EPIDEMIOLOGICAL AND AETIOLOGICAL ASPECTS OF DIARRHOEAL DISEASE IN THE EASTERN CAPE.

THESIS

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ABSTRACT

Diarrhoeal disease is a major cause of mortality in children in developing countries. It also remains a serious problem among all age groups throughout the world.

Whereas studies to determine the epidemiological and aetiological factors of diarrhoeal disease have been reported for other parts of South Africa and the world, as yet no information is available for the Eastern Cape. Therefore this study was undertaken to determine the factors for this area. Enteropathogens were compared for the different ages in the various population groups and, where possible, seasonal and geographical differences were emphasised.

A total of 7 278 faecal samples were examined by six laboratories in the Eastern Cape during the period November 1988 to October 1990. Data was recorded noting the age, sex and population group of the patients. The towns selected were Port Elizabeth, Uitenhage, Cradock, Grahamstown and their surrounding areas.

The isolation rates for the pathogens studied in the various population groups were compared to those reported in similar studies in other countries.

The seasonal incidences of the various selected pathogens were compared with those reported from elsewhere in South Africa. It was thought that the higher temperature of summer may influence the finding in the White population group, while rainfall would play a greater role for the Coloured and Black populations.

The geographical distribution of the pathogens emphasised the difference in living conditions between the different population groups. For example a generally higher infestation rate of ***Helminths*** occurred in rural areas and in the groups living under poorer conditions.

The low isolation rates for certain bacteria and the large percentage of samples from which no pathogens were isolated indicate the need for further research. However, the finding should be valuable for determining Public Health priorities and in the management of outbreaks of diarrhoeal disease.

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CHAPTER 1

1. INTRODUCTION

Diarrhoeal disease is a serious problem in both developed and developing countries. It is a major cause of mortality in children living under poor conditions[1,2] in developing countries and sickness amongst all age groups world-wide[3]. The high morbidity rate also has serious financial consequences with the loss of many hours of work.

In South Africa the White, Asian and Coloured infant mortality rate is below the target of the WHO (World Health Organisation) of <50 per 1000 live births. The average infant mortality rate for Blacks has dropped since the 1960's, but is still higher than the target of the WHO. In 1986 the second highest number of deaths in South African infants was caused by "Intestinal infections" which represented 27,5% of the number where cause is known per annum[4]. In the years 1986 to 1989 close on 30 000 deaths were reported in children under the age of 5 years. One fifth of these were the result of preventable diarrhoeal infections, i.e. about 6 000 deaths. It must be pointed out that the registration of deaths among the Black population group is incomplete to the extent of roughly 50 percent and these figures do not include the independent National States[5].

In the United States an average of 500 children died annually from diarrhoeal disease from 1979 through to 1983. These diarrhoeal deaths were most common among Black children younger than one year of age, living in the South[6].

In Egypt diarrhoeal disease is considered to account for 60% or more of all child deaths throughout the year[7].

Over the years many papers have been published discussing various outbreaks of diarrhoea and aetiological studies have been done world-wide by many investigators who are concerned about this global problem[2,3,8,9,10,11,12,13].

The Eastern Cape has been subjected to severe drought conditions for the last few years (1984-1991). This has led to various problems and unemployment. Many of the people in the rural communities have moved into the towns and cities to seek employment. Squatter settlements started at different places, have added to the overcrowded living conditions already existing in certain areas. These poor housing conditions do not provide adequate health protection due to lack of ventilation, adequate sanitation and water supplies. Bucket latrines are over used allowing fly breeding, the emission of offensive odours and the spread of faecal matter. Often the nearest water supply is an excessive distance away from the household which may lead to unhygienic storing of domestic water. Further, the water may be collected from a polluted

source e.g. a nearby stream, giving rise to the spread of diarrhoeal disease. In the Port Elizabeth area alone more than 280 000 people are living on the city periphery in what is termed "informal housing settlements". The Walmer township in Port Elizabeth has 11,623 residents. The water supply in the area varies according to the different types of housing. In some areas 80 people may share a tap, but the figure can rise to 412. Some of the "permanent residents" have their own bucket latrines and 90% of the 730 squatter shacks now have their own bucket facilities. Those without, use their neighbours' facilities or "bush" sanitation. In other settlements the conditions again vary and some people are without any form of sanitation or water supplies. Problem areas are being tackled to upgrade the living standard, but the presence of the above conditions enhances the spread of diseases especially diarrhoea. (Personal Communication, P.E. Municipality)

The unhygienic preparation and storage of food, illegal slaughtering and sale of contaminated meat products increase the spread of enteropathogens. Within the Eastern Cape, as elsewhere in South Africa, both "First World" and "Third World" standards of health and hygiene occur within the same area.

The climate in the Eastern Cape varies between the different areas. The coastal area does not experience such severe temperatures changes.

A need exists for a study of the aetiology of diarrhoeal disease under these circumstance. Studies concerning the prevalence of enteropathogens in developed and developing countries have been undertaken but the actual situation in the Eastern Cape is largely unknown and a number of questions arise. Do the epidemilogical patterns recorded elsewhere also apply to the Eastern Cape? Do the different living conditions and resultant hygiene levels affect the findings of the laboratories to which their samples are sent? How are the different population groups and age groups affected by their different circumstances? As the occurrences of certain enteropathogens appear to be seasonal a comparison of the situation in the Eastern Cape, with patterns in the other areas that have been previously investigated could prove informative.

During the last 10 years or more several 'new' organisms have been isolated and recognized to cause diarrhoea and further research is ongoing[139,140]. With improved methods of isolation, it is becoming within the reach of the general laboratory to isolate many of these bacteria routinely. This study was undertaken to compare the aetiology of diarrhoeal disease at different ages in the various population groups. Where possible seasonal and geographical differences are emphasised.

Children in day care centres who may harbour many enteropathogens pose a specific problem within the context of the larger problem. A study was undertaken at

different day care centres in the Eastern Cape to consider the special aetiological factors involved at these centres.

The following enteropathogens were determined as the ones to be used in this study. *Salmonella species, Shigella species, Yersinia enterocolitica, Aeromonas hydrophila, Campylobacter species,* Enteropathogenic *Escherichia coli,* helminths, protozoa, *Cryptosporidium* and *Rotavirus.*

1.1. SALMONELLA SPECIES

Salmonellae cause a wide range of human enteric disease, from self-limited gastroenteritis with mild symptoms of short duration, to severe gastroenteritis with or without bacteremia, to typhoid fever, which is a severe, debilitating and a potentially life-threatening illness[14].

Salmonellosis is an important public health problem in many parts of the world[15]. Over the past five years *Salmonella enