* spp. Although these are free-living, they are capable of causing severe pathogenic conditions in man (Smyth, 1994). *Naegleria fowleri* is transmitted through heated water (in which it exists as a flagellate) such as stagnant ponds, swimming pools or factory effluents (Fripp, 1995). Trophozoites in the flagellate form are then taken with the water into the body through the nasal cavity and once in here, they change into amoeboid forms and invade the olfactory mucosa. The trophozoites then migrate to the central nervous system where they can cause fatal primary amoebic meningoencephalitis (Lastovica, 1977; Fripp, 1995).

Although no human cases were reported, *Naegleria fowleri* was isolated from a heavily polluted area of the Eerste River in the Western Cape (Fripp, 1995). *Acanthamoeba*

spp., which do not have a flagellate form in their lifecycle, have been implicated as agents of eye infections (Fripp, 1995; Niszl and Markus, 1998), chronic encephalitis, skin ulcers and keratinitis (Smyth, 1994). Like *Naegleria*, *Acanthamoeba* occurs in waterbodies and has also been found in the Eerste River. It has also been isolated from air conditioning systems and kidney dialysis machines (Smyth, 1994). In Johannesburg, Markus and Niszl (1990) cultured *Acanthamoeba* from contact lenses of people who did not have ocular disease. These authors advise contact lens users not to use home-made saline, non-sterile distilled water, tap water or saliva for cleaning, storing or inserting their contact lenses as these do not kill or inactivate amoebic cysts. Niszl and Markus (1991) isolated another free-living amoebo-flagellate, a species of *Mastigina*, from an infected eye of a patient in a Johannesburg hospital.

2.1.4 Coccidia (Phylum Apicomlexa)

The gut Coccidia are members of a large, varied, and exclusively intracellular group of protozoan parasites. Four genera of this group (*Sarcocystis, Cryptosporidium*, *Isospora* and *Cyclospora*) are human pathogens (Ackers, 1997). Another genus, *Toxoplasma*, is a zoonotic protozoon, the cat being the principal reservoir host. These protozoan parasites have asexual and sexual stages within intestinal epithelial cells of the host and produce an environmentally resistant cyst stage, the oocyst. Infections are acquired either by the ingestion of these oocysts in contaminated food or water; or by ingestion of the cysts in raw or slightly cooked meat. These parasites have become a major problem with the coming of the AIDS pandemic where their infections lead to severe enteritis and chronic diarrhoea (Amenata *et al.*, 1999). In immunocompetent individuals, they cause a self-limiting diarrhoea which normally lasts for a few days only.

Parasites of the genus *Isospora* cause intestinal disease in several mammalian host species. Human intestinal isosproriasis is caused by *Isospora belli* and symptoms of infection in immunocompetent individuals include chronic diarrhoea, stearrhoea (loss of body fat) and weight loss. Symptoms are more severe in AIDS patients, with the diarrhoea being more watery (Lindsay *et al.*, 1997). Elsdon-dew and Freedman (1953) encountered several cases of infection by *Isospora* species in KwaZulu-Natal. These

were described as *I. belli*, *I. rivolta* and *I. hominis*. *Isospora hominis* was however, later indicated as a stage in the life-cycle of *Sarcocystis* species of cattle and pigs (Markus, 1977).

Sarcocystis spp. also have indirect life-cycles, with herbivores (both wild and domestic) acting as intermediate hosts and carnivores (including man) as the definitive hosts. Man may occasionally act as an intermediate host. Human infection is through the ingestion of the oocysts in contaminated food or water, or from undercooked meat. Although *Sarcocystis* cysts have been found in muscles of different animals, man is the definitive host for *S. hominis* which he acquires by eating infected, undercooked beef and *S. suihominis* which he gets from infected pork. The symptoms of intestinal sarcosystosis in man are nausea and vomiting, stomach pains, diarrhoea and dypnoea (Markus, 1977; Fripp, 1995).

Man can be infected with *Toxoplasma gondii* through the ingestion of infective oocysts passed in the faeces of cats, by congenital transmission or by ingestion of the oocysts in raw or slightly cooked meat (Walker, 1984). Cooks, butchers and anybody who handles raw meat is at risk of getting toxoplasmosis due to the presence of infective oocysts in the muscles of various mammals (Smyth, 1994). Walker (1984) reported that in Australia, a high proportion of sheep from the cool and moist areas of the country are infected with *T. gondii*, probably as a result of ingestion of oocysts passed onto pastures by cats and that eating undercooked mutton in this country can be a source of *Toxoplasma* infection. Cats are the only animals in which *T. gondii* can complete it's sexual development, and all the other mammalian species implicated in it's transmission have been identified as intermediate hosts. In man, toxoplasmosis is almost symptomless although it may result in fever and swelling of the liver and spleen. Smyth (1994), mentioned that the immune status of an individual is an important factor in the pathogenicity of *T. gondii* infections and that in immunocompromised individuals, new and old infections can lead to ocular toxoplasmosis and sometimes to fatal central nervous system disorders.

In South Africa, *Toxoplasma* infections are mild as the local strains appear to be of low virulence (Jacobs, 1977). Toxoplasmosis has been found in Johannesburg (Mason *et al.*, 1974), the Free State (Brink *et al.*, 1975), Kwazulu-Natal (Jacobs, 1977), and the Western and Eastern Cape (Jacobs, 1977). A 10% prevalence rate was also recorded from the Bushmen tribe in Namibia (Jacobs, 1977). Congenital toxoplasmosis occurs when a pregnant woman acquires the disease and although she may be asymptomatic, transmission to the foetus can take place through the placenta leading to premature births, mental abnormalities and sometimes death of the born infant. In a study to determine the occurrence of toxoplamosis in white pregnant mothers in the Johannesburg area, Jacobs (1977) found a prevalence of only 2.2% (37/1700). The mothers were asymptomatic during pregnancy, and their babies had no clinical or serological evidence of infection when examined at four months of age.

The Microsporidia are a phylum of small unicellular obligate protozoa which commonly parasitise invertebrates by penetrating the host's cells (Fripp, 1995). Various genera and species of microsporidia cause disease principally in immunocompromised individuals. Microsporidia produce resistant spores which are excreted in faeces, urine or other body secretions (WHO, 1998). Until recently, microsporidiosis had been considered a rare human disease, but several cases have now been reported in AIDS patients who have renal and vascular pathology, persistent diarrhoea and weight loss due to *Encephalocystozoon cuniculi* (Carr *et al.*, 1998) and severe diarrhoea due to *Septata intestinalis*, another microsporidian (Fripp, 1995). Pol *et al.* (1993) and Carr *et al.* (1998) have reported microsporidiosis cases due to *Enterocytozoon bieneusi* in HIV infected people.

Another recently discovered coccidian is *Cyclospora*. Bendall *et al.* (1993) reported the existence of cyanobacterium-like bodies (CLB) which were associated with prolonged severe and sometimes fatal diarrhoea and weight loss. These bodies were seen mainly in travellers and immunocompromised patients and they are now known as *Cyclospora cayetanensis*. In Johannesburg, Markus and Frean (1993) reported *C. cayetanensis* infection in two children who had diarrhoea. Infections have also been reported from

various other places such as Peru (Madico *et al.*, 1993) and United Kingdom (Bendall *et al.*, 1993).

Blastocystis hominis is a bowel protozoan that was initially considered as a yeast but has recently been found to be a protozoan (Fripp, 1995). It is generally considered to be a commensal although it can very rarely cause a mild diarrhoea, especially in immunocompromised people (Logar *et al.*, 1994; Juckett, 1996; Morgan *et al.*, 1996). Its pathogenicity is therefore still a controversial issue (Horiki *et al.*, 1997). At the Amoebiasis Research Unit (Medical Research Council, Durban), *B. hominis* often grows and multiplies in culture when the Robinsons medium is used to culture amoebae from stool specimens (C. Anderson, pers comm.). Usually, the organism is not seen in microscopic examination of the stools before the culture medium is added but rapidly multiplies in culture. This resulted in the introduction of acriflavine to the culture medium by this unit, which effectively kills *B. hominis*.
Cryptosporidium will be dealt with in detail in Chapter 4.

2.2 Protozoan parasites and HIV infection

Blastocystis hominis, *Cryptosporidium parvum*, *Isospora belli*, microsporidia, *G. intestinalis*, *E. histolytica*/*E.dispar*, *Toxoplasma* and *Sarcocystis* have all been found in HIV-infected patients (Sarvillo *et al.*, 1990; Brandonisio *et al.*, 1999; Pakianatban and McMillan, 1999; Fontanet *et al.*, 2000). Immunity against protozoan infections is predominantly T-cell mediated and therefore these opportunistic infections in immunocompromised people are a result of the weakened immune system by HIV (Canning, 1990). The virus binds to $CD4^+$ receptors on T-cells, destroying the cells and decreasing T-cell mediated immunity of the host. The gastrointestinal tract, lungs, nervous system and skin are regarded as common areas in which opportunistic infections occur in people with AIDS (Granziano and Lemanske, 1989). The loss of $CD4^+$ cells is considered as the most accurate marker of HIV infection (Carr *et al.*, 1998) and can be expressed as an absolute value, a percentage, or a ratio of CD4 to CD8 cells (Lachman, 1990).

In healthy individuals, infection with pathogenic protozoons usually leads to a transient diarrhoea, whereas there is no pathogenicity associated with commensal protozoons. Mendez *et al.* (1994) stated that both pathogenic and commensal protozoan infections can be life threatening in immunocompromised patients, particularly those with AIDS. Lachman (1990) also mentioned that opportunistic infections associated with HIV are commonly seen in a more severe form and are characterized by a high density of organisms.

In a study done in the USA (Chaisson *et al.*, 1998), 14% of HIV infected patients died due to opportunistic infections. Toxoplasmosis and cryptosporidiosis, among other infections, were significantly associated with the risk of death. When these patients were followed-up, the average monthly loss of $CD4^+$ cells was almost double that of patients without opportunistic pathogens, suggesting that $CD4^+$ cells decline during or after infection with these pathogens. The authors suggested several mechanisms for the association of opportunistic infection and death in AIDS patients. Some infections can result in direct mortality of the host due to morbidity associated with these infections. They can also cause an upgrading in HIV replication and therefore result in higher viral loads, a possibility also suggested by Elson-Dew (1977). The ways in which opportunistic infections upregulate HIV expression may include: antigen mediated activation of latently infected cells; activation of uninfected T-cells, making them more susceptible to HIV infection; and promotion of viral replication by pro-inflammatory cytokines.

2.3 Intestinal protozoons of non-human primates

Since the widespread use of non-human primates (baboons and monkeys) in biomedical research, it has become necessary to gain knowledge about the diseases common to and hosted by these animals that are potentially transferable to Man. Parasitology research has been done on different wild and captive (zoos and laboratories) non-human primates. Since many parasites (e.g. several protozoons and the whipworm, *Trichuris*) harboured by non-human primates and man appear to be similar at the light microscope level, parasitologists believe that they might be identical (Myers and Kuntz, 1968).

However, it is possible that differences may be found between them using the electron microscopy, and by biochemical and molecular techniques.

The implication of zoonotic protozoan infections is that the increased contact between man and non-human primates would encourage the transmission of pathogenic protozoons from one to the other. Contact between man and non-human primates may occur when they share the same ecosystem, the animals are captured to be sold or used for food or research, and when humans get close enough to touch the animals, as is the case with pet owners and when people visit zoos (Brack, 1987). McConnell *et al.* (1974) mentioned that in the Kruger National Park, the highest densities of baboons were along tourists' roads and human habitats (temporary camping and working sites) where they become accustomed to traffic and soon learn to beg for food. Myers and Kuntz (1968) also realized that the baboons included in their study in Kenya had contact with indigenous people as well as with handlers during trapping.

This human-animal interaction which exposes people to pathogens harboured by non-human primates has been reported in the vervet monkey (*Cercopithecus aethiops*). Due to their food raiding activities in human habitats, these animals are considered as pests (Brennan *et al.*, 1985). At the Amboseli National Park in Kenya, vervets have become accustomed to eating food from human garbage disposal areas and being hand-fed by tourists and residents (Brennan *et al.*, 1985). These lodge vervets are considered as "nuisances" as they frequently attack and injure tourists and residents, exposing these people to pathogens. Interaction between man and vervet monkeys has also been reported from a residential suburb in Blantyre, Malawi (King and Lee, 1987) where the monkeys raid gardens of residents for maize, beans and other crops, damaging the crops. The raiding of residential areas by monkeys is also common here in Durban where these monkeys often get into houses through open windows in search for food.

Parasitism of non-human primates by intestinal protozoa is dependent primarily on eating habits and ecological habitat (Kuntz and Myers, 1972; Rowland and Vandenbergh, 1965). These animals become prone to parasites due to their omnivorous eating habits, their curiosity which encourages the handling of a wide range of food and

most importantly, the fact that they are coprophagous (i.e. they eat faeces) as this brings them into contact with contaminated faeces passed by fellow members or by other animals (Kuntz *et al.*, 1968). Although the same range of protozoan infections has been found in both free-ranging and captive animals of the same species, Myers and Kuntz (1968) noted a decrease in the prevalence of intestinal protozoa of the primates as they pass from free-ranging to captivity. This change is partly due to the drastic alteration in the diet.

In Southern Africa, studies have been done on the parasitology of the Chacma baboon (*Papio cynocephalus ursinus*) (Table 3) mainly because of its large size and availability as it occurs in a wide variety of habitats (Jackson *et al.*, 1990). The prevalences of infection by the various intestinal protozoons in the Chacma baboon are in the following ranges: (Powell and Elson-Dew, 1961; McConnell *et al.*, 1974; Goldsmid and Rogers, 1978; Myers *et al.*, 1971; Appleton *et al.*, 1986; Appleton and Henzi, 1993):

Amoebae

Entamoeba coli	17 - 78 %
Entamoeba histolytica	3 - 14 %
Entamoeba hartmanni	8 - 11 %
Entamoeba chattoni	15 %
Endolimax nana	4 - 16 %
Iodamoeba butschlii	1 - 22 %

Flagellates

Chilomastix mesnili	4 - 17 %
Trichomonas spp.	4 %

Ciliates

Balantidium coli	20 - 80 %

Coccidia

Isospora spp.	3 %

In a study in Karkloof, KwaZulu-Natal, cysts of five protozoan species were recovered from faecal samples of the Samango monkey, *Cercopithecus mitis labiatus* (Appleton *et al.*, 1994). The species were identified as *Entamoeba coli*, *E. hartmanni*, *E. chattoni*, *Endolimax nana* and *Iodamoeba butschlii*. *Balantidium coli*, which is regarded as a

natural parasite of non-human primates (Myers and Kuntz, 1968), has been found more frequently in stools of baboons and other non-human primates than *E. coli* (McConnell *et al.*, 1974; Kuntz *et al.*, 1968; Myers *et al*, 1971; Goldsmid and Rogers, 1978; Kessler *et al.*, 1984). No clinical symptoms are associated with *B. coli* infection in the lower primates although the parasite can cause watery-mucoid diarrhoea and muscular weakness in the great apes (Brack, 1987; Jackson *et al.*, 1990). Non-human primates are also natural hosts of *E. histolytica* (Brack, 1987). This author mentions that pathogenesis of *E. histolytica* in this group of animals depends on the host species, with New world monkeys and great apes being more prone to clinical amoebiasis than Old World monkeys because of the differences in gastric morphology and physiology.

Table 2.3. Intestinal protozoons found in the South African baboon (*Papio cynocephalus ursinus*).
(The baboons were free ranging in game reserves)

Protozoan										Locality
Entamoeba coli	*Entamoeba histolytica*	*Endolimax nana*	*Entamoeba hartmanni*	*Iodamoeba buetschhlii*	*Entamoeba chaltoni*	*Chilomatix mesnili*	*Balantidium coli*	*Trichomonas spp.*	*Isospora spp.*	
+	+	+	+	+	–	–	–	–	–	Durban (Powell and Elson-Dew, 1961)
+	–	+	–	+	–	+	+	–	–	Giant's Castle Game Reserve (Appleton *et al.*, 1986)
+	–	+	–	+	–	–	+	–	–	Mkuzi Game Reserve (Appleton and Henzi, 1993)
+	–	–	–	+	–	–	+	–	+	Kruger National Park (McConnell *et al.*, 1974)
+	+	+	+	+	+	–	–	–	–	Johannesburg (Myers *et al.*, 1971)
+	–	–	–	+	–	+	+	+	–	Zimbabwe (Goldsmith and Rogers, 1978)

+ = present , – = absent

Generally, the same range of parasites has been found from studies done on the parasitology of non-human primates in other parts of the world. These include studies done on Rhesus Monkeys (*Macaca mulatta*) by Kessler *et al.* (1984) on the island of Cayo Santiago, Puerto Rico; McGrew *et al.* (1989) in Tanzania and Senegal; Rowland and Vandenbergh (1965) in La Cueva and Guayacan islands, Puerto, Rico; Kuntz and Myers (1972) in South America; on the Taiwan Macaque (*Macaca cyclopis*) by Kuntz *et al.* (1968); and on Costa Rican squirrel monkeys by Appleton and Boinski (1991). In

addition to the common intestinal protozoons of non-human primates and Man found in South Africa, the following protozoons have been found in other parts of the world; *Iodamoeba williamsi*, and *Entamoeba chattoni* (Rowland and Vandebergh, 1965), *Trichomonas hominis* (Kuntz and Myers, 1972; Kuntz *et al.*, 1968), *Embadomonas intestinalis* (Kuntz and Myers, 1972), *Troglodytella abrassarti* and other *Troglodytella* spp. (McGrew *et al.*, 1989; Imai, 1990), *Dientamoeba fragilis* (Myers and Kuntz, 1968).

Entamoeba chattoni is a commensal of the large intestine of the monkey (Burrows 1972; WHO, 1998), and is rarely diagnosed in man (Beaver *et al.*, 1984; Brack, 1987; Juckett, 1996). Morphologically, *E. chattoni* resembles *E. histolytica* and has frequently been isolated both from wild and captive animals while *E. histolytica* infections have mainly been from captive animals (Jackson *et al.*, 1990). In a study done in the Kruger National Park, Jackson *et al.* (1990) found that amoebiasis due to *E. histolytica* infection is truly zoonotic, the Chacma baboon being the reservoir host, as the baboons chosen for the study were from an area considered to be optimally isolated from humans. The possibility that the baboons used in the study were infected by contact with human carriers was however, not ruled out.

Mild infection with *Dientamoeba fragilis* does not produce symptoms in man and non-human primates, but heavy infections can cause diarrhoea, excessive mucus production and irritation of the intestinal mucosa (Burrows, 1972). *Embadomonas intestinalis* is mainly a parasite of man although it has been described in non-human primates (Beaver *et al.*, 1984). Infection rate of *E. intestinalis* is usually so low that infections are normally missed. The ciliate *Tronglodytella abrassarti* is very rare in both humans and non-human primates (Smyth, 1994). *Tronglodytella* spp. have been found in fresh faeces of wild gorilla in Gabon, West Africa, (Imai *et al.*, 1990). Recently, *Cryptosporidium* oocysts have also been found in stools of Vervet monkeys and Olive baboons (Muriuki *et al.*, 1997) and in a wide range of other non-human primates (Muriuki *et al.*, 1998).

The presence of both pathogenic and non-pathogenic protozoa in non-human primates has significant implications both for public health and animal agriculture and as

Munene *et al.* (1998) say, "intervention against protozoa and other agents will improve primate health and also increase safety to animal handlers".

CHAPTER 3

CHANGES IN THE PREVALENCE AND INTENSITY OF INTESTINAL PROTOZOAN INFECTIONS IN CHILDREN AFTER RECEIVING ANTHELMINTIC TREATMENT

3.1 INTRODUCTION AND BACKGROUND

Multiple infestations with intestinal parasites have been studied more frequently than other types of poly-parasitism because of their widespread distribution and the relative ease with which stool specimens can be obtained for parasitological examination. There is however, very little understanding of the interactions between intestinal parasites and their role in affecting disease control programmes. Control programmes have usually been concerned with either a single disease or a certain group of parasites, and very little concern has been given to other health problems in the same area. Intestinal helminths usually co-exist with intestinal protozoons in infected individuals, and although the helminths have successfully been brought under control by chemotherapy, very little has been done to determine the effect of this treatment on the protozoons.

In a deworming study in northern Bangladesh, Rousham (1994) found that although the prevalence of *Ascaris lumbricoides* significantly decreased from 74% to 9% after treatment with mebendazole, there was a significant increase of *Giardia duodenalis* prevalence from 5% to 31%. An increase in the number of *Giardia* infections after helminth treatment was also noted by Taylor *et al.* (1995) in a study in southern KwaZulu-Natal. This increase in the prevalence of *Giardia* is clearly an undesirable outcome of any public health programme.

Research on interactions between intestinal worms and protozoons is important to public health administrators who have to be aware of the size and complexities of the effect of helminth chemotherapy on intestinal protozoan parasites and be able to implement proper control strategies. This chapter reports on a study done to monitor changes in the prevalence and intensities of intestinal protozoan infections in school

children after receiving anthelmintic drugs. Attention was focused on primary school children as this age-group usually has the highest infection rates and harbours higher worm burdens than other age-groups, and drugs can easily be administered through the schools' infrastructure (WHO, 1996).

The initial design of this study involved a drug trial whereby biscuits containing a standard dose (400mg) of mebendazole (Vermox®) would be given to primary school children who were infected with intestinal nematodes. However, because of ethic reasons, the Medicine Control Council did not approve the use of these biscuits and therefore these could no longer be used in the treatment. Moreover, the company that had promised to provide the biscuits was not prepared to provide the drug. The children had been promised treatment and therefore the Centre for Integrated Research was obliged to provide treatment and the drugs used in the treatment were ultimately obtained from the Department of Health. All these resulted in a delay in treating the children, and as can be seen in Table 3.1 below, the first treatment was only executed four months after the initial survey.

3.2 MATERIALS AND METHODS

3.2.1 Study area

Stool samples were collected from children attending Carrington Heights Junior Primary School. The school is located in South-Central Durban and is located at an altitude of 120m above sea level, a latitude of 29°52'57.9" S and longitude of 30°58'5.58" E (GIS). It mostly serves learners living in central Durban (city centre, Chesterville, Cato Manor/Crest, SeaView, Bellair) and southern Durban (Umlazi, Lamontville, Woodlands etc.) areas (Collett van Rooyen and O'Brien, 1999).

Carrington Heights Junior Primary School was chosen as the study area for the following reasons: (1) the school is relatively close (6 km) to the University of Natal and therefore collected specimens could quickly be transported to the laboratory for parasitological examination, (2) junior primary school children (grades 1 to 3) are more

co-operative than older children in providing stool samples (3) most of the learners are from the Cato Manor informal settlement (urban slum) area and the populations in such areas tend to have high infestations of parasitic diseases because of sub-standard living conditions (T.I. Mosala, pers. comm.). However, it should be pointed out that although most pupils are from the Cato Crest area, the school serves pupils from very mixed backgrounds with regard to living conditions. Permission to do the study was granted by the Principal of the school and all children with informed consent from parents (Appendix A) were included in the study.

3.2.2 Study design and population sample

A total of four surveys, involving collection of stool samples for the determination of prevalence and intensities of infection were carried out from February 1999 to November 1999. Survey 1 involved the initial collection of 301 stool samples in order to determine the prevalence and intensity of infection by intestinal worms and protozoons. This included 173 females and 128 males, 73 children were from Grade I, 107 from Grade II and 101 from Grade III and the ages ranged from 6 to 11 years (mean ± SD, 7.7 ± 1.1 years). Children who were infected with at least one nematode species were given a single oral dose (400mg) of albendazole (Zentel®). The drug has a broad-spectrum activity and is effective against most nematode species. It is easily administered, as the dosage is the same regardless of age or weight.

Survey 2 was done a month after Survey 1, and it involved 174 children. It was carried out to determine drug efficacy and the effect of treatment on protozoan infections. Although some individuals were not successfully treated, another dose of the drug could not be given due to time limitations imposed by the school term. Survey 3 consisted of 172 children and was carried out three months post-treatment. It involved the collection of one more stool sample in order to determine re-infection rates. Ash *et al.* (1977) recommended that post-treatment checks should be delayed until at least one month after completion of treatment for nematode infections. Re-infected individuals and those with old infections were given another dose of albendazole. The last survey involved 160 children and was done to determine drug efficacy and the effect of the second

treatment on protozoan infections. Table 3.1 below gives an outline of the overall study design.

Table 3.1. Outline of study design

Survey 4										√ *
Treatment 2									√	
Survey 3								√ *		
Survey 2						√ *				
Treatment 1					√					
Survey 1	√ *									
	Feb. 1999	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov. 1999

√ = time of survey * = stool sample taken for microscopy

3.2.3 Sample collection

Samples were collected at ±08h30 so that stools could be passed with ease after breakfast though some children who do not have any breakfast at home could not pass any stool. The school has a feeding scheme and provides the learners with sandwiches during break time (10h00 - 10h30) and after the break some of the learners who were initially unable to pass out stool usually managed to do so. Subjects who still could not provide any stool were followed up for the next 3-4 days.

The initial collection was done by the author (who attended to the female pupils), a female colleague and a male colleague (who attended to the male pupils); subsequent collections were done by the author and the male colleague. Each individual was allocated a number and in each collection, issued a corresponding numbered specimen bottle for a single stool sample. The pupils were told to first pass out urine into the toilet before passing stool onto the brown paper provided. This was done to avoid mixing stool with urine because of the occurrence of the different parasites in the two metabolic by-products. In order to avoid any 'exchange' of stool between subjects, the transfer of

the stool sample from the brown paper into the specimen bottles was done by the field workers. The samples were then transported to the laboratory for parasitological examination.

3.2.4 Laboratory methods

In the laboratory, the stool samples were classified by consistency based on the classification by Cheesbrough (1981): formed (F), semi-formed (SF), mushy (M), mushy-diarrhoeic (MD), diarrhoeic (D) or watery (W). Two sub-samples (0.5g - 1.0g) were then weighed into 15ml polypropylene tubes and 7ml of 10% formalin (Appendix B) was added into each tube. Stools remaining in the specimen jars were also preserved in 10% formalin and stored for future use. Formalin fixes the parasites and therefore preserves their morphology. The weighed sub-samples were then processed using the modified formol-ether concentration technique as outlined by Allen and Ridley (1970) (Appendix C). The technique gives a good concentration of parasitic cysts, eggs and larvae in fresh or preserved faeces and is recommended as the "best overall technique for the concentration of parasitic infections" (Cheesbrough, 1981). The sediments obtained were then examined as wet mounts in isotonic saline (Appendix B) and iodine using an Olympus BX40 light microscope fitted with a camera.

Iodine is of value in the identification of protozoan cysts as it stains organelles such as nuclei and glycogen vacuoles. The numbers of eggs obtained per slide (eps) under the 10X objective were converted into the number of eggs per gram (epg). To account for the different water content in stools of different consistencies (see above), correction factors were applied to the egg counts as follows: F(1.0); SF(1.5); M(2.0); MD(3.0); D(4.0); W(4.5). Geohelminth and *S. mansoni* intensities were classified according to Renganathan *et al.* (1995) and protozoan infections were assessed by the number of cysts per concentrated sample or per field of vision under the 40X objective (C.E. Archer, unpublished data) as outlined in Table 3.2.

Table 3.2. Scoring of protozoan intensities and classification of helminth intensities

Protozoons (C.E. Archer, unpublished data)

Qualitative	**Quantitative**
Occasional (occ.)	≤ 3 cysts per concentrated sample (p.c.s.)
Scanty (sc.)	>3 but ≤ 30 cysts p.c.s. **OR** 1 cyst per field
+	>30 but ≤ 800 cysts p.c.s. **OR** 1 -2 cysts per field
++	>800 but ≤ 20 000 cysts p.c.s. **OR** 2 - 50 cysts per field
+++	>20 000 but ≤ 80 000 cysts p.c.s **OR** 50 - 200 cysts per field
++++	>80 000 cysts p.c.s. **OR** >200 cysts per field

Schistosoma mansoni (Renganathan *et al.*, 1995)

Light infection	<100 epg
Moderate infection	101 - 400 epg
Heavy infection	>400 epg

Geohelminths (Renganathan *et al.*, 1995)

	A. lumbricoides	*T. trichiura*	Hookworm
Light infection	1 - 4 999 epg	1 - 999 epg	1 - 2 999 epg
Moderate infection	5 000 - 49 999 epg	1 000 - 9 999 epg	3 000 - 9 999 epg
Heavy infection	≥ 50 000 epg	≥ 10 000 epg	≥ 10 000 epg

Although *Blastocystis hominis* is a commonly occurring protozoan, it was not reported in the stool samples examined. The organism is usually found in diarrhoeal/loose stools and most of the stools examined in this study were formed as they were from healthy individuals. In addition to this, the recovery of *B. hominis* cysts is usually done on fresh stool samples by the direct wet mount method (Horiki *et al.*, 1997) which was not possible in this survey as the samples were collected in the mornings and the afternoons were used for formalin preservation. Examination of the samples for *B. hominis* and protozoan trophozoites immediately after collection was not possible as the collection, preservation, processing and examination were all done by the same individual.

3.2.5 Statistical analysis

Prevalence (number of infected individuals/total number of examined individuals x 100) and intensity (number of eggs per gram of stool) of infections were then determined for each parasite in the four surveys. In order to assess any changes in the prevalence and intensities of infection, only those subjects who were successfully followed up in at least three surveys were included in the analysis.

Summary statistics were used to determine the prevalence and intensity of infection, and polyparasitism (multiple species infections in an individual) in the four surveys done at Carrington Heights Junior Primary School. The study population was classified into three age groups: < 7years, 8-9 years and > 10 years.

The paired samples *t*-test was used to compare the mean intensities of infection by geohelminths after the two treatments. This computes the Student's *t* test for significant differences in mean intensities before and after treatment. The χ^2 test was used to compare the difference in prevalence and intensity of infection by protozoons and for sex and age distributions of intestinal infections.

For the determination of changes in prevalence and intensities of intestinal parasites in children after anthelmintic chemotherapy, additional pre- and post-treatment data from four schools in Health Region A of KwaZulu-Natal were included in the analysis.

Health Region A is in the south coast of KwaZulu-Natal and the data used were from the KwaZulu-Natal Parasite Control Programme. The samples were collected from the schools by field workers after which they were processed and examined in the laboratory by a trained laboratory technician using the diagnostic method that is similar to the one used for samples collected from Carrington Heights Junior Primary School. The age-groups were re-classified into 6-8 years, 9-11 years and > 12 years in order to include age and sex data from the four additional schools.

3.3 RESULTS

Protozoan infections are diagnosed by the presence of cysts in stool samples. Iodine-stained cysts of protozoons can be distinguished from each other on the basis of morphological features such as shape and size, granularity and cytoplasmic inclusions, and morphology and number of nuclei. Amoebic pre-cystic stages lack cyst walls and therefore do not have a definite shape, this makes identification to species level difficult and unwise. Helminth infections are diagnosed by the presence of eggs or larvae in the stool. The shape and size of the egg, thickness of the eggshell, and other morphological features characterize different helminth species. Although helminth eggs can be seen without any stain, iodine makes them more conspicuous.

A total of 14 parasite species including: seven protozoons; one cestode; two trematodes and four nematodes, were found in the study. Unidentified medium-sized precystic stages of amoebae were also seen. The only pathogenic protozoons found in the study were *Entamoeba histolytica/dispar* and *Giardia intestinalis*. A guideline of the identification of intestinal protozoons is given in Appendix D. The morphological diagnostic features of protozoan cysts and helminth eggs found in the study are described as below (Brack, 1987; Cheesbrough, 1981; Smyth, 1994; Fripp, 1995; WHO, 1998). The plates are original unless otherwise stated.

3.3.1 Protozoons

i) Amoebae (Plate 3.1)

Entamoeba histolytica/E. dispar cysts are spherical in shape, small (measuring 10-15 µm in diameter), and have a granular cytoplasm. Immature cysts have 1 - 2 nuclei and often contain 'sausage-shaped' chromatid bars which are usually seen in saline preparations but do not always stain with iodine. Mature cysts have 4 nuclei.

Entamoeba coli cysts vary greatly in shape and size, ranging from 10-30 µm in diameter. Immature cysts contain 2 - 4 nuclei and glycogen vacuoles which stain brown with iodine. The cysts also have thin, pointed chromatid bars. Mature cysts have 8 nuclei and the cytoplasm is usually retracted from the cyst wall.

Entamoeba hartmanni - The cysts are round in shape, contain 1 - 4 nuclei and like *E. histolytica/dispar* cysts, have a granular cytoplasm. They are however smaller than those of *E. histolytica/dispar*, measuring 5-10 μm in diameter. There are small, blunt ended chromatid bars in the cytoplasm.

Iodamoeba butschlii cysts measure 9-15 μm in diameter and show great variation in shape and size. They only have 1 nucleus and large, well defined glycogen vacuoles that stain brown with iodine.

Endolimax nana cysts (Plate 3.2A) are small, measuring only 7-9 μm in diameter; they are round or oval in shape and contain 1 - 4 very small nuclei which appear as small 'dots' on one side of the cyst. They do not have glycogen vacuoles or chromatid bars in the cytoplasm.

ii) Flagellates

Giardia intestinalis. Mature cysts (Plate 3.2B) are oval and measure 7-12 μm x 5-7 μm, and they have 4 nuclei that are usually located towards one end of the cyst. The cysts are easily recognized in iodine preparations as the axonemes stain well.

Chilomastix mesnili cysts (Plate 3.2C) are small, measuring 6-10 μm in length and have a thick cyst wall. They contain a single nucleus, remains of flagella, cytostome and have a characteristic 'nipple' on one end, giving them a lemon-shaped appearance.

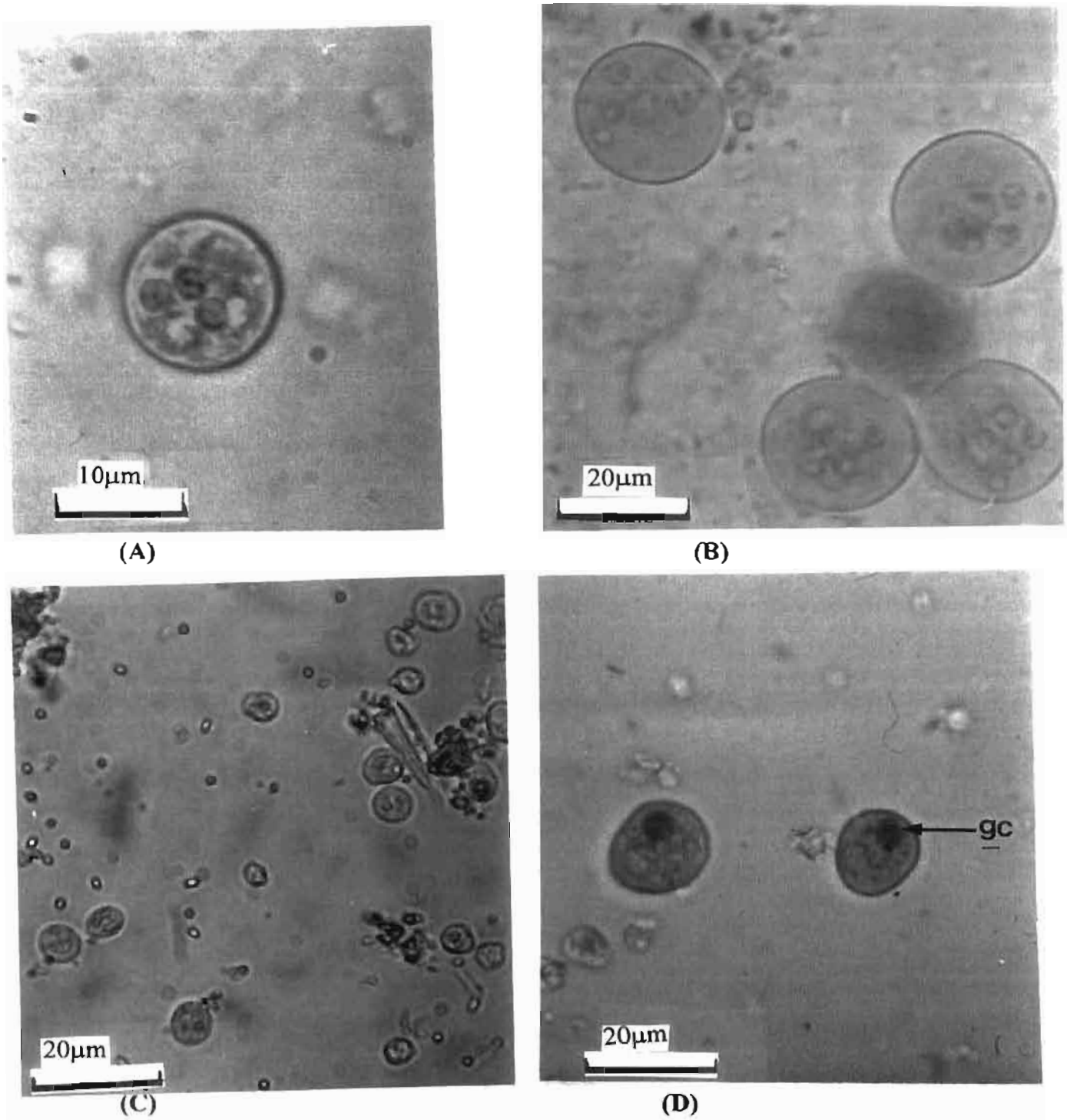
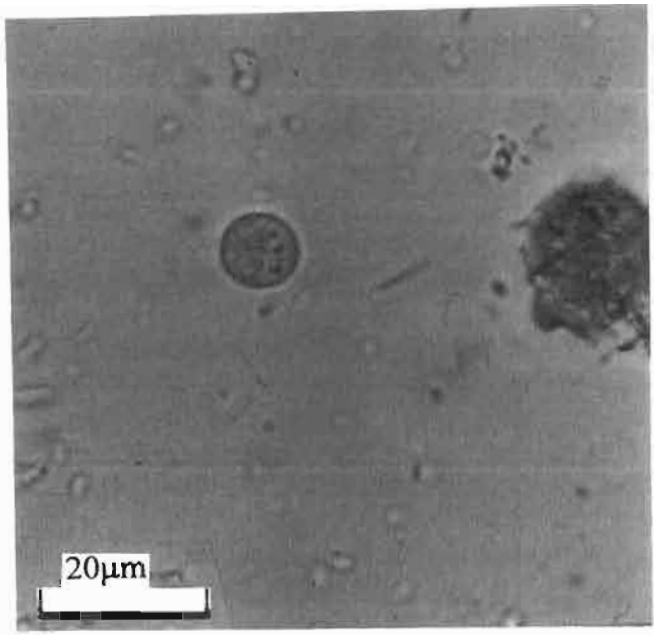
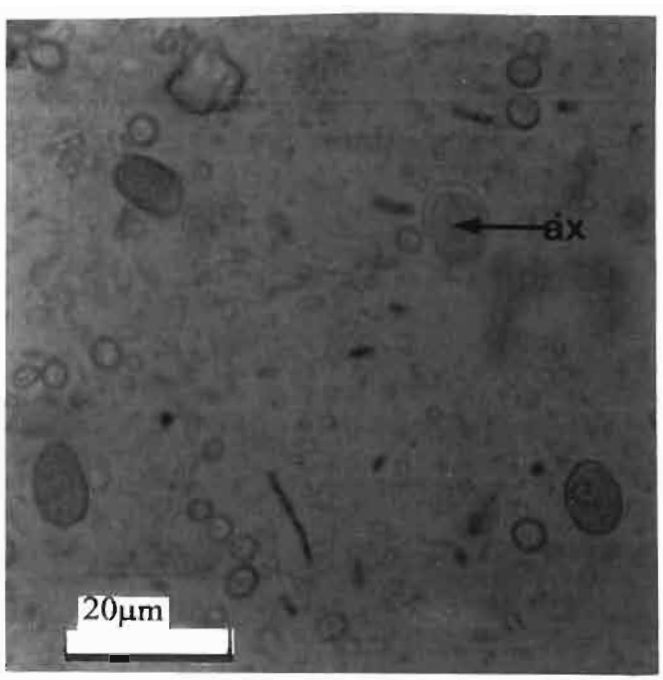


Plate 3.1 Amoebic cysts in iodine preparations. **(A)** *Entamoeba histolytica/dispar* showing 3 nuclei; **(B)** *Entamoeba coli* cysts showing up to 8 nuclei; **(C)** *Entamoeba hartmanni* cysts showing the granular cytoplasm and one nucleus; **(D)** *Iodamoeba butschlii* cysts with darkly stained glycogen vacuoles (gc).
(**A** and **D** are from WHO; **C**, courtesy of T.I. Mosala)



(A)



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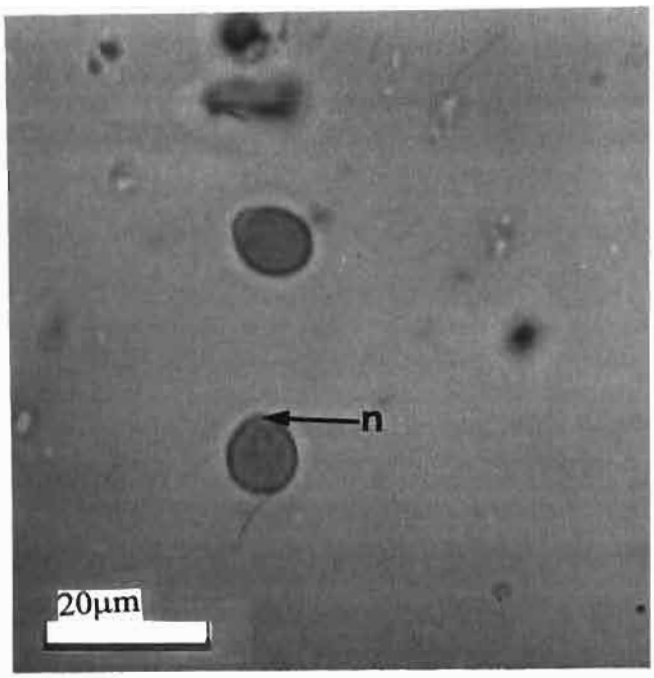


(C)

Plate 3.2 *Endolimax nana* cyst **(A)** with 'dot'-like nuclei; **(B)** oval *Giardia intestinalis* cysts showing axonemes (ax); **(D)** lemon-shaped cysts of Chilomastix mesnili with 'nipples' (n)
(**A** and **C** are from WHO, 1998)

3.3.2 Helminth parasites that were found co-occurring with protozoons in the study area

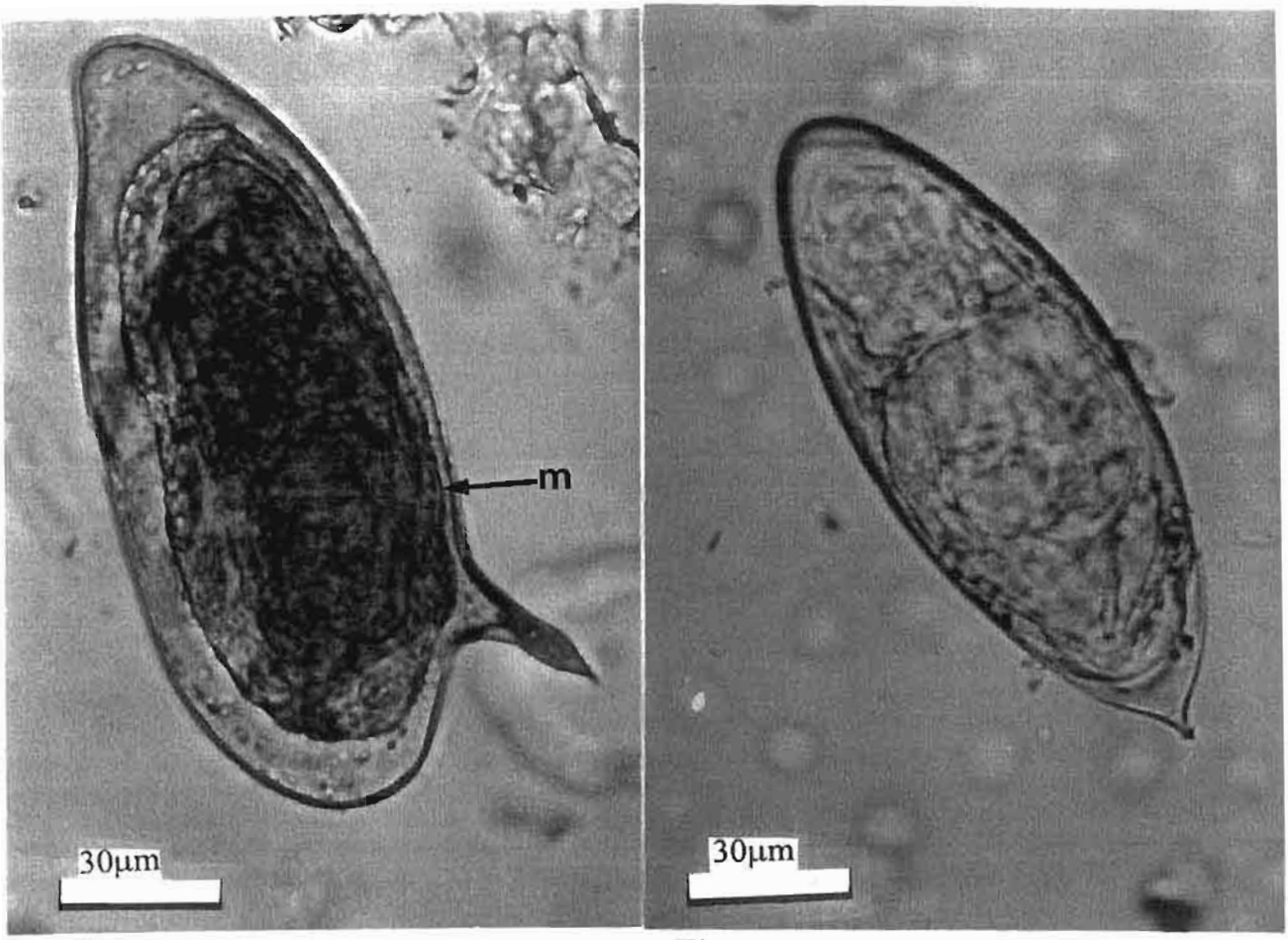
i. Cestodes

Taeniid tapeworms were the only cestodes found in the study. Eggs of *Taenia solium* (pork tapeworm) and *Taenia saginata* (beef tapeworm) are identical (Plate 3.3C). They measure 35- 43μm in diameter, are spherical, have a thick, radially-striated shell, and contain a single oncosphere larva which has three pairs of hooklets.

ii. Trematodes

Schistosoma mansoni (intestinal bilharzia) eggs are large (measuring 114-175 μm in length and 45-70 μm in width), yellowish-brown in colour and are elongated. They have a thin shell and a characteristic lateral spine. The egg is usually embryonated when passed out with stool (Plate 3.3A).

Schistosoma haematobium (urinary bilharzia) eggs (Plate 3.3B) are usually found in urine but may occasionally be found in faeces. They are large (112-170 μm by 40-70 μm) and have a terminal spine. As with *S. mansoni*, the eggs contain developed miracidia.



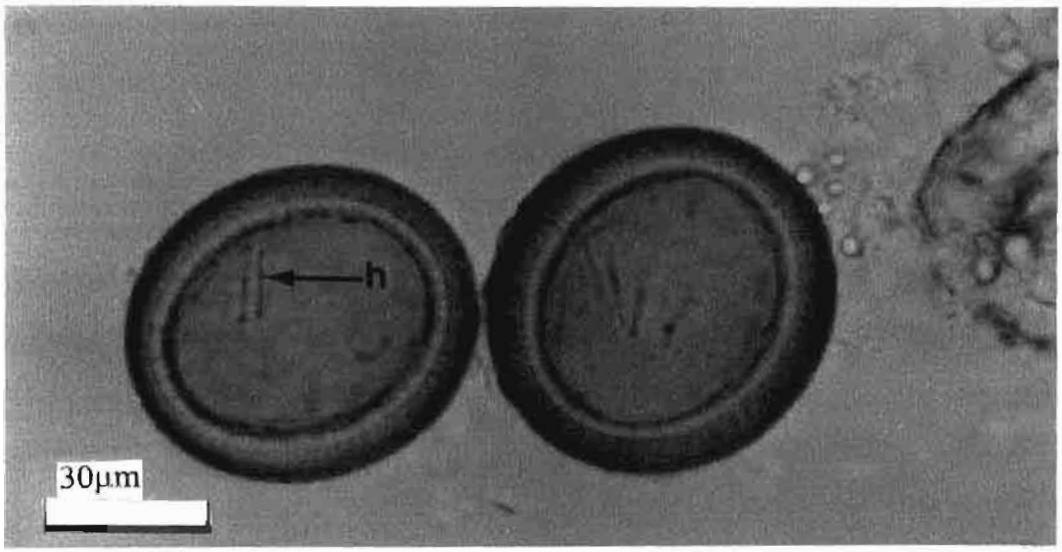


Plate 3.3 **(A)** the egg of *Schistosoma mansoni* has a lateral spine and contains a fully developed miracidium (m); **(B)** *Schistosoma haematobium* egg showing the terminal spine; **(C)** thick walled taeniid eggs containing embryos with pairs of hooklets (h)
(Photographs are from WHO, 1998)

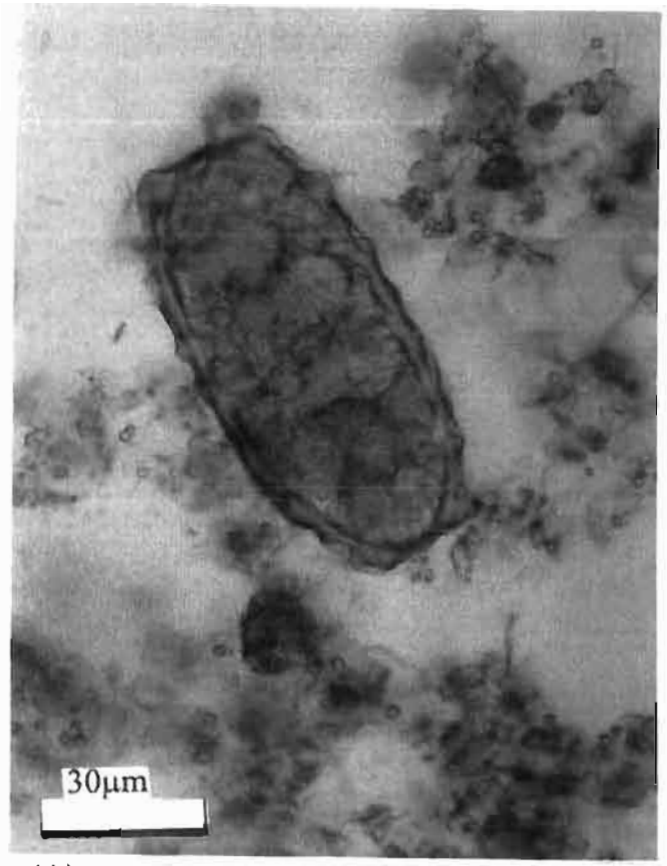
iii. Nematodes

Ascaris lumbricoides (common roundworm) eggs (Plate 3.4) may vary slightly in their shape and size. A typical fertilized egg (Plate 3.4B) has three shell layers and measures 55–75 µm x 35–50 µm. It is brown in colour (tanned), contains a single egg cell and the shell has conspicuous bumps called mamillations. Occasionally, unfertilised eggs are produced (Plate 3.4A); these are larger than the typical fertile eggs (90 µm x 45 µm) and are usually filled with a granular mass. Sometimes eggs that lack the mamillated layer are produced. These are called decorticated eggs and are about the same size as the normal fertilised eggs (Plate 3.4C).

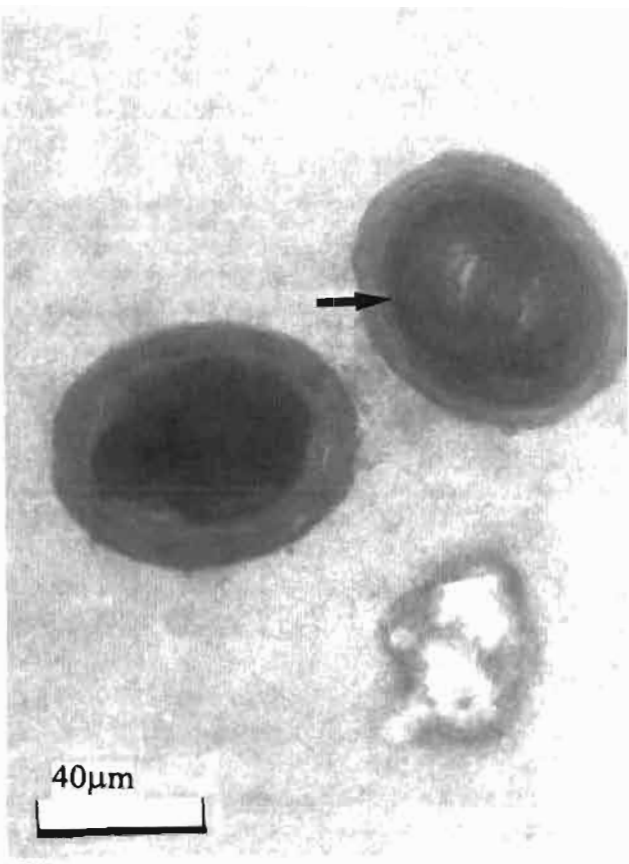
Trichuris trichiura (whipworm) eggs (Plate 3.5A) are smooth, oval, yellow-brown, and have mucoid plugs on each end. They are slightly smaller than *Ascaris* eggs, measuring 50–55 µm x 22–24 µm.

The hookworms *Necator americanus* and *Ancylostoma duodenale* are medically important human parasites. Although adult worms of these two species can easily be identified on the basis of possession of cutting plates (*N. americanus*) or teeth (*A. duodenale*), their eggs are identical. The typical hookworm egg (Plate 3.5C) measures 60–75 µm x 36–40 µm, it has a single thin shell, is almost colourless and usually contains a 4 or 8 celled morula larva. Although *N. americanus* is by far the most common hookworm species in KwaZulu-Natal (Appleton *et al.*, 1999), nematodes such as *Ternidens diminutus*, *Trichostrongylus* sp. and *Oesophagostomum apiostomum* have "hookworm-like" eggs (Goldsmid, 1967). Egg sizes of these nematodes overlap with those of hookworm and *T. deminutus* in particular, has been recorded from Zimbabwe and in some parts of South Africa (Goldsmid, 1968). A more reliable diagnosis is that of culturing nematode eggs by use of the Harada-Mori test-tube cultivation technique (coproculture) (Goldsmid, 1967). The method is however time consuming and cannot be used for routine examination in hospital laboratories (Goldsmid, 1968). Differentiation of hookworm eggs from those of *T. diminutus* was initially done on the basis of their volume ($l \times b^2$) but can now be done more accurately on the basis their area ($l \times b$) (Goldsmid, 1972).

Enterobius vermicularis (pinworm) eggs (Plate 3.5B) are oval, thin-shelled and flattened on one side. They measure about 55 µm x 30 µm in length and are usually embryonated when laid. *Enterobius* eggs are occasionally passed out with stool as the female worms migrate out of the anus and lay their eggs on the perianal skin. The most efficient method of recovery of the eggs therefore is by the Scotch-tape method where a transparent adhesive tape is placed on the perianal area, removed, placed on a slide and then examined under the microscope.



(A)



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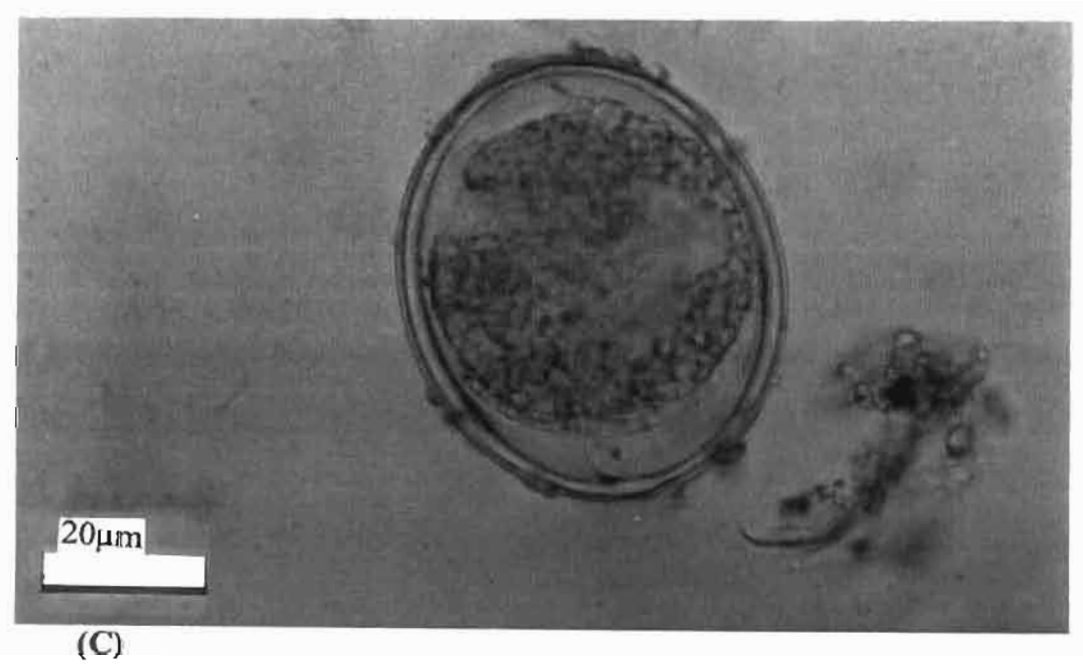
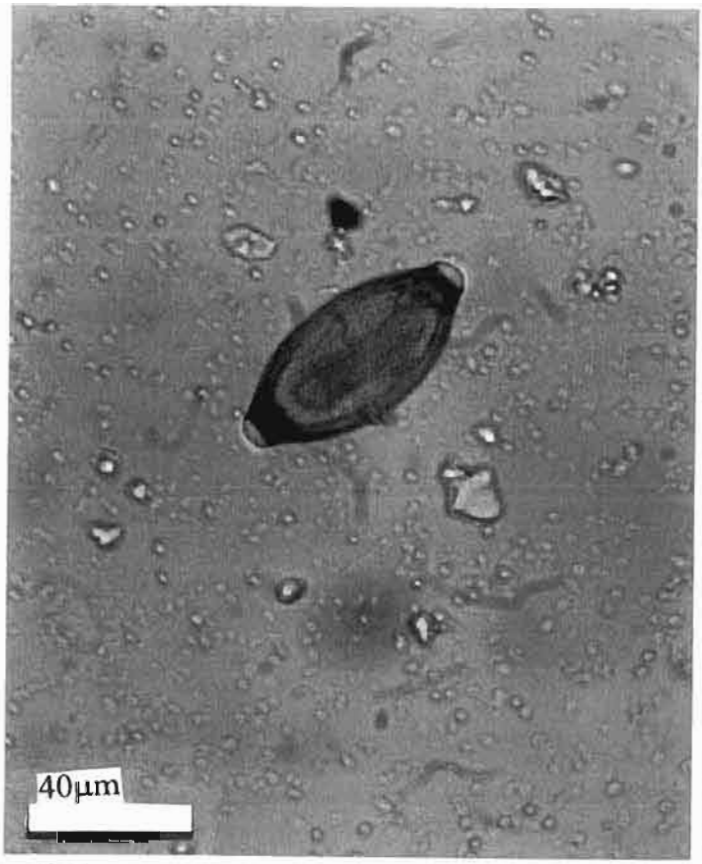
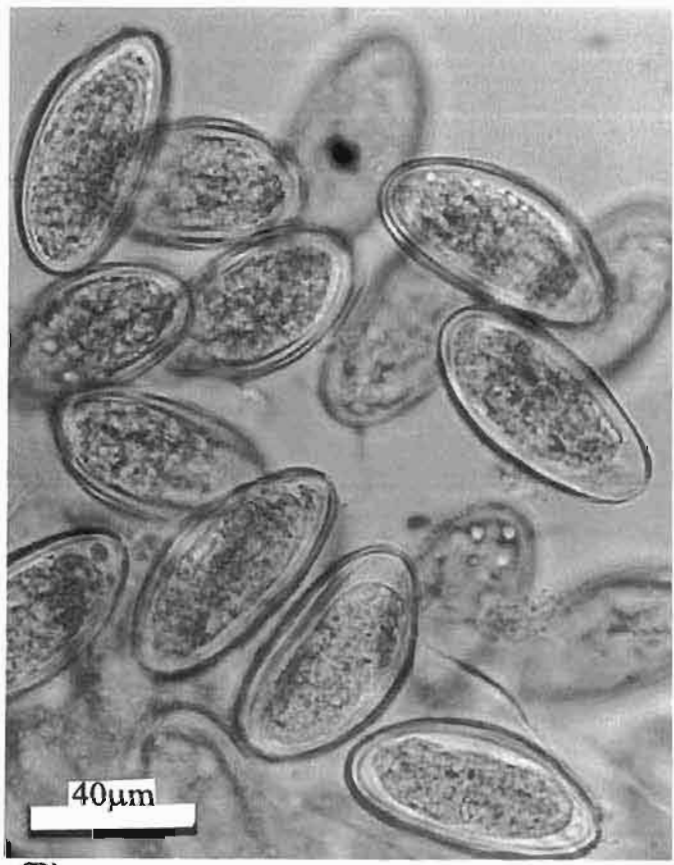


(C)

Plate 3.4 Eggs of *Ascaris lumbricoides*. **(A)** an unfertilized egg; **(B)** fertilized eggs, one of the eggs contains a larva (arrow); **(C)** a decorticate egg (**A** and **C** are from WHO, 1998).



(A)



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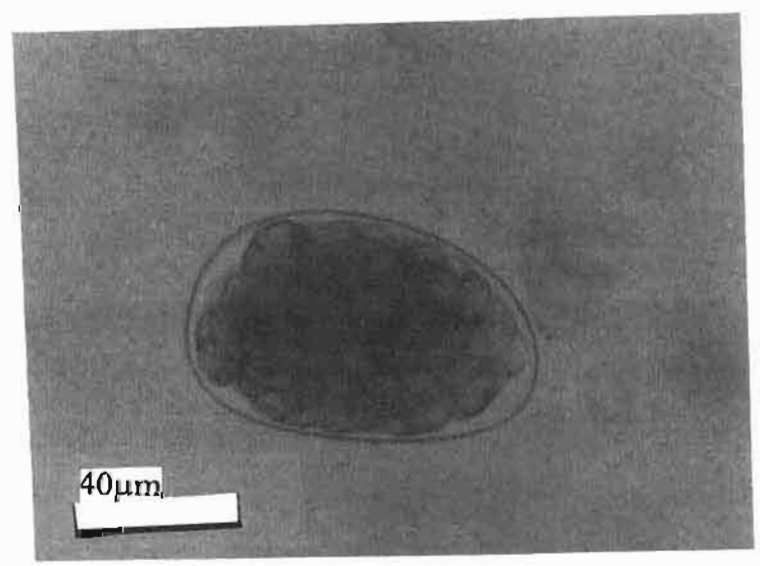


(C)

Plate 3.5 Eggs of **(A)** *Trichuris trichiura*; **(B)** *Enterobius vermicularis* and **(C)** hookworm.

3.3.3 Prevalences of intestinal parasites

Prevalences of all parasites recorded from the study were generally low to moderate (Table 3.3). The most commonly found helminth species were *A. lumbricoides* and *T. trichiura*; and the most commonly found protozoons were *E. coli* and *E. nana*.

Table 3.3. Prevalence (%) of intestinal parasites found in the study

Parasite	Survey 1 N = 301	Survey 2 N = 174	Survey 3 N = 172	Survey 4 N = 153
Protozoons				
Entamoeba coli	17.9	29.3	26.7	32.0
*E. histolytica/dispar/dispar**	0.3	4.6	0.6	0.7
E. hartmanni	0.6	5.7	3.5	1.3
Endolimax nana	1.7	20.1	13.4	13.1
Iodamoeba butschlii	2.0	6.3	7.6	10.5
Giardia intestinalis	3.3	5.2	5.2	3.3
Chilomastix mesnili	1.7	4.0	1.2	1.3
Unidentified amoebae	5.0	6.9	2.9	2.6
Cestodes				
Taenia sp.	1.7	1.1	0.6	0
Trematodes				
Schistosoma mansoni	1.7	2.3	0.6	0.7
Schistosoma haematobium	0	0	1.2	0
Nematodes				
Ascaris lumbricoides	47.2	6.4	6.4	3.3
Trichuris triciura	45.2	32.7	54.7	43.8
Necator americanus	3.0	0	0	0
Enterobius vermicularis	3.0	0	0.2	0.7
No Parasite Seen (NPS)	26.6	21.8	25	31.4

* - cysts of the two species are indistinguishable under light microscopy

3.3.4 Intensities of protozoan infections

Intensities of protozoan infections recorded from the study (Table 3.4) were generally low. Heavy (i.e. +++) infections were only found in children infected with *E. coli* in surveys 2, 3 and 4 respectively, *E. hartmanni* in survey 2, and *E. nana* in surveys 3 and 4.

Table 3.4 - Intensities of protozoan infections in the four surveys done at Carrington Heights Junior Primary School

Survey 1								
Intensity	***E.coli***	***E.histo/ dispar***	***E.hart.***	***E.nana***	***I.butc.***	***G.lamblia***	***C.mesn***	***UMSPA***
occasional	9	-	1	-	1	3	2	5
scanty	9	-	1	5	5	4	2	6
+	18	1	-	1	-	1	1	4
++	18	-	-	-	-	2	-	-
+++	-	-	-	-	-	-	-	-
Survey 2								
occasional	6	1	3	3	5	1	2	1
scanty	12	3	6	6	3	5	-	5
+	14	2	-	9	2	2	1	2
++	16	2	-	17	1	1	4	4
+++	2	-	1	-	-	-	-	-
Survey 3								
occasional	6	-	1	3	4	2	-	-
scanty	15	-	2	3	4	2	2	5
+	12	-	1	3	5	3	-	-
++	11	1	2	8	-	2	-	-
+++	2	-	-	6	-	-	-	-
Survey 4								
occasional	8	1	1	1	4	2	2	1
scanty	9	-	-	5	7	3	-	3
+	16	-	-	8	4	-	-	-
++	14	-	1	5	1	-	-	-
+++	2	-	-	1	-	-	-	-

UMSPA = unidentified medium-sized precystic amoebae

3.3.5 Intensities of helminth infections

The number of eggs per gram (epg) obtained in the study were categorized as light, moderate and heavy according to Table 3.2. Mean egg counts of helminths (excluding *E. vermicularis*) recorded from the study are shown in Table 3.5.

Table 3.5. Mean intensities (epg) and classification of helminth infections found in the four surveys

Survey	N	*A. lumbricoides*	*T. trichiura*	hookworm	*Taenia* sp.	*S. mansoni*
1	301	3140	564	134	101	18
2	174	208	264	0	112	14
3	172	440	365	0	106	06
4	153	1268	147	0	0	04
Classification of intesity						
Light		17.8 %	44.5 %	1.1 %		1.4 %
Moderate		3.5 %	3.6 %	0		0
Heavy		0	0.3 %	0		0

Classification of intensity of infection by the three parasites is based on the classification by Renganathan *et al.* (1995). There is no recommended way of classifying *Taenia* infections and therefore there are no figures given for this parasite. Intensities of infection found in the four surveys were mostly light. Heavy infections of *A. lumbricoides*, hookworm, or *S. mansoni* were not found in any of the surveys. Heavy *T. trichuris* infections were recorded only in survey 1 and these were significantly reduced after the treatment. The Formol-ether concentration method does not give an accurate indication of the prevalence and intensity of *E. vermicularis* infections and therefore recording of *E. vermicularis* intensities was done qualitatively as with protozoan intensities. Of the nine children infected with *E. vermicularis* in survey 1, three had "scanty" infections and six had one plus (+) infections. In survey 3, one child

had an "occasional" infection while one had a "scanty" infection. No *E. vermicularis* infections recorded were recorded from surveys 2 and 4.

3.3.6 Polyparasitism in the study area

Seventy two percent of the children were infected with at least one parasite species. However, most children were infected with only one species and very few harboured four or more species (Figure 3.1). In the whole study, only one individual from survey 1 had six parasite species. Although multiple infections were more common in the 8 - 9 year age-group (Appendix Ei), the difference in multiple infections with regard to age was not significant. Multiple infestations were also more common in females than in males, although not significantly so (Appendix Eii). The prevalences of helminth infections were significantly reduced after the two treatments although the reduction in multiple infections is not very apparent from Figure 3.1 due to the fact that protozoan infections were not treated. Plate 3.6 shows different combinations of multiple infections from WHO (1998) and this study.

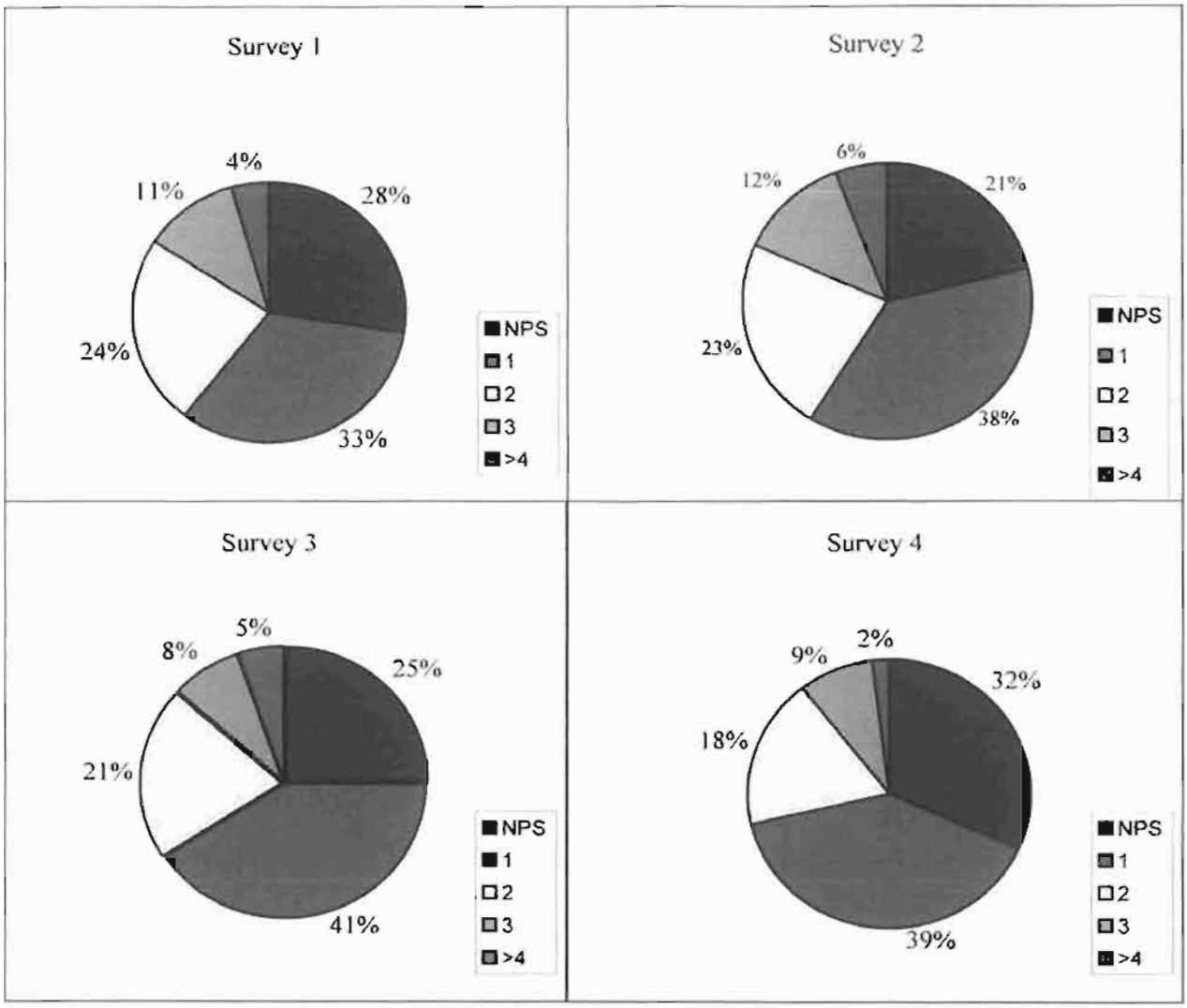
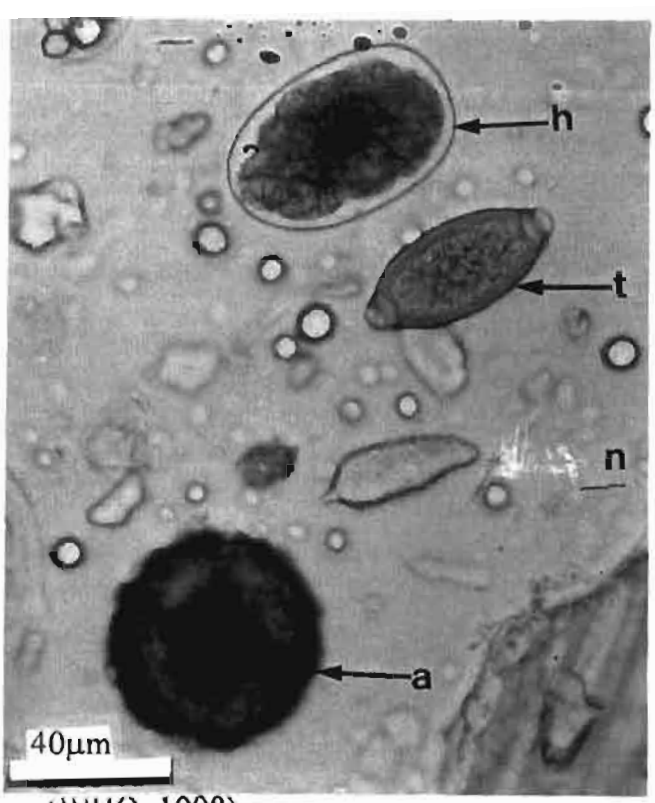
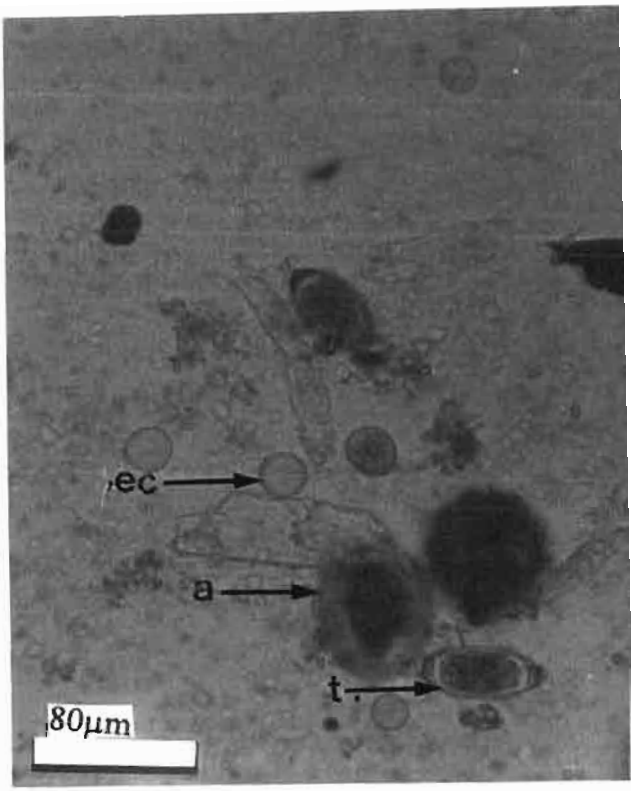


Figure 3.1. Polyparasitism in school children in surveys 1 - 4
(Numerals in the legend indicate the number of parasite species)





(WHO, 1998)

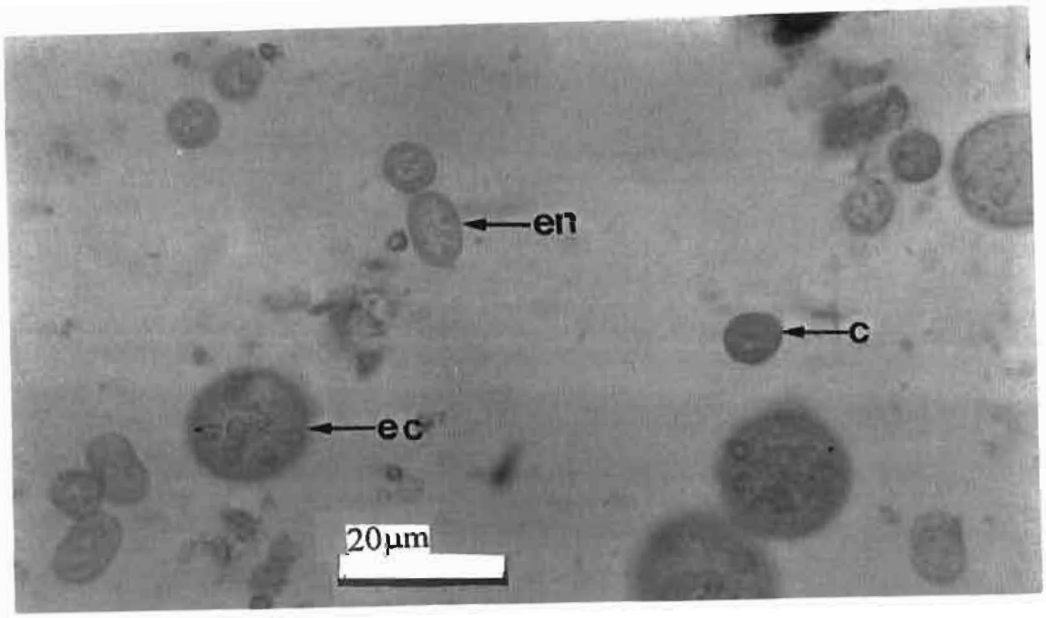


Plate 3.6 Multiple infections by *A. lumbricoides* (a), *T. trichiura* (t), hookworm (h), *E. coli* (ec), *E. nana* (en) and *C. mesnili* (c).

3.3.7 Changes in prevalence and intensity of infection in children after geohelminth treatment

Data analysed in order to determine changes in prevalence and/or intensity of infection after chemotherapy were from individuals who were successfully followed up in at least 3 surveys. Surveys 1 and 2 included pre- and post-treatment data from the five schools in Health Region A. The second treatment was administered only at Carrington Heights Junior Primary School (School 1) and therefore data from surveys 3 and 4 were obtained only from this school. Prevalences of protozoan infections were low in most cases and for statistical analysis protozoan parasites were grouped together. *Enterobius* was excluded from the analysis.

i) Changes in prevalence

Ascaris lumbricoides eggs were found in 75.2% (236/314) of all subjects before treatment and in only 9.7% (29/298) children after the first treatment. This difference was highly significant ($\chi^2 = 37.638$; $p = 0.0001$). *Trichuris trichiura* prevalence dropped from 77.7 % to 62.1 % ($p = 0.004$) and hookworm from 12.7 % to 1.0 % ($p = 0.004$). In three schools, all the children had been successfully treated for hookworm infections. Figure 3.2 shows the changes in prevalence of intestinal geohelminths and protozoons after helminth treatment.

Although the prevalence of *A. lumbricoides* and *T. trichiura* decreased after the second treatment, the decrease was not significant. Hookworm infections were not recorded from these surveys 3 and 4. The prevalence of intestinal protozoons increased significantly ($\chi^2 = 10.840$; $p = 0.001$) from 33% to 50.3% after the first treatment and from 35% to 42% after the second treatment. The drop from 50.3% to 33% is probably due to seasonal variations as the treatment was done in winter. Mosala (1995) has noted a decrease in the prevalence of protozoan infections in winter.

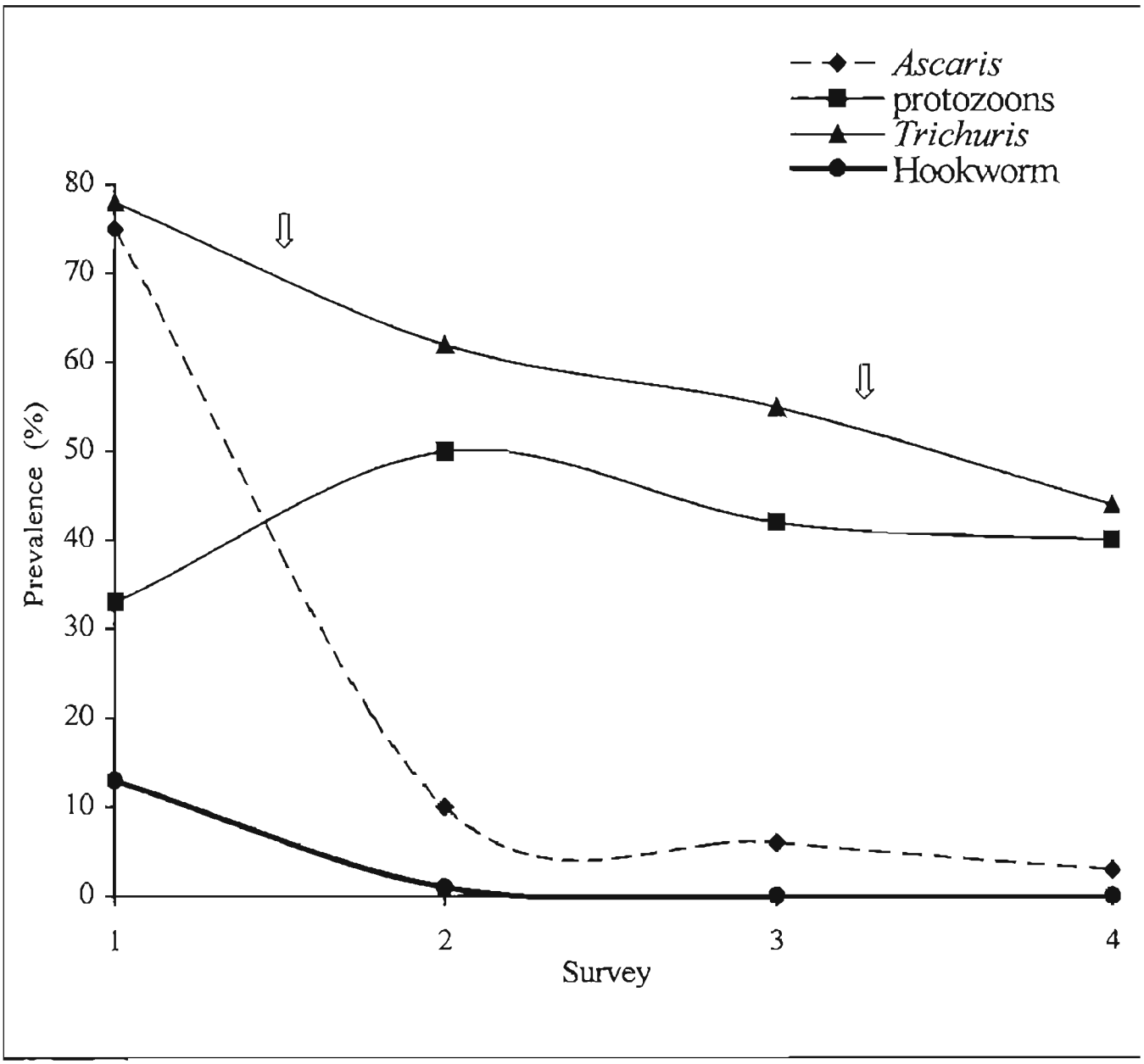


Figure 3.2. The prevalences of *A. lumbricoides*, *T. trichiura*, hookworm and protozoons in surveys 1 – 4. (Arrows indicate treatment 1 and 2)

ii) Changes in intensity

T-test analysis of data pooled from the five (Carrington Heights and Health Region A) schools showed significant differences in mean intensities of *Ascaris* from 3934 to 440 epg ($p = 0.0001$), *Trichuris* from 840 to 147 epg ($p = 0.001$), and hookworm from 100 to 37epg (0.004) before and after treatment. The differences in mean intensities by school are shown in Table 3.6. Generally the intensities of infection by the three parasites significantly ($p < 0.05$) decreased after the first treatment in all five schools, and the second treatment in Carrington Heights Junior Primary School. However in School 3, an increase in the intensity of hookworm infection was observed after treatment (Table 3.6). This is due to the fact that one learner, who was probably not treated, had a much higher hookworm egg count than the rest of the children and this therefore increased the mean egg count.

Significant changes in intensity of infection after treatment in the five schools were also found in the following protozoan species: *E. coli* ($p = 0.001$); *E. nana* ($p = 0.004$); *E. hartmanni* ($p = 0.001$) and *G. intestinalis* ($p = 0.01$).

Table 3.6. Comparison of differences in geohelminth mean intensties (epg) by school before and after the two treatments. (The first treatment was after Survey 1 while the second treatment was after Survey 3)

School	Survey 1	Survey 2	Survey 3	Survey 4
Ascaris lumbricoides				
1	3189	208	1268	440
2	7051	1194		
3	5788	1860		
4	3589	106		
5	3035	no infections		
Trichuris trichiura				
1	560	264	369	147
2	344	231		
3	640	481		
4	2065	679		
5	83	no infections		
Hookworm				
1	159	no infections	no infections	no infections
2	133	no infections		
3	98	149		
4	83	no infections		
5	63	12		

3.3.8 Prevalence and intensity in relation to age and sex

More females than males were infected by both protozoons and helminths but this difference was not significant. The 6 - 8 year age group was the least parasitized while the greater than 12 year age group was the most frequently parasitized (Figure 3.3a and b). Significant differences ($p = 0.047$) in prevalence between the different age groups were obtained with *Ascaris* in survey 2 where 8% of infected children belonged to the 6 - 8 year age group and 23% belonged to the greater than 12 year age group. It should however be pointed out that the number of children in the different age-groups was not the same and could have had an effect on the observed age and sex distributes. Mean intensities of helminth infections between different age groups, and in males and females were not significantly different.

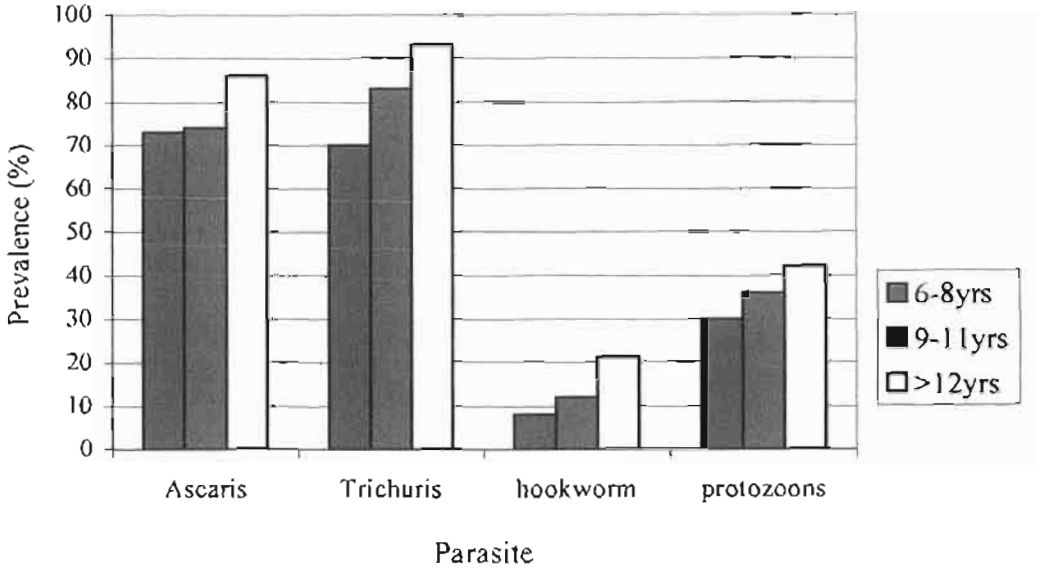


Figure 3.3a Parasite prevalence by age in Survey 1

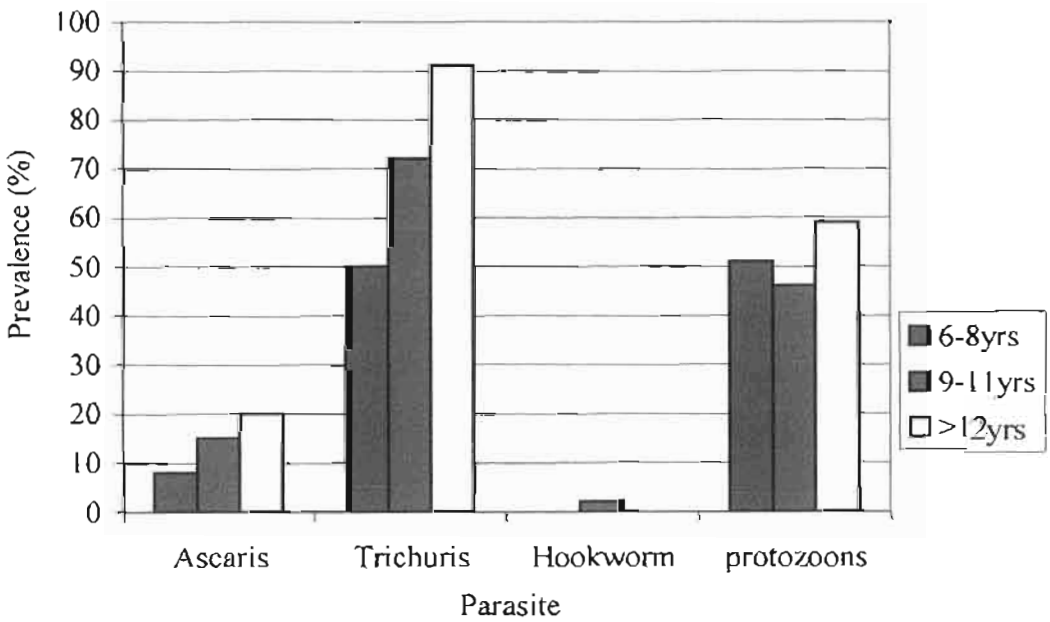


Figure 3.3b Parasite prevalence by age in Survey 2

3.4 DISCUSSION

3.4.1 Prevalences of intestinal protozoan infections

The range of intestinal protozoons found in the present study is similar to that found in surveys done on children in other parts of KwaZulu-Natal (Table 2.1). Although the prevalences obtained in the different studies differ, in most cases *Entamoeba coli* was most common commensal while *Giardia intestinalis* was the most common protozoan pathogen. This is usually the case even in studies done elsewhere in the world (Goldin *et al*, 1990; Chacin-Binilla *et al.*, 1992; Garcia, 1999). With the exception of *E. coli*, the prevalences of intestinal protozoons found in the present study and in the different surveys done in other parts of southern Africa are usually low (Table 2.1) and seldom exceed 10%. *Balantidium coli*, *Isospora hominis* and *Trichomonas hominis* were not found in the present study. Infections by these three protozoons have previously been found in children and adults in Durban (Elson-Dew and Freedman, 1952; Powell *et al.*, 1961; Kvalsvig *et al.*, 1991) and northern KwaZulu-Natal (Shuttle *et al.*, 1981). In Zimbabwe, intestinal protozoan infections were found in 70% of the studied children (Mason *et al.*, 1986). Protozoan infections usually occur throughout the year as the development of infective stages occurs inside the host (Farthing *et al.*, 1986).

The epidemiology of *E. histolytica/dispar* and *G. intestinalis* has particularly been noted because of the pathogenicity (see Chapter 2) of these protozoons. *Giardia* infections were found in 38% of primary school children in Santiago, Chile (Goldin *et al.*, 1990). In The Gambia, *E. histolytica/dispar* cysts were found in 13.7% and 52.3% of the inland and coastal populations respectively (Bray and Harris, 1977). In Zambia, Feacham *et al.* (1983) found *E. histolytica/dispar* infections in 26% of the children studied. Schutte *et al.* (1977) recorded a *E. histolytica/dispar* prevalence of 22% in children from Amanzimtoti, south of Durban, and the highest prevalence (60.5%) of *E. histolytica/dispar* recorded in South Africa was by Schutte *et al.* (1981) in northern KwaZulu-Natal. This should however be re-examined in terms of the proven differences between *E. histolytica/dispar* and *E. dispar* (see Chapter 2.1.3). In Egypt, *E. histolytica/dispar* and *G. intestinalis* infections were found in 97% and 40%

respectively, of the study population (Lawless *et al.*, 1956), and *Giardia* infections were found in 7.1% of school children in the USA (Harter *et al.*, 1982). The occurrence of *Giardia* cysts in the stools of school children in the USA was related to drinking untreated water.

As in the present study, more protozoan infections were found in females than males (Appendix Eii), Chacin-Bonilla *et al.* (1992) also found a higher prevalence of *E. histolytica* in females than in males. In other studies (Mason *et al.*, 1986; Walker-Smith, 1988) however, prevalences of *E. histolytica/dispar* and *G. intestinalis* were higher in males than in females. In the present study, the differences seen in protozoon infections in the different age-groups may have been due to the different numbers of individuals in the three age-groups. Most of the children were aged between 6 and 8 years while a smaller number were 12 years or older. The age-range was also narrow as the study group was made up of only junior primary school children. A broader range would perhaps provide a more accurate picture of this aspect of the epidemiology of intestinal protozoons.

Breast-feeding is thought to offer some resistance to *Giardia* infections (Gillin *et al.*, 1983; Farthing *et al.*, 1886) and therefore giardiasis is more common in pre- and primary school children, with peak prevalences in the 5-10 year age-group, than in breast-feeding infants (Schutte *et al.*, 1981; Hossain *et al.*, 1983). In older children, the prevalence is usually lower due to acquired immunity and less contact with contaminated objects. With *E. histolytica/dispar*, a higher prevalence is usually found in adults than in children (Schutte *et al.*, 1981; Gillin *et al.*, 1983; Kravitz *et al.*, 1993). This suggests that infection with this parasite does not necessarily provide resistance to subsequent infections.

3.4.2 Polyparasitism

Polyparasitism is important both to the clinician and to the epidemiologist. To the clinician, it is the presence of multiple infections in the same individual while to the epidemiologist, it is the co-occurrence of parasitic infections in a population (Buck *et al.*, 1978). It is a reflection of living standards and is often associated with poverty,

illiteracy, poor sanitation and high risks of exposure to infective organisms (Chacin-Bonilla *et al.*, 1992). Polyparasitism is of more significance when an individual is infected with hookworm, bilharzia and malaria as all these parasites feed on blood and therefore infection results in a progressive anaemia due to blood loss (Buck *et al.*, 1978).

Although up to six parasite species were found in some individuals, most people were infected with only one parasite species, with very few people harbouring four or more species (Figure 3.1). Double infections with *A. lumbricoides* and *T. trichiura* were the most common. Polyparasitism is therefore not of much significance in children attending Carrington Heights Junior Primary School, and the treatment given was effective in reducing multiple infections. In Survey 1 (pre-treatment), 13 people were infected with four to six parasite species and this was reduced to only three children harbouring four species after the second treatment (Appendices Ei and Eii).

Polyparasitism by intestinal parasites has been recorded from various studies in South Africa (Elson-Dew and Freedman, 1952; van Niekerk *et al.*, 1979; Freeman and Grunewald, 1980; Kvalsvig *et al.*, 1991; Gunders *et al.*, 1993; Bradley and Buch, 1994). As with the present study, anthelmintic chemotherapy was effective in reducing polyparasitism in infected children.

3.4.3 Associations between intestinal protozoan and helminth infections

Intestinal helminth infections in children are associated with morbidity such as impaired physical and mental development resulting in poor academic performance (Nokes *et al.*, 1991; Taylor *et al.*, 1995) and school-age children are the most affected as they usually have greater worm burdens (Renganathan *et al.*, 1995). High prevalence and intensities of *Ascaris lumbricoides, Trichuris trichiura* and hookworm have commonly been found in children (mostly from coastal areas) in South Africa. High infestation rates by these nematodes have been recorded from children in KwaZulu-Natal (Schutte *et al.*, 1981; Maurihungirire, 1993; Taylor *et al.*, 1995; Appleton and Gouws, 1996; Mabaso, 1998; Appleton *et al.*, 1999), in black and coloured children in the Eastern and Western Cape

(van Niekerk *et al*, 1979; Freeman and Grunewald, 1980; Millar *et al.*, 1989; Gunders *et al.*, 1993), and in the Bushman tribe in Namibia (Evans *et al.*, 1990).

In most instances, as is the case in the present study, these worms have successfully been treated with broad-spectrum anthelminths such as albendazole (Zentel®) and mebendazole (Vermox®), or by iron supplementation in hookworm infections. This usually results in a decrease in both the prevalence and intensity of infection, and therefore an improvement in the mental and intellectual abilities of the treated children. However in the present study the reduction in prevalence of *T. trichiura* was not significant though the intensity of infection was significantly reduced. Deworming has a greater effect on the intensity of infection than on the prevalence as marked changes in intensities may show only small changes in prevalence (Anderson and May, 1985; Hall *et al.*, 1992; Gunders *et al.*, 1993). Holland *et al.* (1989) suggested that prevalence should be regarded as a "relatively insensitive measure" of drug efficacy and re-infection.

Although elimination of intestinal worms by chemotheraphy reduces mortality and morbidity due to these parasites, the increase in both prevalence and intensity of intestinal protozoons after anthelmintic treatment is certainly not a desirable outcome of any control programme. In healthy individuals, *G. lamblia* and *E. histolytica/dispar* infections are usually asymptomatic, with very little epithelial damage, and in such cases the parasites are usually present in very low numbers. However, if this balance is upset and the number of infective organisms increased, the pathogenicity of these parasites increases and this results in greater epithelial damage (Wright, 1979). Bradley (1972) also mentioned that the likelihood of a parasite causing disease is related to the parasite load in the host, and that excessive parasite numbers (even in the case of slightly pathogenic species) may kill the host.

Some authors have suggested possible mechanisms involved in the interactions between nematode and protozoan infections. Christensen *et al.* (1987) mentioned that the suppression of protozoan infections by helminth infections and that of helminth infections by protozoan infections is due to induced changes of the intestinal mucosa by one infection, thereby inhibiting the establishment of the other. This probably explains

the situation seen in hosts infected with both intestinal protozoan and helminth species. Helminths induce suppression of protozoan infections, and therefore the absence of helminth infections (after anthelmintic treatment) results in proliferation of protozoan infections, as reflected by increased cyst excretion.

Another view by Dobson (1985) is that suppression of one group of parasites by another is due to competition. Intestinal helminths and protozoons occupy the same niche in the host and therefore compete for nutrients and attachment sites. Elimination or a reduction in the population of helminths will obviously favour the proliferation of protozoan species. This author further mentioned that the most effective competition mechanism used by parasites is their pathogenicity. Hookworm for example, attach to the mucosal wall of the host using cutting plates (*N. americanus*) or teeth (*A. duodenale*) and the change brought about by this infection on the intestinal mucosa would neither favour attachment by *G. intestinalis* nor invasion by *E. histolytica/dispar.* Similarly, protozoan infections can modify the intestinal wall, making parasitism by other parasites impossible. Competition between *A. lumbricoides* and/or *T. trichiura* with protozoons will mostly be for the host's nutrients as these helminths do not attach themselves to the host's intestinal wall and are thus unlikely to cause changes to the mucosal membrane.

In another study, Murray *et al.* (1977) noticed a low prevalence of malaria in children who were heavily infected with *Ascaris*, and an increase in *Plasmodium falciparum* infections after the treatment of *A. lumbricoides*. These authors suggested that heavy *Ascaris* infection might lead to a deficiency of nutrients that are needed for the multiplication of *Plasmodium*. With helminths, the co-existence of several species in the same host is common as the presence of one species has little influence on the entry and development of another species (Buck *et al.*, 1978; Beaver and Jung, 1985). In this group of parasites, one infection usually increases the host's susceptibility to infection by another species (Keita *et al.*, 1981).

This interspecific competition is determined by the number of specimens/individuals of the parasite, and as each species becomes more aggregated in distribution, intraspecific

competition becomes important in regulating population densities of the parasite in the host.

CHAPTER 4

ENVIRONMENTAL AND SOCIO-ECONOMIC FACTORS THAT DETERMINE THE PREVALENCE OF PROTOZOAN INFECTIONS IN KWAZULU-NATAL

4.1 INTRODUCTION

KwaZulu-Natal is situated in the eastern part of South Africa at approximately 28° 30` S and 30° 30` E. The Drakensberg Mountains form its western border with the Free State province and Lesotho. The eastern border is the Indian Ocean shoreline, the northern one is shared with Mpumalanga province, Swaziland and Mocambique. The Eastern Cape province borders the southern side. The climate in the province ranges from temperate to sub-tropical and is characterised by warm low-lying valleys, breezy uplands and windy mountains. The air on the coast is humid and warm, it is dry and cool in the midlands, and cold in the highlands. The annual rainfall decreases from the coast to the midlands and then increases towards the Drakensberg escarpment where the highest amount of rainfall in KwaZulu-Natal occurs (Schulze, 1982, Schulze *et al.*, 1997). Several studies (Appleton and Gouws, 1996; Appleton *et al.*, 1999; Mabaso, 1998) have been done on the distribution of the common soil-transmitted nematodes (geohelminths) with regard to this topography and to the different climatic conditions experienced in KwaZulu-Natal.

The present study was aimed at determining associations between the prevalence of intestinal protozoan species and environmental factors in KwaZulu-Natal, and is therefore an addition to studies previously done on the distribution of intestinal parasites in this province. Intestinal protozoons commonly occur with intestinal nematodes, and as Appleton and Gouws (1996) mentioned, knowledge of the distribution of intestinal infections with regard to the country's topography would be of great use in the planning of cost-effective parasite control programmes.

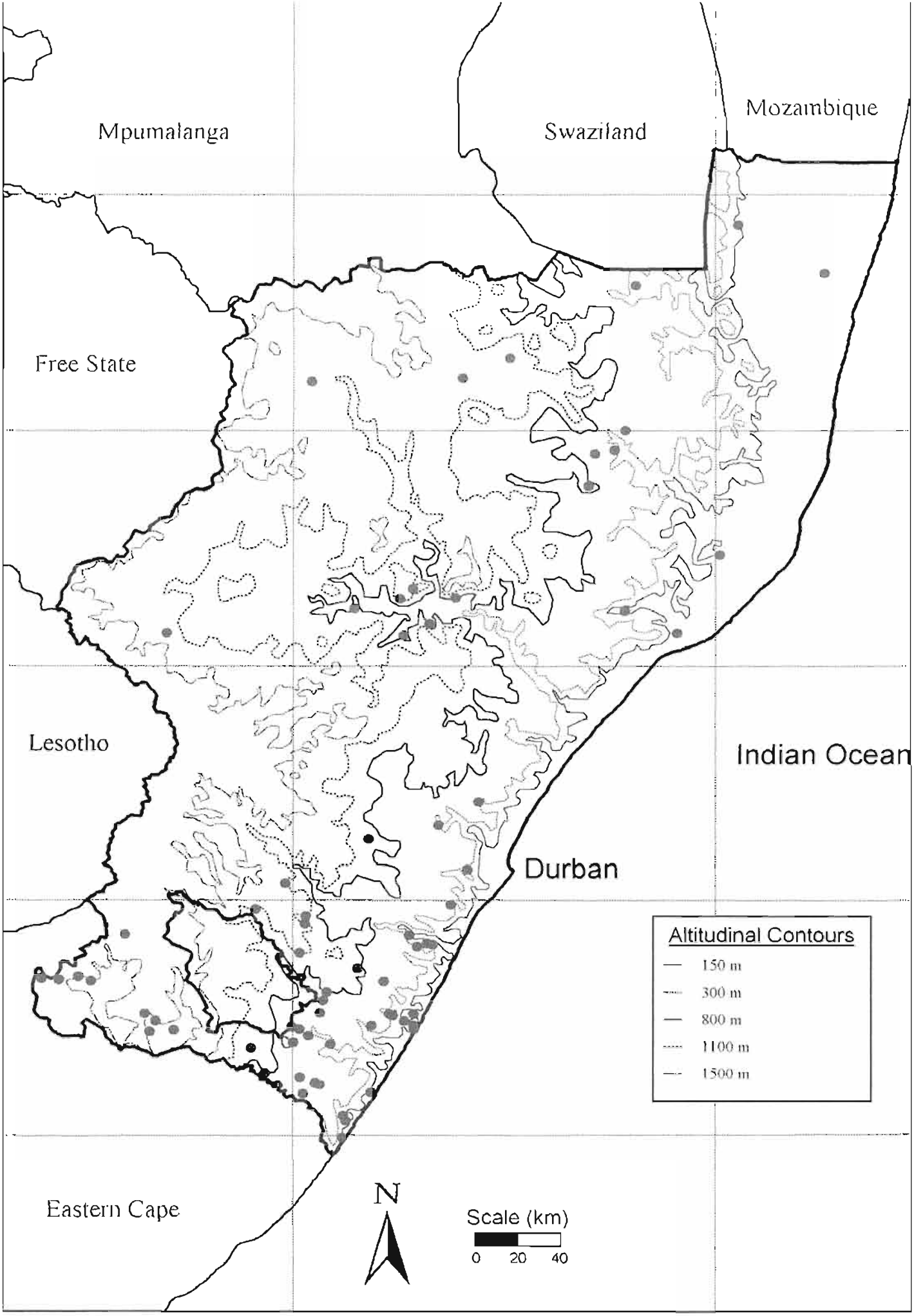
Although geohelminths have direct life cycles, the development of infective stages (eggs or larvae) takes place in the soil. Transmission of these parasites mostly depends on climatic factors and on the physical and chemical properties of the soil. Intestinal protozoons can be transmitted through contaminated water, from person-to-person by the faecal-oral route, and by infected animals. As protozoan cysts are immediately infective once released from the body of the host, socio-economic factors are likely to play a much more important role than environmental factors in the transmission of protozoan infections. This chapter reports on a study done to test this hypothesis by determining significant associations between the prevalence of the commonly found intestinal protozoons and selected environmental and socio-ecomonic factors.

4.2 MATERIALS AND METHODS

4.2.1 Source and mapping of data

Data on the prevalence of intestinal protozoons in children were obtained from primary school data of samples collected from Health Regions A to H by the KwaZulu-Natal Parasite Control programme, and from the altitudinal transect study by Appleton and Gouws (1996). The data from the health regions were pre-treatment data that was collected in February to September 1998 and was part of the KwaZulu-Natal Parasite Control Programme and the transect data were collected in 1994 from six schools in KwaZulu-Natal. Stool samples from both sets of data were collected from primary school children and these were examined using the Formal-ether concentration technique as outlined by Allen and Ridley (1970).

A total of 71 schools located at altitudes ranging from 0 - 1643 metres above sea level were selected. The altitudes, latitudes and longitudes of these schools were obtained from the Geographical Information Systems (GIS) database of the School of Life and Environmental Sciences, University of Natal, Durban. Figure 4.1 is a map of KwaZulu-Natal showing the sampling locations. The socio-economic variables were obtained from the 1996 Census database of The Education Foundation (also available at the School of Life and Environmental Sciences), while rainfall and temperature variables


Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
N
Scale (km)
0 20 40

were extracted from the maps given by Schulze (1982). The altitudes of the study locations and the prevalences of the intestinal protozoons in the different areas are shown in Appendix F. Prevalences of intestinal protozoons were plotted on the map of KwaZulu-Natal using the GIS Atlas programme.

4.2.2 Statistical analysis

Data were analysed using version 6.12 of the SAS statistical package. Normal probability plots and box plots were made to see the distribution of the data. Scatter diagrams were also plotted to determine any apparent associations between the occurrence of protozoan infections and the selected environmental and socio-economic factors. Basic summary statistics were generated for all variables. The climatic factors chosen are representative of temperature and rainfall in the summer and winter months, and of mean annual recordings. The selected socio-economic variables are related to population density, education, hygiene, employment and income status.

Since the data used were not normally distributed, a transformation was performed to normalize the data and stabilize the variance using the following equation:

$$y_i = \arcsin\left(\sqrt{p_i}\right)$$

where: y_i = dependent variable; p_i = proportion (i.e. prevalence/100)

Univariate analysis was carried out to determine correlations between protozoan prevalence and the individual variables and a univariate stepwise regression was performed on the transformed variables in order to determine those that combined best to explain the distribution of protozoan infections. In the multivariate analysis, all the variables were simultaneously considered.

4.3 RESULTS

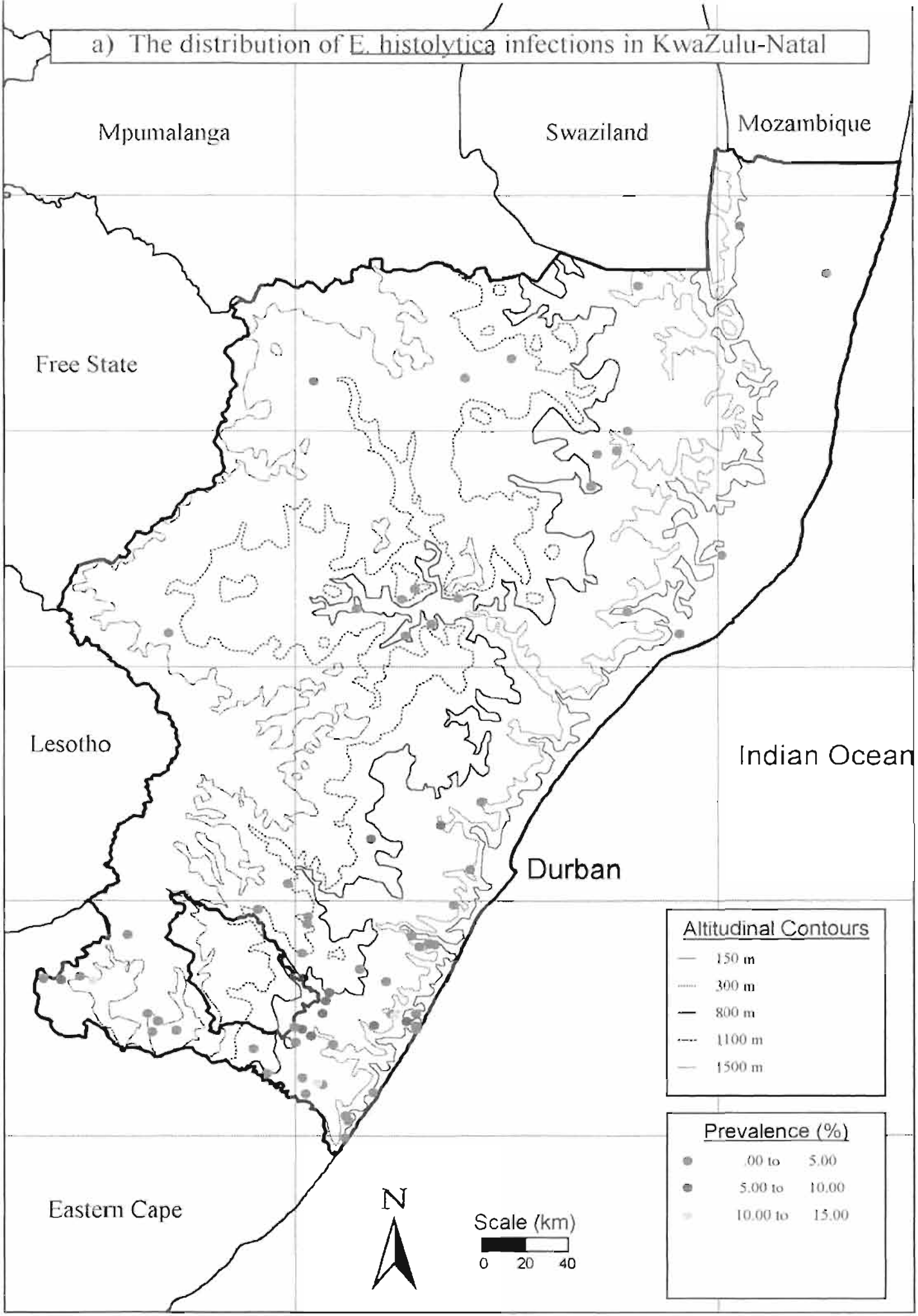
No apparent associations were seen between the distributions of protozoan species with altitude from the obtained maps (Figures 4.2a - g). With the exception of *E. coli*, prevalences were low (mostly below 10%), and all species were found throughout the whole province. As can be seen from the maps, these low prevalences were found at both low and high altitudes. Similarly, moderate prevalences (10-40%) have been recorded at both low and high altitudes. Prevalences above 50% were only found with *E. coli* and *E. nana* infections and although these were found in very few localities, they were only present at altitudes above 300m. This relationship is more apparent with *E. nana* (Figure 4.2d) infections.

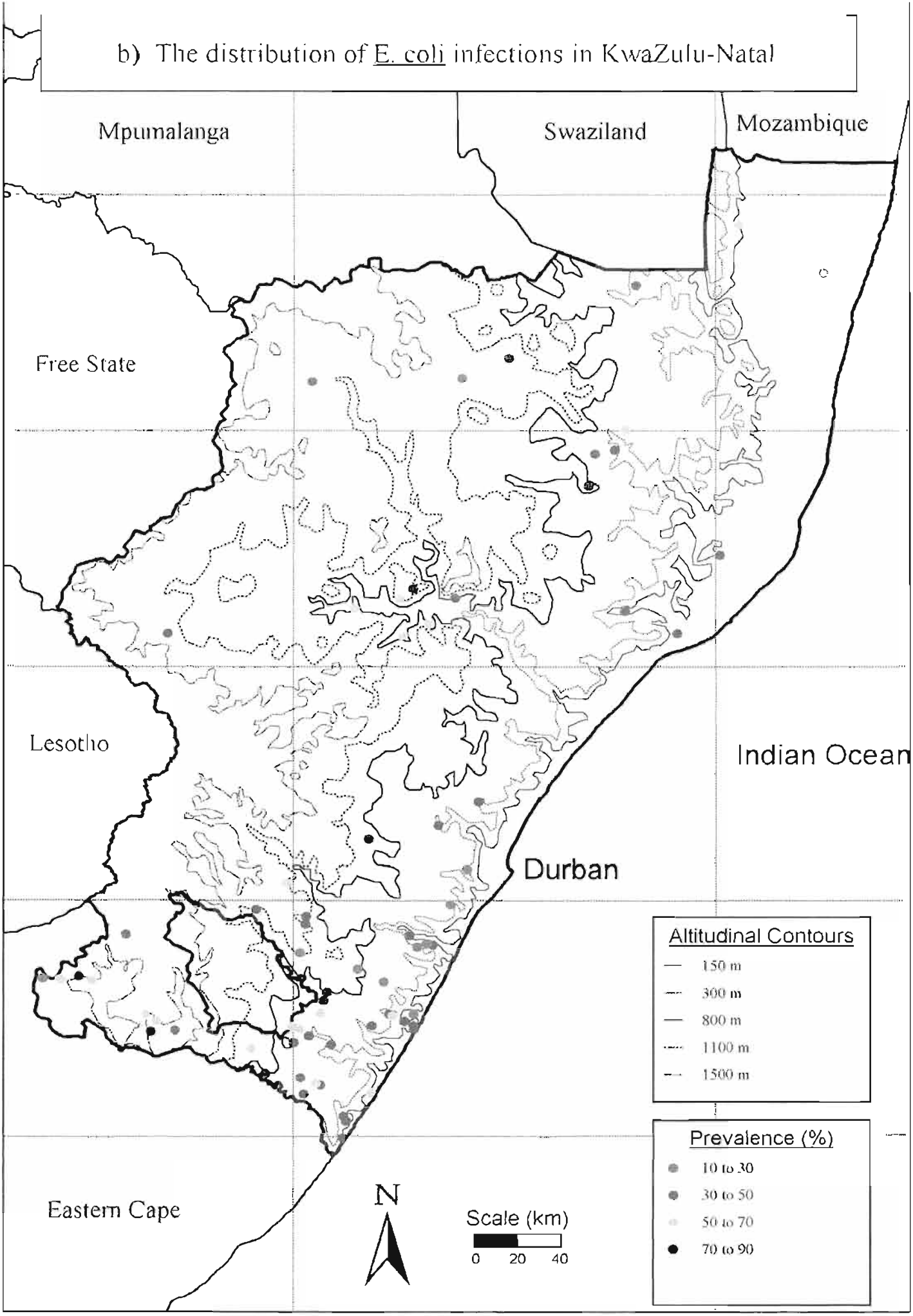
4.3.1 Summary statistics

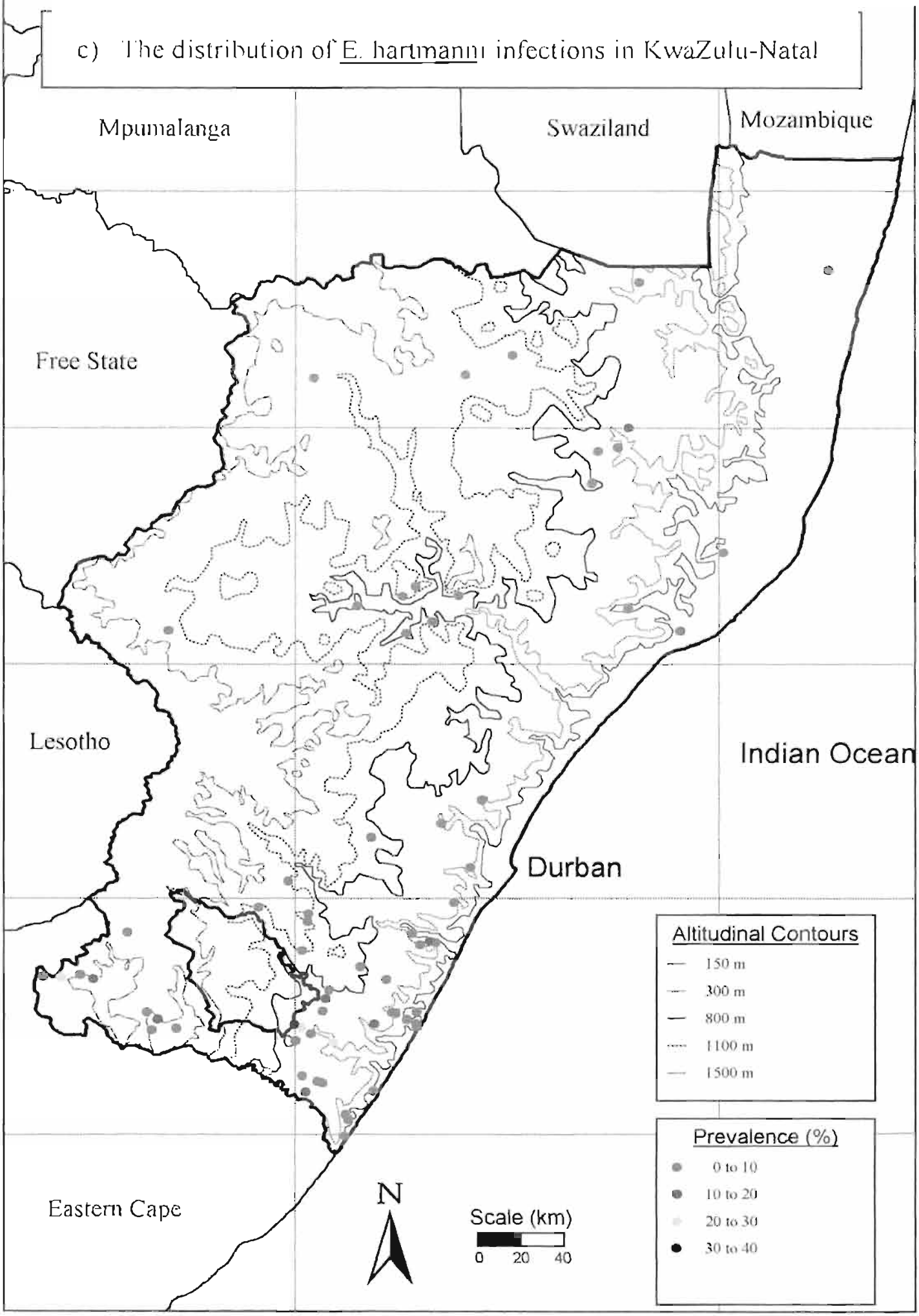
Summaries of the prevalences of protozoan species (dependent variables) and the socio-economic and environmental factors (independent variables) used in the study are given in Tables 4.1, 4.2 and 4.3 below.

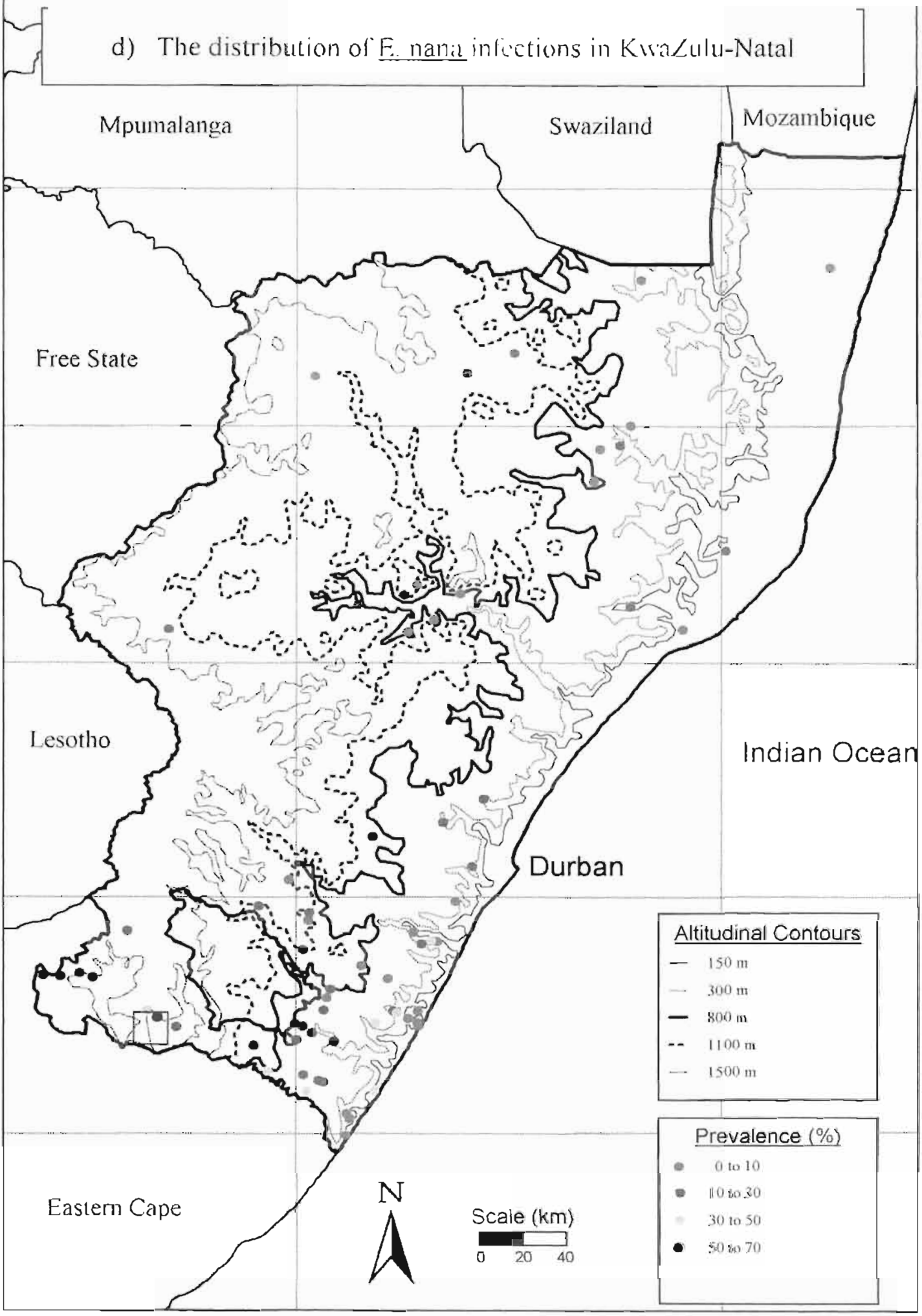
Table 4.1. Summary of protozoon prevalences recorded from the 71 localities in KwaZulu-Natal.

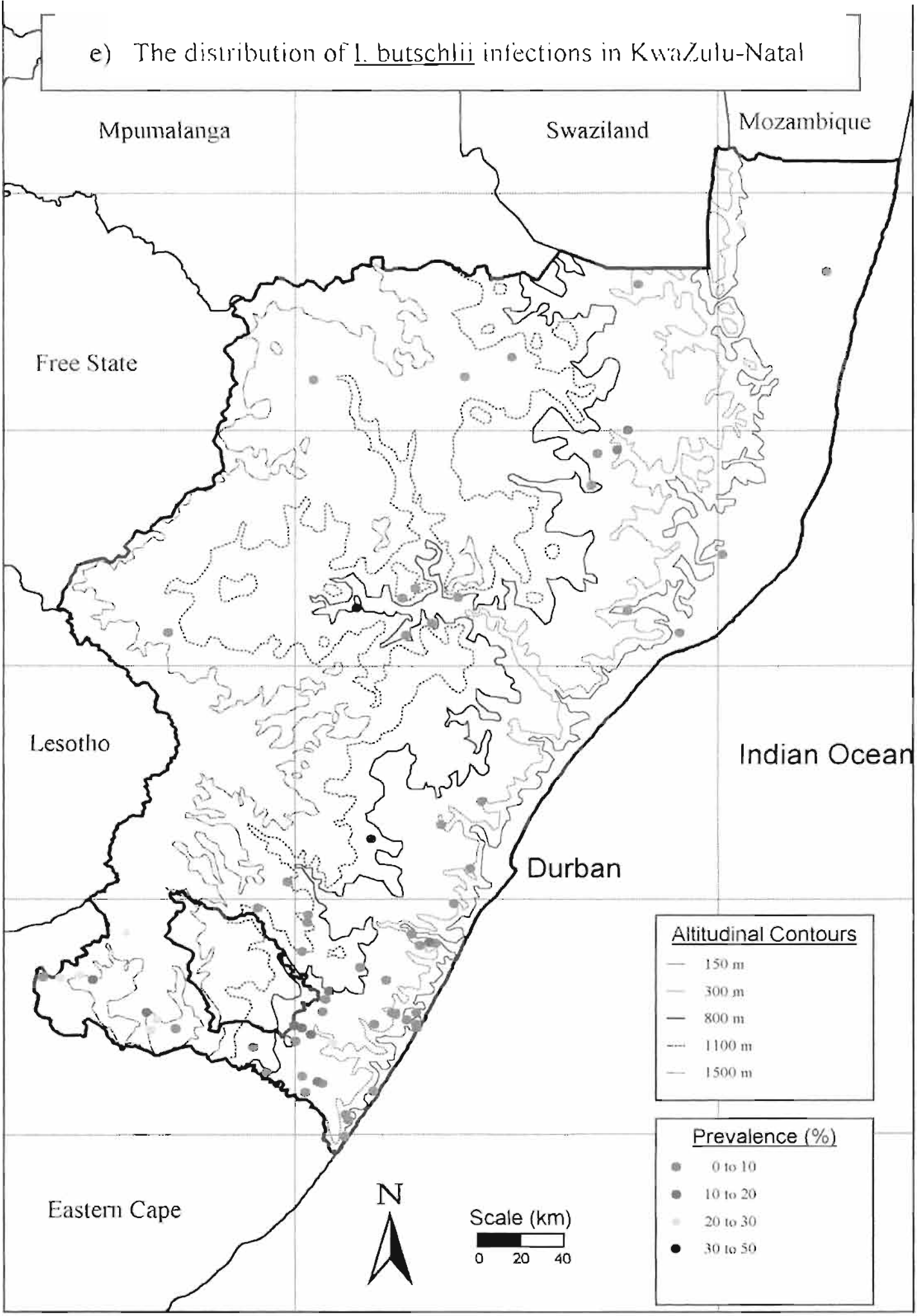
Protozoon	% Prevalence (Mean ± sd)	Range (%)
Entamoeba histolytica/dispar	3.5 ± 3.9	0 - 15.0
Entamoeba coli	44.4 ± 14.3	15.0 - 81.1
Entamoeba hartmanni	7.4 ± 8.1	0 - 33.3
Endolimax nana	23.3 ± 18.9	0 - 70.1
Iodamoeba butschlii	8.9 ± 9.4	0 - 43.1
Giardia intestinalis	7.3 ± 6.9	0 - 25.0
Chilomastix mesnili	5.4 ± 5.1	0 - 20

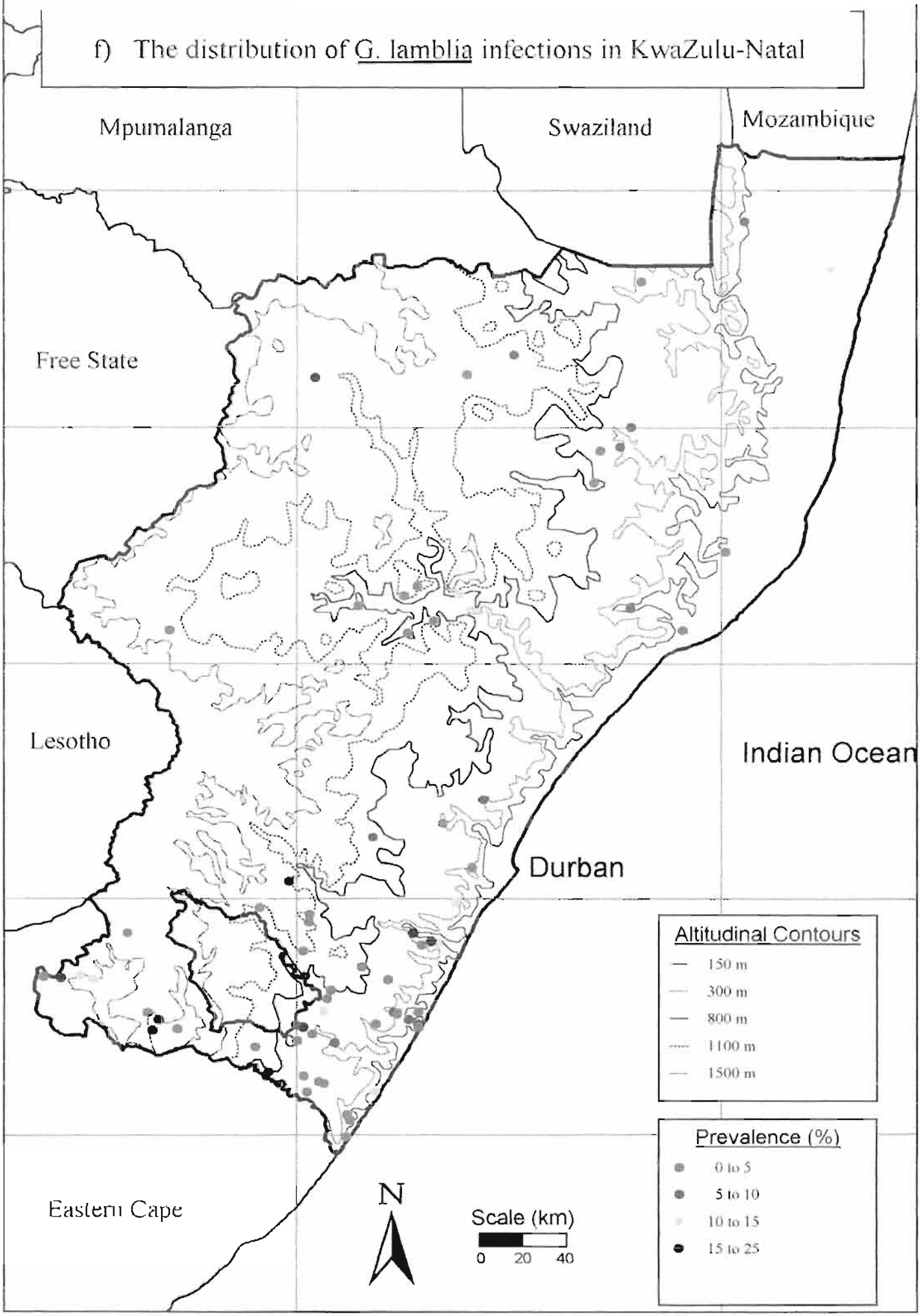

a) The distribution of E. histolytica infections in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
N
Scale (km)
0 20 40
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
.00 to 5.00
5.00 to 10.00
10.00 to 15.00


b) The distribution of E. coli infections in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
10 to 30
30 to 50
50 to 70
70 to 90
N
Scale (km)
0 20 40


c) The distribution of E. hartmanni infections in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
N
Scale (km)
0 20 40
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
0 to 10
10 to 20
20 to 30
30 to 40


d) The distribution of E. nana infections in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
0 to 10
10 to 30
30 to 50
50 to 70
N
Scale (km)
0 20 40


e) The distribution of I. butschlii infections in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
0 to 10
10 to 20
20 to 30
30 to 50
N
Scale (km)
0 20 40


f) The distribution of G. lamblia infections in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
0 to 5
5 to 10
10 to 15
15 to 25
N
Scale (km)
0 20 40

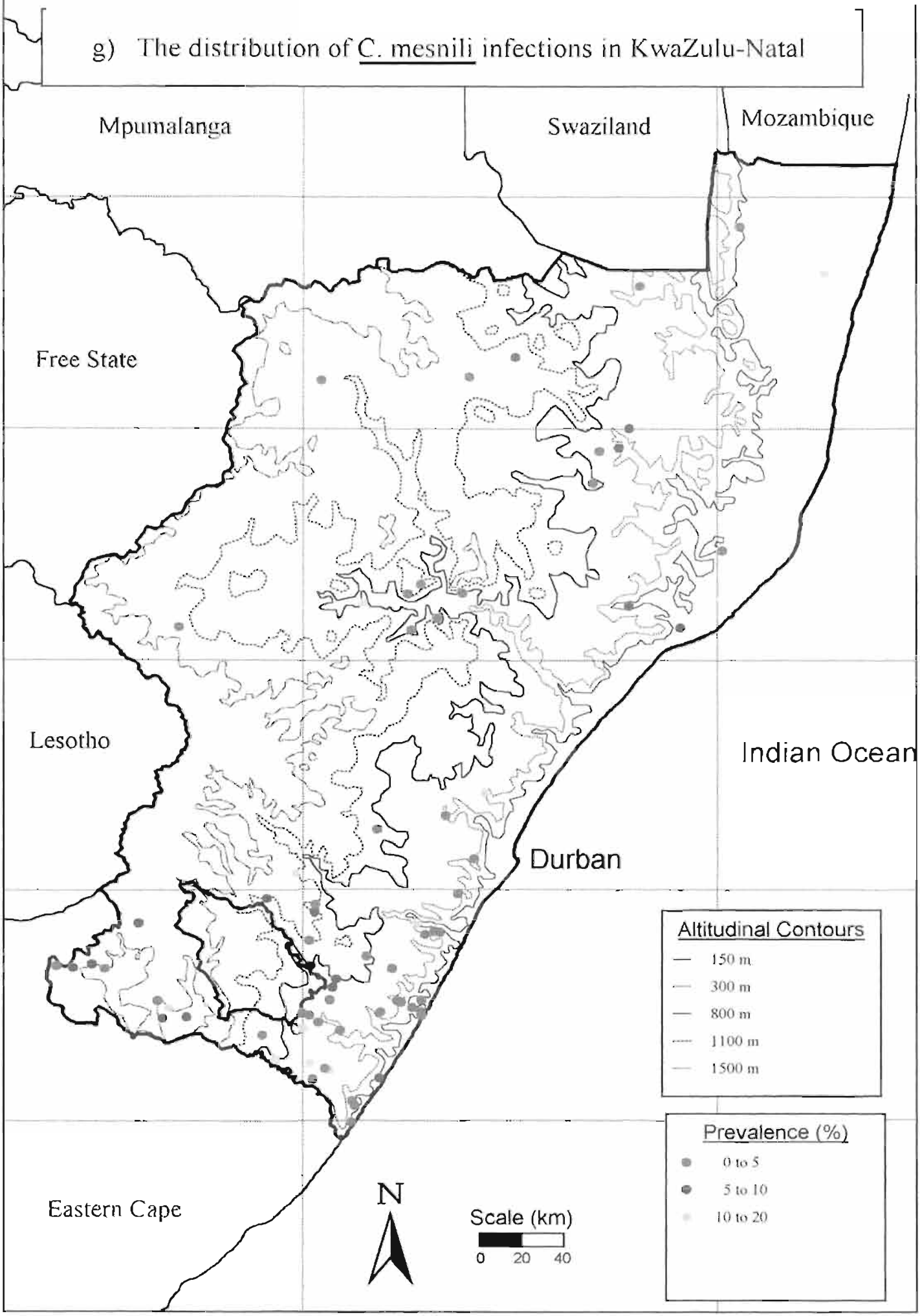

g) The distribution of C. mesnili infections in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
Free State
Lesotho
Indian Ocean
Durban
Eastern Cape
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
0 to 5
5 to 10
10 to 20
N
Scale (km)
0 20 40

Table 4.2. Summary of climatic factors for the 71 study locations (data extracted from Schulze, 1982)

Variable	**Mean ± sd**	**Range**
Altitude (m above sea level)	683 ± 465	0 - 1643
Temperature-based variables (°C)		
Mean Annual Temperature (MAT)	18.4 ± 2.1	14 - 22
Mean Monthly Temperature for January (MMTJan.)	22.2 ± 1.8	19 - 26
Mean Monthly Temperature for July (MMTJuly)	13.9 ± 3.1	7 - 18
Rainfall-based variables (mm)		
Mean Annual Precipitation (MAP)	898.6 ± 135.5	600 - 1400
Mean Monthly Precipitation for January (MMPJan.)	126.0 ± 10.6	100 - 150
Mean Monthly Precipitation for July (MMPJuly)	22.3 ± 4.7	15 - 30

Table 4.3. Summary of socio-economic variables for the study area (Education Foundation, 1996 census)

Variable	**Mean ± sd**	**Range**
population density (no. of people/km^2)	444 ± 1168	3 - 8761
% population aged >18 years with Grade 6 or higher	47.4 ± 16.0	4.1 - 91.7
% population employed	14.4 ± 14.9	0 - 70.6
population with no income	646 ± 546	0 - 3318
% households classified as formal	34.6 ± 33.3	0 - 100
% households classified as informal	1.2 ± 3.6	0 - 18.2
% households classified as traditional dwellings	61.7 ± 35.8	0 - 100
% homeless people	0.02 ± 0.11	0 - 0.7
% households with electricity	29.0 ± 33.2	0 - 98.3
% households with flush or chemical toilets	12.5 ± 26.3	0 - 99.2
% households with piped water	19.7 ± 32.6	0 - 99.2

4.3.2 Univariate associations

Univariate analysis involved the calculation of the Spearman Correlation Coefficient (r) for correlations between the prevalence of intestinal protozoon infections and the selected climatic and socio-economic variables. No strong associations were obtained, Tables 4.4i) and ii) show the significant ($p < 0.05$) moderate correlations found. The prevalence of *E. coli* was found to increase with an increase in altitude and a decrease in MAT, MMT for January and July, MAP, and MMP for July. Similarly *E. nana* prevalence increased with an increase in altitude, a decrease in MAT, MMT for January and July, and MMP for July. The prevalence of *I. butschlii* was only significantly correlated with MMT for January. Although these correlations were significant, the associations seen were weak as most of the points were scattered far from the line of fit. No significant correlations were obtained between the prevalence of the other protozoons and any of the factors.

Significant correlations with socio-economic factors were only obtained with *E. coli*, *E. histolytica/dispar* and *I. butschlii*. The prevalences of these three species were negatively correlated with population density. *Entamoeba coli* prevalence was positively correlated with the presence of traditional households while negative correlations were obtained with literacy, and informal and traditional households. A positive correlation was obtained between the prevalence of *E. histolytica/dispar* and traditional households, while formal households and number of households with flush toilets were negatively correlated with this protozoon. A negative correlation was obtained between the prevalence of *I. butschlii* and population density.

Table 4.4. Univariate associations between protozoan prevalences and i) environmental, ii) socio-economic factors (significant at $p < 0.05$)

i)

Protozoon	Variable	r	p
E. coli	Altitude	0.31399	0.0077
	MAT	-0.39974	0.0006
	MMT Jan.	-0.41334	0.0003
	MMT July	-0.36321	0.0054
	MAP	-0.30398	0.01
	MMP July	-0.35764	0.0022
E. nana	Altitude	0.35486	0.0024
	MAT	-0.39927	0.0006
	MMT Jan.	-0.52260	0.0001
	MMT July	-0.34649	0.0031
	MMP July	-0.32248	0.0061
I. butschlii	MMT Jan.	-0.38531	0.0009

ii)

Protozoon	Variable	r	p
E. coli	population density	-0.33904	0.0054
	% pop. with at least Grade 6	-0.40507	0.0007
	% informal households	-0.34314	0.0048
	% traditional households	0.35124	0.0038
E. histolytica/ dispar	% formal households	-0.32702	0.0074
	% traditional households	0.34522	0.0045
	% households with flush toilets	-0.35667	0.0033
I. butschlii	population density	-0.33868	0.0054

$r \geq 0.7$ = strong correlation; $0.3 \leq r < 0.69$ = moderate correlation; $r < 0.3$ = weak correlation (E. Gouws, 2000 (pers. comm.))

4.3.3 Univariate stepwise regression

Univariate stepwise regression procedure on the transformed variables involved the calculation of the coefficient of determination (R^2) and the F-statistic. Although some significant associations were obtained between protozoan prevalence and some of the selected factors, these were weak as in all cases the value of R^2 was below 0.5. The significant associations ($p < 0.10$) obtained are shown in Table 4.5.

4.3.4 Multivariate analysis

When all the variables were simultaneously considered, the only significant factor was the number of households with electricity. The result for this test is as follows:

Wilks' Lambda value	0.77816866
F-statistic (7 degrees of freedom)	1.8733
p-value	0.0960

Table 4.5. Significant ($p < 0.10$) associations between prevalence and selected factors in the stepwise regression procedure

Variable	Parameter Estimate	Standard Error	F-value	p
E. coli ($R^2 = 0.3841$)				
MMT January	-0.04133	0.00871	22.54	0.0001
% informal houses	-0.01277	0.00432	8.77	0.0043
% households with flush toilets	-0.00151	0.00061	6.05	0.0167
E. histolytica/dispar ($R^2 = 0.2451$)				
% pop. with Grade 6 or above	0.00253	0.00108	5.49	0.0224
% households with electricity	0.00091	0.00055	2.73	0.1033
% households with flush toilets	-0.00139	0.00067	4.28	0.0429
% traditional households	0.00165	0.00583	8.08	0.0061
E. hartmanni ($R^2 = 0.1863$)				
MMT January	-0.04478	0.01289	12.07	0.0009
MMP January	-0.00706	0.00229	9.47	0.0031
population with no income	-0.00007	0.00004	3.27	0.0754
E. nana ($R^2 = 0.3780$)				
MMT January	-0.06191	0.01337	21.43	0.0001
% population employed	0.00322	0.00181	3.15	0.0806
% traditional households	0.00218	0.00072	9.09	0.0037
I. butschlii ($R^2 = 0.4178$)				
Altitude	-0.00011	0.00006	3.50	0.0662
MMT January	-0.06382	0.01534	17.31	0.0001
% informal households	-0.00889	0.00549	2.62	0.1108
% households with flush toilets	-0.00478	0.00141	11.36	0.0013
% households with piped water	0.00236	0.00122	3.73	0.0581
G. intestinalis ($R^2 = 0.0513$)				
MMP January	-0.00346	0.08541	3.46	0.0674
C. mesnili ($R^2 = 0.2257$)				
% formal households	0.00120	0.00055	4.85	0.0313
% households with electricity	-0.00234	0.00055	18.28	0.0001

4.4 DISCUSSION

4.4.1 Protozoan prevalence and environmental factors

Univariate and multiple regression analyses indicated that except for *E. nana*, the topography is not important in determining the distribution of the common intestinal protozoan species in KwaZulu-Natal. The prevalences of most species were low and all species were found throughout the province. Most species were significantly correlated with climatic factors, particularly mid-summer temperature (MMT for January) and rainfall (MMP for January). Although these correlations were moderate, they show that climatic factors do have a contribution in the distribution of intestinal protozoons.

The high prevalence of *E. nana* at high altitudes as observed from the univariate analysis has previously been noted from other studies (Appleton and Gouws, 1996; Mosala, 1995). In a study on the occurrence of intestinal parasites in baboons, a higher prevalence of *E. nana* was obtained in baboons living in montane habits than in those in the coastal lowland (Appleton and Henzi, 1993). This protozoon therefore survives better at high than at low altitudes. High altitudes are characterized by low temperatures, and Farmer (1980) mentioned that cooler temperatures are less harmful to protozoan species than higher temperatures due to the fact that the levels of dissolved oxygen in water decrease with increasing temperature, and as most protozoan species are aerobic, they would therefore survive best in a cooler environment due to a high content of dissolved oxygen. The prevalence of this protozoon is also influenced by a combination of mid-summer temperature and living in traditional houses.

In the present study, no altitudinal preference was found for the other protozoan species. Intestinal protozoons can be directly transmitted from person to person and have therefore been found in a wide altitudinal range. This is in contrast to the distribution of geohelminths in KwaZulu-Natal as the prevalence of these nematodes has been significantly correlated with both altitude and climatic factors. It is now well known that the prevalences of *Trichuris trichiura*, *Ascaris lumbricoides, Strongyloides stercoralis* and the hookworm, *Necator americanus*, are all highest on the coast of KwaZulu-Natal

and decrease with increasing altitude (Maurihungirire, 1993; Appleton and Gouws, 1996; Mabaso, 1998, Appleton *et al.*, 1999). In Kenya, Ashford and Oppenheimer (1993) also noted that people living furthest from the coast and in dry areas are generally free from intestinal nematode infections. This is not the case with intestinal protozoons as protozoan infections have been recorded from places that are far from the coast, some of which are totally free of nematode infections. These places include the inlands of the Eastern Cape (Heinz, 1973; Segal *et al.*, 1981), Qwa-Qwa area in the Free State (Mosala, 1995), and Lesotho (Esrey *et al.*, 1989; Kravitz *et al.*, 1993) where the climate is temperate, with long dry summers and very cold winters. In other temperate countries such as the USA and the UK, *G. intestinalis* is ranked the most prevalent intestinal parasite (Faubert, 1988).

In the univariate analysis, prevalences of *E. coli* and *E. nana* were negatively correlated with temperature and rainfall-derived variables. The lack of positive associations between the occurrence of protozoan infections with rainfall-derived variables was unexpected as moist environments increase the survival of both free-living and encysted parasitic protozoa, regardless of the location and temperature (moist soil, a variety of water bodies, even in snow) (Farmer, 1980). Encysted protozoons can survive in cold water for several months (Farmer, 1980; Walker-Smith, 1988). The significant associations between mid-summer temperature and rainfall with most protozoan species (see Section 5.3.3) raises the possibility of seasonal transmission. Increased summer transmission would occur in cases of water-borne transmission due to increased rainfall.

Survival and transmission of intestinal protozoons, which occur under a broad range of environmental conditions, are due to the formation of tough, resistant cysts. These are formed under adverse conditions of desiccation, nutritional deficiencies, and chemical and physical changes in the environment (Farmer, 1980). Although cyst formation varies in the different species, most protozoan cysts are made up of a very tough, thick wall that is only dissolved in the gut of the host (Westphal and Muhlpfordt, 1976). Some cysts even have multiple layers to ensure their survival under adverse environmental conditions. Cysts of *I. butschlii* are made up of a dense, thick layer and another layer of lower density (Westphal and Muhlpfordt, 1976) while the cysts of *E.*

histolytica/dispar and *E. coli* are formed from a single hyaline wall of keratinized protein (Farmer, 1980). The cyst wall of *G. intestinalis* is composed of chitin (Walker-Smith, 1988), and *Giardia* cysts are also resistant to disinfectants such as chlorine, hydrogen peroxide and ozone (Goldin *et al.*, 1990; du Preez and Gericke, 1999). *Giardia* cysts are however readily killed by temperatures above 35° C and therefore the only effective way of inactivating them is by heating (boiling and pasteurization) (Farmer, 1980; du Preez and Gericke, 1999).

In the case of geohelminths, the development of the life-cycle stages takes place in the soil, and therefore rainfall facilitates growth by providing moisture for the developing larvae. Survival and transmission of the geohelminths with thick shells (*A. lumbricoides* and *T. trichiura*) is mostly influenced by rainfall while a combination of high temperatures, moderate rainfall and sandy soil facilitate the transmission of *N. americanus* and *S. stercoralis* (Mabaso, 1998; Appleton *et al.*, 1999). It is this developmental phase in the soil that distinguishes the distribution requirements of geohelminthic from those of protozoan infections.

4.4.2 Protozoan prevalence and socio-economic factors

The fact that moderate significant correlations were obtained between the prevalence of protozoan species and some of the socio-economic factors used in the present study shows that these are to some extent important in the distribution of protozoan infections. As discussed below, the importance of socio-economic factors in the transmission of intestinal protozoons, especially the pathogenic species, has also been indicated in several studies. Stepwise regression analysis showed that a combination of environmental and socio-economic factors is also important in determining the prevalence of protozoan infections.

Population density was negatively and moderately correlated with the prevalence of *E. coli*, *E. histolytica/dispar*, and *I. butschlii* in the univariate analysis, however, this association between population density and prevalence was not significant for these protozoons in the regression analysis. The risk of parasite infection is proportional to

human population density, and in cases where the mode of transmission is person-to-person, overcrowding facilitates rapid transmission of faecally-transmitted parasites (Chacin-Bonilla *et al.*, 1992). However, in rural areas population densities are usually low and person to person transmission would be insignificant as there is no overcrowding.

Literacy, that is the number of people with Grade 6 education or above, was negatively correlated with the prevalence of *E. coli*. This shows the importance of education in preventing transmission of protozoan infections. Educated communities are more conscious of the values of basic hygiene practices that prevent the spread of infection than illiterate ones. The education level of the mother is also very important as it affects the socio-economic status of the whole family, the use of health services, understanding of proper nutrition, and the general ability of the mother to provide a healthy environment (Kustner, 1989). The association between the prevalence of *E. coli* and literacy, together with the mean monthly temperature for January and informal households, contributed 37% of the variation in the prevalence of *E. coli* in the studied communities.

The prevalence of *E. histolytica/dispar* was negatively correlated with the presence of flush toilets in the univariate analysis, and this was the only significant association in the multiple regression analysis. This illustrates the importance of proper sanitary facilities in controlling protozoan infections. An outbreak of amoebiasis in a farming area in Philippi, Cape Town, was related to living under poor hygienic conditions as the majority of the farm workers had no toilet facilities and used open water sources for their potable supply (Whittaker *et al.*, 1994). Feachem *et al.*(1983) also noticed that the prevalence of *E. histolytica* was higher in families with poor sanitary facilities than in families without sanitary facilities, indicating that poorly constructed and/or maintained sanitary facilities can therefore be a source of contamination. In addition to sanitation, the presence of flies, contact with contaminated objects and water-bodies, large families, unhygienic practices, have all been significantly associated with a high prevalence of *E. histolytica/dispar* (Connell and French, 1939; Mackie *et al.*, 1956; Bradley, 1978; Feachem *et al.*, 1983).

Traditional houses are usually build out of mud bricks and sometimes cow dung is used for decorations on the inside and outside of the house and therefore the positive correlation between traditional housing and the prevalences of *E. coli* and *E. histolytica/dispar* could be expected as the moisture in mud and dung provide an ideal place for survival of protozoan cysts. Informal houses can be made of a variety of materials including planks, wood or cardboard which can also retain moisture. *Giardia intestinalis* has been associated with living under low socio-economic conditions (Feachem *et al.*, 1983; Walker-Smith, 1988; Gurses *et al.*, 1991).

When all the variables were simultaneously considered, the presence of electricity was the only factor that contributed significantly to the variations observed. The presence of electricity in a household is important as it determines the overall hygiene of the family. In most families where there is no electricity, there is also no refrigerator. Therefore prepared food is kept in the open where it can easily be contaminated.

Several studies have also focused on associations between socio-economic factors and the prevalence of intestinal nematodes. As with intestinal protozoons, intestinal nematodes are transmitted through the faecal-oral route and are also found in communities with poor living standards and therefore the epidemiology of the two groups of parasites might be similar in regard to socio-economic factors that aid in their transmission. In Tanzania (Renganathan *et al.*, 1995) the high prevalence and intensity of helminths in one school was associated with high population density and lack of sanitation, and in Venezuela multiple intestinal infection was associated with low hygiene and overcrowding (Chacin-Bonilla *et al.*, 1992). Holland *et al.* (1988) found higher prevalences of single and multiple helminth infections in children living in houses made of wood or bamboo than in those living in houses made of concrete blocks. In places where there are no sanitary facilities, habits like defaecating in damp, shaded areas increase the transmission of helminth infections. The thermal heat due to fermentation in properly constructed sanitation systems kills helminth ova and therefore prevent transmission (Hawkins and Feachem, 1978).

In the present study *G. intestinalis* was only associated with mid-summer rainfall, this further shows that importance of rain in the transmission of protozoan infections which are normally high in summer due to increased rainfall. No association was found between *G. intestinalis* prevalence with any of the selected socio-economic factors. This finding is similar to results found in some studies where associations were not obtained between the prevalence of this protozoon with neither the presence of piped water (Mason *et al.*1986), nor the use of unhygienic water sources, improved water supplies, and proper sanitary facilities (Esrey *et al.*, 1989). The tough, thick cyst wall of *Giardia* enables it to survive in a wide range of environments. In Zimbabwe, improvement in sanitation did not change hookworm reinfection rates (Bradley *et al.*, 1993), and in Tennessee (USA) the white community had higher prevalence rates of intestinal parasitic infections than the rural Negro community despite a higher level of sanitation and cleanliness in the former (Jones *et al.*, 1954). In comparing the prevalence of parasitic infections between the informal settlement of Cato Manor (Durban) and the adjacent formal settlement of Chesterville, Elson-Dew (1953) found similar prevalences of protozoan infection in both settlements despite the differences in living conditions in these two areas. These observations and the lack of strongly significant correlations in the present study imply that in addition to socio-economic factors, there are other factors that may have an effect on the distribution of intestinal protozoons.

Human behaviour and activities, socio-cultural practices and human interactions with domestic animals have previously been associated with the transmission of protozoan infections and may have a more significant impact. However, these were not included in the present study and further investigations would need to be undertaken to establish their significance. Human activities that have been found to aid in the transmission of protozoan infections include the disposal of human faeces, animal faeces from abattoirs and sewage sludge onto land (du Preez and Gericke, 1999). Zoonotic transmission, particularly with *Giardia intestinalis*, has been suggested by several authors (Bemrick and Erlandsen, 1988; Faubert, 1988; Olson *et al.*, 1994; Wallis, 1994). This factor would be important in rural communities where domestic animals roam about freely and infected ones can contaminate water-bodies that are used as sources for domestic use.

Basic hygienic practices that prevent transmission of protozoan infections include washing of hands before handling food and after defaecation, proper water storage in areas where there is no running water, and proper cleaning and maintenance of sanitary facilities (Bradley, 1978). Pescod (1978) pointed out that the provision of clean water, proper housing and sanitation are not adequate in themselves since it is human behaviour that dominates in the spread of parasitic infections. Some authors (Goyder, 1978; Wolman, 1978; Palmer Development Group, 1993; du Preez and Gericke, 1999) have suggested that there should be increased primary health awareness by involving communities in the planning and development of control programmes. Educating the community on infections is essential so that the effort and resources that the government puts in providing facilities such as running water and proper sanitation can have a positive impact.

This study has some limitations that may have had an effect on the results. The data used were obtained from rural communities where living standards and prevalences of infections would generally be similar and therefore fall within a narrow range. Most of the study locations were in the Health Region A (see Figure 4.1) and therefore there was very little variation in the data on environmental variables. The data used are good and appropriate for investigating environmental variables because they are from similar communities. This fact, however, gives them limited use for looking at socio-economic variables. Another limiting factor was the fact that no protozoan infections (i.e. 0% prevalence) were found in children in a lot of schools (see Appendix F). More significant correlations would have probably been obtained if the data used were spread over a wider range in terms of both protozoan prevalences and the socio-economic and environmental factors.

CHAPTER 5

OCCURRENCE OF *CRYPTOSPORIDIUM* INFECTION IN CHILDREN

5.1 INTRODUCTION AND BACKGROUND

Cryptosopridium infection was first reported in laboratory mice in 1907, but the pathogenicity of this parasite in animals was only recognised in 1955 (Beaver and Jung, 1985; Trees, 1997). The occurrence of *Cryptosopridium* in man and its recognition as a human pathogen was only recently discovered (Meisel *et al.*, 1976; Nime *et al.*, 1976). The first report on *Cryptosporidium* infection in South Africa is by Smith (1985) where oocysts were found in stools of diarrhoeal children admitted to King Edward VIII hospital in Durban. Cryptosporidiosis is found more frequently in developing than in developed countries (Hojlyng *et al.*, 1984; Addy and Aikins-Bekoe, 1986), and the increase in the number of people infected with the HIV has increased the prevalence of this disease (Diagnostics Pasteur, 1989; Griffiths, 1998). *Cryptospridium* is now established as one of the five common pathogens (including viral and bacterial enteropathogens) associated with acute watery diarrhoea in children (Tziprori *et al.*, 1983; Bogaerts *et al.*, 1984; Mata *et al.*, 1984; Fripp *et al.*, 1991; Hussey, 1998). In some areas, it has replaced *Giardia intestinalis* as the most commonly found gastro-intestinal protozoan parasite in children (Fripp *et al.*, 1991). Although cryptosporidia are gastrointestinal coccidian parasites of numerous wild and domestic animals, human intestinal cryptosporidiosis is caused only by *C. parvum* (Trees, 1997; Griffiths, 1998).

This chapter reports on the occurrence of *Cryptosporidium* oocysts in diarrhoeal stool samples obtained from the gastroenteritis paedriatric patients at King Edward VIII Hospital in Durban, South Africa.

5.1.1 Transmission of *Cryptosporidium*

Cryptosporidosis is highly infectious as the oocysts are infective when discharged in the stool (Bogaerts *et al.*, 1984) and the infectious dose of oocysts for humans is very small. In an experiment on healthy human volunteers, as few as 30 oocysts could establish an infection (DuPont *et al.*, 1995). Human infection is through contaminated water, person to person contact, interaction with infected animals, and through sexual activities. Although oocysts have been isolated from some foodstuffs, food-borne transmission is not yet fully established due to the small numbers of oocysts in foodstuffs (Griffiths, 1998). Sexual transmission of cryptosprodiosis is common in homosexual men and in people who practice penile-anal sex, and the risk is increased in HIV-infected individuals (Ma and Soave, 1983; Manabe *et al.*, 1998).

Besides man, *C. parvum* infects cats, dogs, cattle, sheep and other mammals (Diagnostic Pasteur, 1989). These mammals therefore serve as reservoirs for this parasite, and because of the close ecological association between humans and these domestic animals, zoonotic infections are common (Newman *et al.*, 1993; Webster and MacDonald, 1995; Griffiths, 1998). Cryptospridiosis is responsible for about 55% of epidemic diarrhoea cases in animals (Diagnostic Pasteur, 1989). As the oocysts shed in freshly passed stools from animals are immediately infective, people have been infected after visiting or working on farms and from handling and caring of infected animals in laboratories (Koch *et al.*, 1985; Trees, 1997).

Cryptosporidiosis outbreaks due to person-to-person transmission have been reported from day-care centres (Walters *et al.*, 1988), family units (Chacin-Bonilla *et al.*, 1993), and in hospital patients and personnel where infection resulted from direct exposure to the patient's faeces, bedding, bedpans and soiled clothing (Stuchler, 1988; Koch *et al.*, 1985). Navarrete *et al.* (1991) reported a paediatric ward outbreak where 82% of children who were exposed to an infant with *Cryptosporidium* infection and AIDS developed cryptosporidiosis.

Water-borne transmission is common in travellers due to drinking of contaminated water (Jokippi *et al.*, 1985). Contamination of water-bodies usually results from run-off of animal or human faeces, accidental spillage of farm slurry from agricultural land, and from animal and human sewage effluent discharges (Madore *et al.*, 1987; Jamey-Swan *et al.*, 2000; Bridgman *et al.*, 1995). Water draining off animal and human faeces containing oocysts is common in the rainy season, and this often leads to human outbreaks. In Milwaukee (USA), MacKenzie *et al.*, (1994) reported a massive outbreak of water-borne cryptospridiosis where 400 000 people were affected. Contamination of swimming pools by recreational bathing is another way in which people get infected with *Cryptosporidium* (Madore *et al.*, 1987) as the oocysts are very resistant to chemical disinfectants such as the chlorinating agents used in water purification and in swimming pools (D'Antonio *et al.*, 1985; Madore *et al.*, 1987; Walters *et al.*, 1988; Jamey-Swan *et al.*, 2000; Sorvillo *et al.*, 1994; MacKenzie *et al.*, 1995). Furthermore, in cases where oocysts are recovered from treated drinking water, current laboratory methods do not enable the determination of the viability or infectivity of the oocysts (Jamey-Swan, 1999).

Cryptosporidium usually occurs with *Giardia* in contaminated water. Samples from selected water-bodies in South Africa have been examined microscopically for the presence of cysts and oocysts. In KwaZulu-Natal, cysts and oocysts were regularly detected in the Midmar dam and its river inflows, and in the KwaGqishi river near Pietermaritzburg (Jamey-Swan, 1999). This author detected high concentrations of cysts and oocysts (0-675 cysts/10 ℓ and 0-1500 oocysts/10 ℓ) in the sludge from wastewater works and lower concentrations (50 oocysts/10 ℓ and 10-40 cysts/ℓ) in untreated water. Up to 520 cysts/10 ℓ and 110 oocysts/10 ℓ were detected in the Darvill final effluent which was discharged into the uMsunduze River (Jamey-Swan *et al.*, 2000). *Giardia* cysts and *Cryptosporidium* oocysts were also found in drinking water samples from unprotected sources serving rural and urban informal settlement communities in the Northern and Northwest provinces (du Preez and Gericke, 1999). Very high concentrations of up to 2000 cysts and 1020 oocysts /10 ℓ of water, were found in sample from these water bodies.

Giardia cysts are usually detected throughout the year whereas the occurrence of *Cryptosporidium* oocysts is sporadic, with high counts after rainfall in summer, particularly after heavy showers (du Preez and Gericke, 1999; Jamey-Swan *et al.*, 2000)

In the USA and UK, *Cryptosporidium* oocysts have been isolated from ground, surface and potable water samples, and from untreated and treated sewage effluents (D'Antonio *et al.*, 1985; Madore *et al.*, 1987; Atherton *et al.*, 1995; Bridgman *et al.*, 1995; Maguire *et al.*, 1995).

5.1.2 Epidemiology and clinical features

Crytosporidiosis is usually acquired by children though adults are also at risk (Tzipori *et al.*, 1983; Bogaerts *et al.*, 1984). *Cryptospridium* has, in many cases been the only pathogen isolated from stool samples of immunocompetent and immunocompromised (AIDS and other immune deficiencies) people with diarrhoea. The association between cryptosporidiosis and diarrhoea has been reported from Europe (Connolly *et al.*, 1988; Palmer and Biffin, 1990; McGowan *et al.*, 1993; Bridgman *et al.*, 1995), Australia (Tzipori *et al*, 1983), North and South America (Mata *et al.*, 1984; D'Antonio *et al.*, 1985; Chacin-Bonilla *et al.*, 1993; Lindo *et al.*, 1998), Asia (Pal *et al.*, 1989), South Africa (Robertson and Spector, 1985; Smith, 1985; Smith and van den Ende, 1986; Walters *et al.*, 1988; Steele *et al.*, 1989; Fripp *et al.*, 1991; Moodley *et al.*, 1991) and elsewhere in Africa (Bogaerts *et al.*, 1984; Hojlyng *et al.*, 1984; Addy and Aikins-Bekoe, 1986). In immunocompetent people, the prevalence of cryptospridiosis is 0.5-5% in developed countries and 3-20% in developing countries (Cohen, 1990). In AIDS patients, the prevalence is 5-15% in developed countries and 30-50% in tropical and developing countries (Diagnostic Pasteur, 1989; Conlon *et al.*, 1990; Manabe *et al.*, 1998).

Unlike other coccidia that infect the host's epithelial cells, *C. parvum* is found in the ciliated brush border of the mucosal epithelium of the intestine (Beaver and Jung, 1985). The parasite is not invasive but can cause focal lesions of the epithelium (Ma and Soave, 1983). Infections range from asymptomatic to a cholera-like illness, with

massive losses of body fluids (Manabe *et al.*, 1998). Other symptoms include headache, nausea, vomiting, fever, anorexia, coughing and malnutrition (Bogaerts *et al.*, 1984; Koch *et al.*, 1985; Connolly *et al.*, 1988; Walters *et al.*, 1988; Casemore *et al.*, 1984). Malabsorption of fat and carbohydrates due to damage of the intestinal brush border membrane is also common (Cohen, 1990.)

In immunocompetent hosts, the disease is usually asymptomatic or presents as a self-limiting diarrhoea (Fripp and Bothma, 1987). In such cases the incubation period is usually short, with an onset of profuse watery diarrhoea accompanied by abdominal cramps. These symptoms usually persist for a few days (5 to 10), after which they usually disappear even without treatment (Fripp and Bothma, 1987).

Cryptosporidisis is aggravated by host genetic factors, gastrointestinal changes (which might be mediated by concurrent opportunistic infections), and the host's immunity (McGowan *et al.*, 1993; Manabe *et al.*, 1998). In immunocompromised hosts the diarrhoea is more severe and can be life threatening. The symptoms are chronic, with fluid loss of up to 20 litres per day (Sturcher, 1988; Fripp *et al.*, 1991) and a frequency of up to 20 stools per day (Connolly *et al.*, 1988). This chronic diarrhoea constitutes one to the diagnostic features of clinical AIDS (Diagnostic Pasteur, 1989). In these patients, infection sometimes affects the biliary tree, pancreas, gall bladder and the respiratory tract (Canning, 1990; Cohen, 1990; Casemore, 1991).

5.1.3 Diagnosis

Diagnosis of cryptosporidiosis is generally by detection of oocysts in faeces, but sometimes identification is by the presence of the tissue stages in biopsy or necropsy tissues (Diagnostic Pasteur, 1989; Casemore, 1991). In tissue material, the parasite can be seen when the tissue is processed and stained by histological stains such as haematoxylin and eosin (Casemore, 1991).

Direct examination of faecal samples can be done on faeces of patients with acute cryptosporidiosis as the number of oocysts excreted is usually high. However, oocysts

cannot be easily distinguished from yeast cells and fungal spores in wet preparations (Casemore, 1991). Concentration methods used in the diagnosis of cryptosporidiosis include the sucrose flotation (such as the Sheather's flotation method) and the modified formol-ether concentration techniques. The original Sheather's floatation method is however, tedious and time-consuming and the oocysts 'pop-up' very quickly in the concentrated sucrose solution used, making their identification difficult (C. Anderson, pers comm.). Concentration with the formol-ether method is usually preferable as the sediment obtained can also be examined for ova and cysts of other parasites (Casemore, 1991).

Staining methods used in cryptosporidiosis diagnosis include the modified Ziehl-Neelsen method, Giemsa, aurimine, and safranin-methylene blue techniques (Fripp and Bothma, 1987; Diagnostic Pasteur, 1987; Casemore, 1991). Most of these methods are tedious and require the use of a fluorescent microscope and this is not always available in small routine laboratories (Fripp and Bothma, 1987). Sometimes oocysts fail to stain (Collignon, 1987), and fungal particles (which have the same size and shape as oocyts) can take up the stain, resulting in false-positives (Bogaerts *et al.*, 1984; Casemore, 1991; Fripp *et al.*, 1991). Detection and identification of oocysts in stool can also be done using immunologically based methods such as monoclonal antibody immuno-flourescence (IFAT) and ELISA (Griffiths, 1998). These methods are however expensive and therefore their use is usually restricted to research laboratories and not in routine examination of stool samples in hospital laboratories.

5.1.4 Treatment

There is no effective drug treatment for cryptosporidiosis (Fripp and Bothma, 1987; Canning, 1990) but loss of fluid and nutrients can be replaced by fluid and electrolyte replacement therapy (Fripp and Bothma, 1987; Walters *et al.*, 1988). Antibiotics such as erythromycin and spiramycin, and anti-diarrhoeal agents reduce the severity of the diarrhoea (Connolly *et al.*, 1988). These are however ineffective in advanced AIDS cases (Cohen, 1990). Antiretroviral agents such as zidovodine (AZT) have been administered to cryptosporidiosis patients and in most cases, the diarrhoea was resolved

(Connolly *et al.*, 1988; Manabe *et al.*, 1998). In order to prevent infection by *Cryptosporidium*, Canning (1990) suggests that AIDS patients should boil their drinking water, avoid contact with farm animals and domestic pets, and avoid homosexual or bisexual activities which facilitate faecal-oral transmission.

5.2 MATERIALS AND METHODS

5.2.1 Patients

Stool samples were collected from paediatric patients at King Edward VIII Hospital in Durban. This is a teaching and referral hospital for outlying hospitals and clinics in Durban and the surrounding areas and it predominantly serves the black community (Smith and van den Ende, 1986). Permission to collect the samples was granted by Drs S. Ramjee and M. Chuggon (Paedriatric Outpatient and Gastroenteritis Inpatient Departments, King Edward VIII Hospital) and by the mothers of the children.

A total of twenty-two fresh stool samples were collected. Four of the specimens were collected from children in the paediatric outpatients and gastroenteritis rehydration units, and 17 samples were from children admitted to the gastroenteritis ward. The ages ranged from 18 weeks to 48 months (mean (SD) =18 (11) months. Ten of the children were males and twelve were females. Specimens were collected within 48 hours of admission to avoid the possibility of studying children with hospital-acquired infections. The collected samples were transported to the parasitology laboratory where they were immediately processed. Seventy formed and semi-formed additional stool samples (preserved in 10% formalin) were also examined for *Cryptosporidum* oocysts. These were remains of samples used in a previous study (Chapter 3).

Some problems were encountered in obtaining the samples from paediatric patients and this resulted in a very small sample size. As permission was required from the mothers to obtain samples from their babies, communicating with the mothers was important. However, most of the mothers could not speak English and linguistic problems were encountered as the researcher is not a Zulu speaker. In most visits to the hospital for

collection of the samples, there would be a different team of nurses on duty, some of whom had some form of difficulty in helping either with talking to the mothers or with collection of the samples. Although the shedding of *Cryptosporidium* oocysts is intermittent and more than one stool sample from each subject is usually needed for diagnosis (Connolly *et al.*, 1988), this could not be achieved in this study due to the reasons already mentioned.

5.2.2 Laboratory methods

The stool samples were concentrated by the Formol-ether Concentration Technique (Allen and Ridley, 1970) and examined for *Cryptosporidium* oocysts using the Sheather's solution. The solution is prepared by dissolving 500g sucrose and 6,5g phenol in 320ml distilled water (Ma and Soave, 1983). This was mixed with the concentrate from the formal-ether preparation on a microscope slide and screened for oocysts under the light microscope using the high-power (40X) objective. This method was recommended by Mrs C. Anderson (Medicine Research Council) and can be used both for formed and unformed stools. Confirmation of oocysts was done under oil immersion (100X objective). A positive concentrate containing cryptosporidial oocysts preserved in 10% formalin (provided by Mrs. C. Anderson) was used for comparison of results. A positive sample prepared by the author was also taken to Mrs Anderson for confirmation. Iodine wet mounts were also prepared from the formol-ether sediments and examined for intestinal protozoan cysts and helminth eggs.

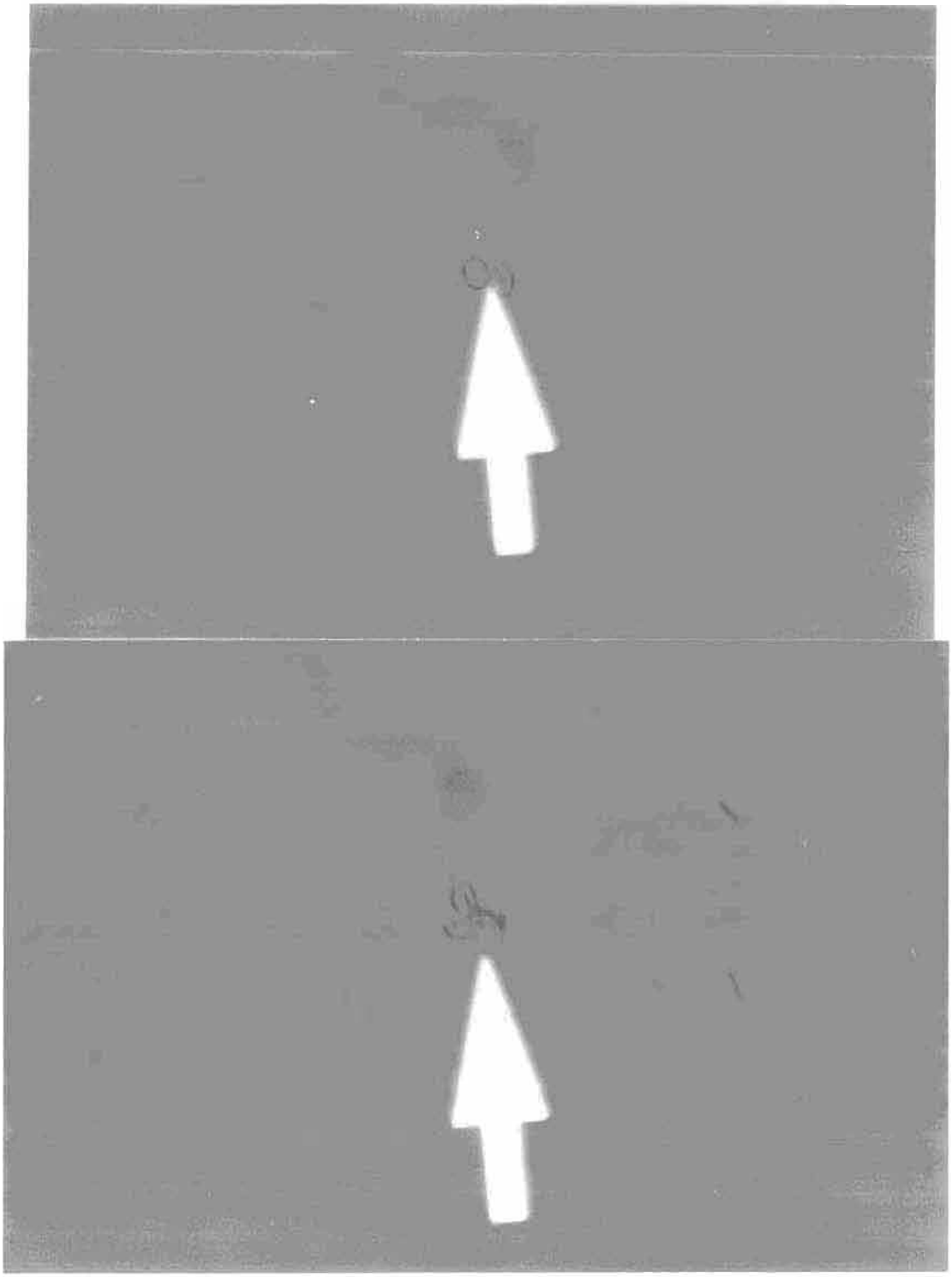
The original Sheather's floatation method involves the concentration of oocysts by centrifugation of stools in the Sheather's solution at 2000 rpm for 5 minutes. A wire loop is then used to transfer a surface drop onto a microscopic slide, covered with a coverslip, and examined microscopically with the 40X objective (Fripp and Bothma, 1987). The limitations of using this method and staining methods are mentioned in Section 5.1.3.

5.3 RESULTS

Cryptosporidium oocysts were recovered from 18.2% (4/22) of the children; one child was from the outpatient department and three were from the ward. All these samples were watery and one sample even contained mucus. The ages of these children were 7, 10, 11 and 14 months. No other parasite was found in the *Cryptosporidium* positive and negative stools. Oocysts were not detected in any of the 70 formed and semi-formed stools examined.

Cryptosporidium oocysts measure 4-5 μm in diameter and are sporulated when released in the faeces (WHO, 1998). In Sheather's solution, the oocysts appear as spherical structures with a light pink colour (Plate 5.1), but because of their minute size, the internal structures, consisting of four crescent-shaped sporozoites and a residual body, are usually not visible by light microscopy (Ma and Soave, 1983; Casemore, 1991).

The immunological status of the peadiatric patients could not be measured and access to their clinical charts was not allowed. It is however likely that there was a certain level of malnutrition and immunodeficiency in these patients as KwaZulu-Natal has the highest prevalence of HIV infections in the whole country (Kustner *et al.*, 1998) and a 26% paediatric HIV prevalence has been recorded from a district hospital in rural KwaZulu-Natal (Yeung *et al.*, 2000).



X1000

Plate 5.1 *Cryptosporidium parvum* oocysts in Sheather's solution from a patient aged 11 months

5.4 DISCUSSION

This study supports findings that *C. parvum* is important as a cause of diarrhoea in children under the age of five years (Smith, 1985). It should however be pointed out that diarrhoea in children can also be caused by bacterial or viral pathogens, or by other parasitic pathogens, as diseases caused by all these have symptoms that are similar to those of cryptosporidiosis (Hojlyng *et al.*, 1984). However, it was outside the scope of this study to determine the presence of bacterial and/or viral infections in *Cryptosporidium* infected children. The 18% prevalence recorded in the present study might be an underestimate of the actual prevalence in the sampled children as the release of *Cryptosporidium* oocysts is often intermittent and low (Tzipori *et al.*, 1983; Fripp and Bothma, 1987). These authors suggest that more than one faecal specimen should be examined in determining *Cryptosporidium* infection rates. In the first survey done on the occurrence of *Cryptosporidium* in South Africa, Smith (1985) found a prevalence of 30.8% in black paediatric inpatients at King Edward VIII Hospital. Other similar surveys done at this hospital are by Smith and van den Ende (1986) and Moodley *et al.* (1991), where prevalences of 11.9% and 9% respectively were obtained. A much higher prevalence of 73% was recorded from infants and toddlers in a day-care centre in Durban (Walters *et al.* 1988) and infection resulted in a cryptosporidiosis outbreak where 10% of the staff members were also infected. This is the highest cryptosporidiosis prevalence recorded in South Africa, especially from otherwise healthy children. The suspected index case was a member of staff (child-minder) whose work included the changing of the infants' nappies.

Surveys on the occurrence of *Cryptosporidium* have been done in other parts of South Africa. In Johannesburg, prevalences of 18.4% and 3.4% were recorded from black children at Chris Hani-Baragwanath Hospital (Berkowitz *et al.*, 1988) and Coronation Hospital (Robertson and Spector, 1985). In Pretoria, cryptosporidial oocysts were found in 6.2% of stool samples (submitted to a pathology laboratory) from white children under the age of five (Steele *et al.*, 1989), and in 4.21% of stool specimens of children and adults at GaRankuwa Hospital (Fripp *et al.* 1991).

The differences in the prevalences found in different parts of South Africa might be due to different geographic locations. The climate in KwaZulu-Natal is sub-tropical and therefore the humidity and high temperatures, which are present most of the year, would result in a higher prevalence of protozoan infections compared to temperate areas where temperatures are generally low. Moodley *et al.* (1991) did not find a correlation between environmental temperature and the occurrence of *Cryptosporidium*, so temperature is not likely to be a cause of the noted differences. The difference in prevalences from studies done in the same area are probably due to different diagnostic techniques.

Black South African children are mostly from communities which lack good sanitation, proper sewage disposal systems, and protected water supplies (Moodley *et al.*, 1991; Pegram *et al.*, 1998). The high prevalence of cryptosporidiosis found in the present study is similar to that found in other developing African countries such as Liberia, 7.9%, (Hojlyng *et al.*, 1984); Rwanda, 10.4% (Bogaerts *et al.*, 1984); and Ghana, 12.9%, (Addy and Aikins-Bekoe, 1986). A lower prevalence of 5.3% was found in Nigerian children (Reinthaler *et al.*, 1987), and in children from developing countries from other parts of the world such as India, 5.6% (Pal *et al.*, 1984); Costa Rica, 4.3% (Mata *et al.*, 1984) and Bangladesh, 4,3% (Shahid *et al.*, 1985). Children from developed nations usually have low infection rates, examples are Poland, 5.6% (Sinski *et al.*, 1987); Australia 4.8%, (Tzipori *et al.*, 1983); and United Kingdom, 5% (Palmer and Biffin, 1990). In all these studies, *Cryptosporidium* was found as the sole pathogen, and oocysts were found in diarrhoeic, watery stools. This is an indication of the strong association between *Cryptosporidium* infections and gastroenteritis. Oocysts were not found in the 70 formed stools examined in the present study, in 94 formed stools submitted for parasitological examination in Rwanda (Bogaerts *et al.*, 1984), and in 39 formed stools of children in Costa Rica (Mata *et al.*, 1986). However, in some occasions, *Cryptosporidium* infection can be asymptomatic, with oocysts either detected in formed stools or by biopsy (Janoff *et al.*, 1990).

Cryptosproridium infection is determined by factors such as age, immune and nutritional status of the host, geographical location, environmental conditions, and seasonality (Bogaerts *et al.*, 1984; Steele *et al.*, 1989). In most studies (Jokipii *et al.*,

1985; Chaisson *et al.*, 1995; Manabe *et al.*, 1998), no association was found between cryptosporidiosis and sex of the subject. An association was however found by Molbank *et al.* (1994), with more infections occurring in boys than in girls. In the USA (Chaisson *et al.* 1995), race was considered unimportant in the occurrence of cryptosporidiosis, however, in South Africa the highest prevalence of the disease was found in the black community (Jarmey-Swan, 1999). This is an indication of the generally poor living standards of the black South African population.

High prevalences have been found in children under two years of age (Hojlyng *et al.*, 1984; Robertson and Spector, 1985; Addy and Bekoe, 1986; Walters *et al.*, 1988; Steele *et al.*, 1989; Moodley *et al.*, 1991) and in children aged between two and three years (Fripp *et al.*, 1991). Mata *et al.* (1984) and Molbank *et al.* (1994) suggested that breast-feeding is protective against cryptosporidiosis in children, but in a study on non-human primates by Miller *et al.* (1991), intentionally infected infants developed the disease regardless of whether the infants had been fed on formula or on breast milk. *Cryptosporidium* oocysts have also been found in infants under the age of one year (Smith, 1985; Addy and Aikins-Bekoe, 1986; Pal *et al.*, 1989; Fripp *et al.*, 1991; Chacin-Bonilla *et al.*, 1993) which, under normal circumstances are breast-feeding. Griffiths (1998) suggested that breast milk may not be particularly protective after exposure to the parasite, but that breast-fed infants are less likely to ingest oocysts from contaminated food and water and are only exposed to oocysts after weaning. In some countries infants are usually carried on the backs of their mothers and this reduces exposure to infection (Moodley *et al.*, 1991).

The seasonal occurrence of cryptosporidiosis could not be determined as the samples were all collected in one month. Seasonal peaks of cryptosporidiosis have usually been found during the warm, humid and rainy season (Tzipori *et al.*, 1983; Mata *et al.*, 1986; Miller and van den Ende, 1986; Steele *et al.*, 1986; Mai, 1987; Reinthaler *et al.*, 1987; Fripp *et al.*, 1991; Moodley *et al.*, 1991). In Calcutta (India) the prevalence of cryptosporidiosis was highest in the monsoon season which is characterized by heavy rainfall (Pal *et al.*, 1989). Although the occurrence of *Cryptosporidium* during the rainy season is an indication of water-borne transmission, Jamey-Swan *et al.* (2000) did not

find any correlation between *Cryptosporidium* infections and rainfall or season of the year, and the authors suggested that socio-economic factors might have contributed to transmission of the parasite.

In the present study, cysts or ova of other intestinal parasitic species were not seen from the stools of *Cryptospridium*-infected children. This is similar to results obtained from a study in Johannesburg (Robertson and Spector, 1985). However, co-infections of *Cryptosporidium* and *Giardia* in children have been found in several studies (Jokippi *et al.*, 1985; Fripp and Bothma, 1987; Reinthaler *et al.*, 1987; Steele *et al.*, 1989; Fripp *et al.*, 1991; Moodley *et al.*, 1991). Double infections with *Giardia* and *Cryptosporidium* would be common in water-borne infections, but unlike cryptosporidosis which occurs mostly during the rainy season, *Giardia* infections are usually evenly spread throughout the year (Fripp, 1989; du Preez and Gericke, 1999). These two parasites have been found occurring in drinking water sources in South African (Jarmey-Swan, 1999; du Preez and Gericke, 1999). Other parasites which have been found co-occurring with *Cryptosporidium* are *Entamoeba histolytica/dispar*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Isospora belli*, *Trichomonas hominis* and *Hymenolepis nana* (Fripp and Bothma, 1987; Reinthaler *et al.*, 1987; Moodley *et al.*, 1991).

Although this study was limited by a small sample size, it confirmed earlier findings that *Cryptosporidium* is an important cause of childhood diarrhoea in South Africa and other developing areas. This survey and most surveys done on the determination of cryptosporidiosis prevalence in South African do not give an accurate picture of this disease in this country, especially in rural and peri-urban communities where living standards are low and therefore infections are likely to occur. Screening of large communities in other countries have revealed that *Cryptosporidium* is a significant cause of diarrhoea in healthy people (Pal *et al.*, 1984; Chacin-Bonilla *et al.*, 1993; Molbank *et al.*, 1994) and the proportion of asymptomatic carriers is high in developing countries (Chacin-Bonilla *et al.*, 1993). The epidemiology of this disease in KwaZulu-Natal and in other parts of South Africa, where a large proportion of the population still has inadequate sanitation and water supplies (Pegram *et al.*, 1998), can only be determined by more detailed surveys.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Different aspects of the epidemiology of intestinal protozoon infections in children were studied. Although intestinal protozoan species that have been found in children and adults living in South Africa are diverse, this study concentrated on infections that commonly occur in school children in KwaZulu-Natal as this age group is usually the mostly parasitized. These are the amoebae; *Entamoeba histolytica/dispar*, *Entamoeba coli*, *Entamoeba hartmanni*, *Iodamoeba butschlii*, *Endolimax nana*, and the flagellates; *Giardia intestinalis*, and *Chilomastix mesnili*. Attention was also focused on the occurrence of the coccidian *Cryptosporidium parvum* in both healthy and diarrhoeic children because of its importance as a cause of fatal, watery diarrhoea in children and immunocompromised individuals.

The prevalences of the common intestinal protozoan species in children attending Carrington Heights Primary School compare with those found in surveys done in other parts of KwaZulu-Natal (Table 2.1). In all studies *E. coli* is the most prevalent species, and with the exception of this protozoon, prevalences of the intestinal protozoan species are generally low. Most pupils had single infections and only a few harboured more than four parasite species, and no seasonal variations in protozoan infections were detected. This study has confirmed the observations by Rousham (1994) and Taylor *et al.* (1995) that, although prevalences of the helminths are significantly reduced after treatment, there is an increase in the prevalence of intestinal protozoan species. Furthermore, an increase in the intensities of these species was also significantly increased after helminth chemotherapy. This increase in protozoan infections is certainly not desirable in any effort aimed at reducing mortality and morbidity associated with parasitic infections as heavy *G. intestinalis* and *E. histolytica/dispar* infections cause more pathogenicity than light infections. Monitoring of protozoan infections after administration of anthelmintic chemotherapy is essential so that anti-protozoal agents such as metrinadole are administered as excessive numbers of

parasites, even in the case of slightly pathogenic species, may be fatal to the host (Bradley, 1972).

Univariate and multivariate analysis of epidemiological data obtained from children in different schools in KwaZulu-Natal showed that the distribution of intestinal protozoan infections in this province is not limited by topography. The only protozoan species that seems to have a different epidemiology is *E. nana* as it thrives better at higher than at lower altitudes. Although reasons for the preference of high altitudes by this species are unknown, the observed association did not occur by chance as it has previously been observed (Appleton and Henzi, 1993; Mosala, 1995; Appleton and Gouws, 1996). Both climatic and socio-economic factors are important in the distribution of protozoan infections and it is suggested that in addition to this, other factors such as the presence of animals and human behaviour might also have a significant impact on protozoon prevalence. These were however not considered in the study and further studies are essential in order to establish their significance.

The 18% prevalence of *C. parvum* found in the present study compares favourably with prevalences found in children under the age of five years in other parts of this country and in other developing countries. Higher prevalences are usually found when there are outbreaks like the one that occurred in a day-care centre in Durban (Walters *et al.*, 1991). The occurrence of *Cryptosporidium* in paediatric patients with diarrhoea shows the importance of this parasite as an enteropathogen. Clinical data obtained from King Edward VIII Hospital Registry shows that from April 1998 to March 2000, nine cases of cryptosporidiosis were recorded from children aged between 6 and 36 months that had been admitted to the hospital. In all nine cases, cryptosporidiosis was not the only diagnosed disease. Five children also had AIDS, two had kwashiorkor and one had *Escherichia coli* infection. Other recorded complications were growth retardation, pneumonia, lung abscess, urinary tract infection and anaemia.

Knowledge of the epidemiology of *Cryptosporidium* is important in the prevention and control of cryptosporidiosis as there is presently no effective treatment against the parasite (Current, 1994), and education must be an important tool in its control.

Although *Cryptosporidium* oocysts were not found in stool samples of pupils from Carrington Heights Junior Primary School, there should be constant monitoring of children in schools and communities to identify sources of infection so as to undertake preventive measures. Testing also allows for the education of patients and facilitates the prediction of outbreaks (MacKenzie *et al.*, 1994). Determination of *Cryptosporidium* infections should not only be done in hospitals but should also include schools and communities as some infections are asymptomatic. Large scale screening of stool samples for *C. parvum* might present a challenge because the diagnostic methods used are different from those commonly used for the other protozoons. It is proposed that this protozoon should be included in routine diagnosis of parasitic infections, particularly in tropical countries.

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Appendix A - Parental consent form

I hereby give consent for my child _____ Grd ___ to take part in the Test-and-Treat Campaign against worms.

Appendix B - 10% Formalin solution (WHO, 1991)

Formalin (neutral formaldehyde, at least 37%)	100ml
Distilled water	900ml

Measure the aqueous formalin solution into a graduated measuring cylinder and pour it into a 1000ml bottle with a glass stopper or screw-cap. Add the distilled and mix well. Label the bottle: 10% FORMALIN and write the date. Store on a shelf or in a cabinet; the solution will remain good for two or more years.

Isotonic Saline solution (WHO, 1991)

Sodium chloride (Nacl)	8.5g
Distilled water	1000ml

Weigh out the sodium chloride into beaker. Measure the distilled water in a clean, glass bottle. Dissolve the sodium chloride in the water and mix thoroughly.
Label the bottle: ISOTONIC SALINE and write the date. Store on a shelf or in a cabinet. Pour some saline into a dispensing or dropping bottle for daily use and write the date on the label. The dispensing bottle should have a pipette with a rubber bulb.

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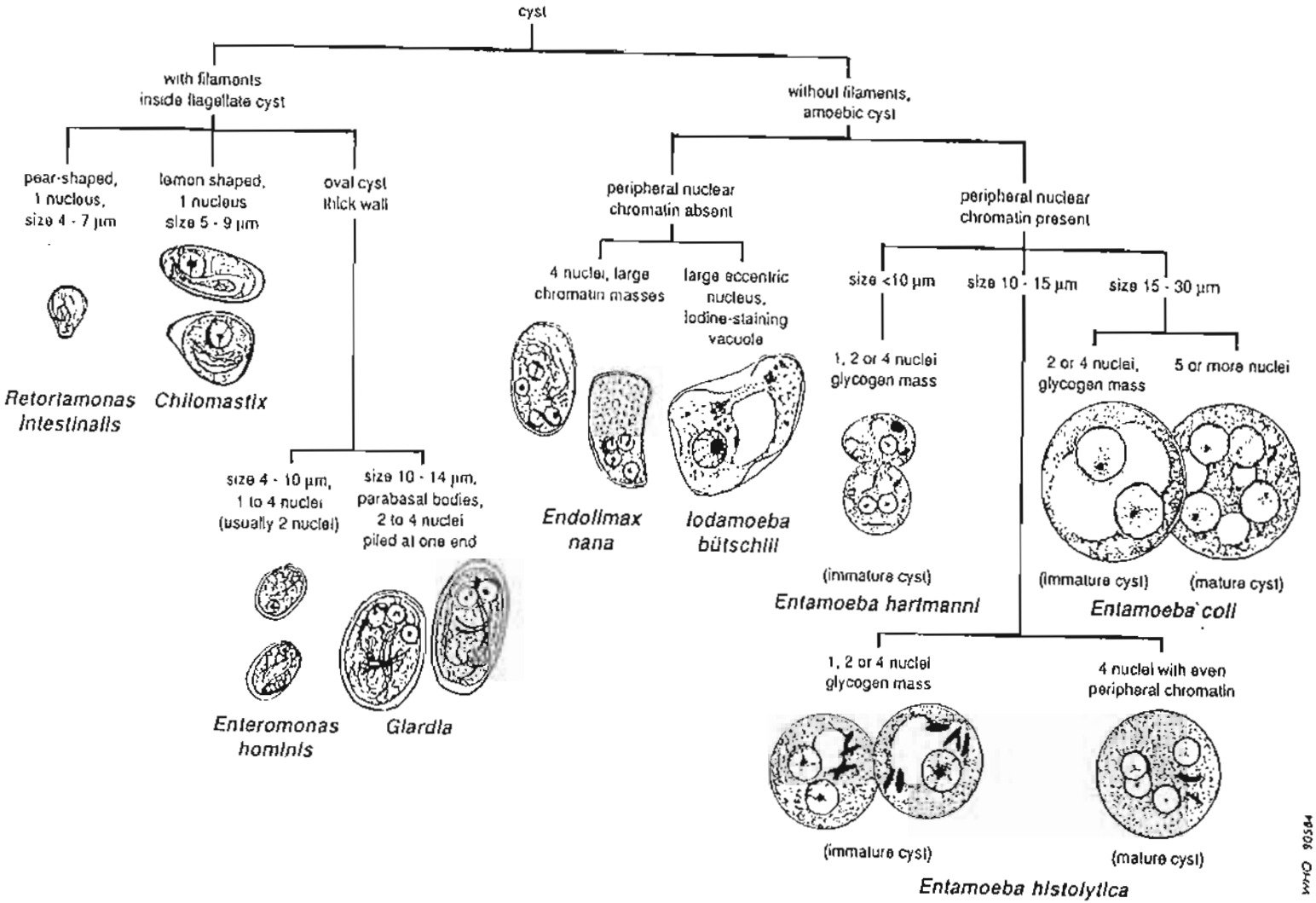
Appendix C - Modified formol-ether concentration method

(Allen and Ridley, 1970)

1. Weigh out between 0.5 g and 1.0 g of faeces into a 15 ml polypropylene conical test-tube and add 7ml formalin (10%) into the tube
2. Using a wooden applicator stick, thoroughly mix the stool sample and filter through a double layer of wetted cotton gauze into a waxed paper cup (Monocup). Wetting the gauze prevents the filtrate from being absorbed by the gauze and reduces the chance of eggs and cysts sticking to it
3. Add a small amount of the 10% formalin to the test-tube to rinse out all the remaining sample by pouring it through the gauze
4. Decant the filtrate into a clean 15ml test-tube, rinse the paper cup with a small amount of 10% formalin to collect any remaining filterate and pour into the test-tube.
5. Allow to stand in an upright position for ± 2 min.; remove excess formalin, leaving ≅ 7ml
6. Add 4ml of diethylether and cover the mouth of the test-tube with a piece of cling-wrap (rubber stoppers were used in the study)
7. Using a thumb, close the test-tube and shake vigorously for 2 minutes, releasing the gas that build-up when necessary during shaking (non-surgical glove should be worn when shaking; there is no need to release gas when rubber stoppers are used)
8. Remove the cling wrap (or rubber stoppers) and centrifuge at 2000 r.p.m. for 2-3 minutes
9. Once centrifuged, the tube's contents are layered: at the bottom of the tube is a pellet containing parasite eggs and cysts; above is a layer of formalin and above this is a plug of debris and on top is the ether with dissolved fat
10. Using a wooden applicator stick, loosen the plug of debris and turn the tube upside down, pouring off all the supernatant and leaving the deposit (sediment) behind. After proper decanting, a small amount of fluid is left on the side of the tube. Whilst still holding the test-tube upside-down, use another applicator stick rolled with tissue paper to wipe of the fluid
11. Add a drop of saline and using a Pasteur pipette and rubber teat draw all the deposit onto a microscope slide and add a drop of iodine for a wet mount

Appendix D – Key to identification of amoebic and flagellate cysts (WHO, 1991)



Appendix Ei. Relationship between multiple infections and age of child at Carrington Heights Junior Primary School

Survey			Polyparasitism						
			0	1 sp.	2 spp.	3 spp.	4 spp.	5 spp.	6 spp.
Pretreatment	Agegroups	<7 years	46	48	29	14	5		1
		8 -9 years	34	46	34	17	5	1	
		>10 years	2	4	7	3	1		
	Total		82	98	70	34	11	1	1
Posttreatment	Agegroups	<7 years	22	26	18	8	3		
		8 -9 years	14	33	17	12	5	1	
		>10 years		5	4	1	1		
	Total		36	64	39	21	9	1	
Re-infection	Agegroups	<7 years	19	33	15	8	1		
		8 -9 years	22	33	18	4	5	1	
		>10 years	2	4	3	2			
	Total		43	70	36	14	6	1	
2nd Posttreatment	Agegroups	<7 years	23	26	11	5	2		
		8 -9 years	24	28	13	8			
		>10 years	1	6	3		1		
	Total		48	60	27	13	3		

Appendix Eii. Relationship between multiple infections and sex of child at Carrington Heights Juniour Primary School

Survey			Polyparasitism						
			0	1 sp.	2 spp.	3 spp.	4 spp.	5 spp.	6 spp.
Pretreatment	Sex of child	male	38	40	31	14	4		
		female	44	58	39	20	7	1	1
	Total		82	98	70	34	11	1	1
Posttreatment	Sex of child	male	11	32	18	8	2		
		female	25	32	21	13	7	1	
	Total		36	64	39	21	9	1	
Re-infection	Sex of child	male	16	37	12	4	2	1	
		female	27	33	24	10	4		
	Total		43	70	36	14	6	1	
2nd Posttreatment	Sex of child	male	20	26	14	3	2		
		female	28	33	13	10	1		
	Total		48	59	27	13	3		

Appendix F. Schools sampled, altitude (m), and the prevalence (%) of the common intestinal protozoan species (N = number of children sampled)

School	N	Alt	*E.coli*	*E.histo*	*E.hart*	*E.nana*	*I.but*	*G.lamb*	*C.mesni*
Matshangule	110	0	30.9	0.0	3.6	4.5	1.8	0.9	5.5
Gobhela	155	30	36.1	0.0	1.9	5.2	4.6	3.9	2.6
Ithongasi	20	30	45.0	5.0	0.0	10.0	0.0	0.0	0.0
Zenzeleni	20	30	15.0	0.0	5.0	5.0	5.0	15.0	15.0
Bangibizo	20	90	35.0	5.0	0.0	5.0	10.0	10.0	0.0
Nontshuntsha	20	107	55.0		5.0	35.0	10.0	15.0	10.0
Kwandabezihle	114	120	32.5	5.3	7.0	25.4	14.4	7.9	0.9
Inyathi	111	120	36.9	0.0	3.6	11.7	0.0	5.5	5.4
Nomakhandzana	20	120	15.0	5.0	5.0	5.0	0.0	5.0	5.0
Kusakusa	20	120	20.0	5.0	5.0	10.0	0.0	15.0	0.0
Pumula	20	133	35.0	0.0	5.0	10.0	0.0	0.0	0.0
Sambane	282	150	56.0	6.7	21.6	47.9	23.8	8.5	4.6
Mhlabulungile	20	180	40.0	5.0	10.0	10.0	10.0	0.0	0.0
Ndunakazi .	254	210	44.1	7.1	2.8	15.7	5.9	3.1	2.4
Kwankukhu	20	210	35.0	10.0	0.0	10.0	10.0	10.0	0.0
Thulubheke	20	215	60.0	15.0	10.0	35.0	5.0	5.0	5.0
Gcilima	20	240	50.0	0.0	0.0	5.0	0.0	0.0	0.0
Tshenkombo	20	270	45.0	5.0	5.0	10.0	5.0	25.0	15.0
Dayeni	20	270	45.0	0.0	0.0	20.0	20.0	10.0	10.0
St. Joachim's	20	312	35.0	5.0	10.0	15.0	5.0	10.0	5.0
Sandanolwazi	20	360	45.0	5.0	0.0	0.0	10.0	15.0	0.0
Mthwana	113	390	45.1	0.0	1.7	11.5	0.9	3.5	4.4
Thandokwethu	20	390	25.0	0.0	15.0	45.0	15.0	25.0	5.0
Igugulamanyoni	20	390	40.0	5.0	5.0	15.0	0.0	10.0	15.0
Umthala	20	390	30.0	0.0	5.0	15.0	10.0	0.0	0.0
Umbalencane	157	420	58.6	7.6	22.3	53.5	19.7	19.1	3.2
Mfabeni	20	420	45.0	0.0	0.0	10.0	0.0	5.0	5.0
Esihosheni	103	470	41.7	1.8	3.9	6.8	2.9	11.7	2.9
Dududu	20	480	25.0	5.0	10.0	25.0	10.0	5.0	0.0
Mahlombe	20	480	65.0	0.0	15.0	5.0	15.0	10.0	10.0
Odeke	20	507	50.0	10.0	15.0	45.0	5.0	5.0	5.0
Munga	20	540	50.0	5.0	5.0	30.0	10.0	5.0	15.0
Mqangqala	20	570	50.0	5.0	30.0	55.0	30.0	10.0	5.0
Mansfield	20	600	65.0	15.0	5.0	25.0	15.0	5.0	0.0
Echibini	20	630	40.0	5.0	15.0	35.0	10.0	0.0	5.0
Esibila	115	660	51.3	0.0	0.9	4.3	0.0	1.7	3.5
Ngongolo	61	690	65.6	1.6	4.9	21.3	43.1	4.9	13.1
Jamengeni Zodah	20	690	45.0	10.0	10.0	25.0	20.0	5.0	0.0
St. Nevard's	20	693	30.0	5.0	0.0	5.0	5.0	5.0	0.0
Jangeni	112	720	51.8	0.0	0.9	4.5	0.0	4.5	1.8
Mlinganiswa	20	720	35.0	0.0	5.0	15.0	5.0	5.0	15.0
Ekhuza	90	750	64.4	7.8	26.7	60.0	19.7	18.9	3.3
Mjika	20	750	45.0	5.0	5.0	40.0	10.0	10.0	15.0
Marshmount	20	780	40.0	0.0	10.0	25.0	10.0	0.0	15.0
Makhowane	20	810	60.0	10.0	0.0	30.0	0.0	15.0	5.0

Appendix F. continued

School	N	Alt	*E.coli*	*E.histo*	*E.hart*	*E.nana*	*I.bue*	*G.lamb*	*C.mesni*
Sakhayedwa	57	840	68.4	1.8	33.3	57.9	17.5	7.0	3.5
Eluphepheni	20	840	40.0	10.0	15.0	10.0	5.0	0.0	10.0
Bhensela	20	840	50.0	0.0	0.0	30.0	5.0	0.0	0.0
Carisbrooke	20	870	20.0	0.0	5.0	15.0	10.0	0.0	0.0
Hluthankungu	20	900	15.0	0.0	0.0	5.0	0.0	0.0	0.0
Masheshisa	19	918	37.8	5.3	0.0	21.1	15.8	0.0	5.3
Vumabakushoyo	20	960	35.0	0.0	5.0	10.0	0.0	5.0	0.0
Ikhwezilamachi	20	1020	55.0	5.0	30.0	60.0	5.0	5.0	0.0
Esibomvine	20	1080	35.0	0.0	0.0	15.0	0.0	0.0	10.0
Bhekuzulu	20	1110	30.0	0.0	5.0	25.0	0.0	5.0	0.0
Mnyawaneni	20	1170	55.0	0.0	0.0	10.0	0.0	25.0	20.0
Ezinyonyana	116	1200	32.8	0.9	4.3	12.1	6.9	2.6	4.3
Nkongolwana	20	1230	45.0	5.0	5.0	25.0	0.0	10.0	10.0
Sikhona	33	1260	36.4	6.1	6.1	9.1	3.0	15.2	3.0
Holwane	114	1290	50.0	0.0	1.8	7.0	2.6	3.5	4.4
Lusiba	20	1290	45.0	0.0	0.0	5.0	5.0	0.0	10.0
Barnhill	81	1320	50.6	0.0	0.0	22.2	2.5	2.5	3.7
Xololo	20	1321	35.0	0.0	10.0	25.0	0.0	0.0	10.0
Oatlands	30	1350	73.3	3.3	6.7	70.1	26.9	15.4	9.6
Kennersley	35	1368	62.9	11.4	20.0	60.0	20.0	11.4	2.9
Khanyanaledi	20	1456	30.0	0.0	0.0	15.0	0.0	0.0	5.0
Matsemula	52	1474	65.4	7.7	23.1	63.5	26.9	15.4	9.6
Meadowbrooke	37	1478	81.1	2.7	8.1	64.9	24.3	13.5	5.4
Tigervlei	105	1500	65.7	2.9	17.1	63.8	28.6	24.8	12.4
Rusfontein	20	1501	60.0	0.0	10.0	35.0	15.0	5.0	5.0
Glen Edward	19	1643	47.4	0.0	5.3	15.8	21.1	0.0	5.3

Alt - Altitude
E. histo. - *E. histolytica*
E. hart. - *E. hartmanni*
I. but. - *I. butschlii*
G. lamb. - *G. lamblia*
C. mesni. - *C. mesnilli*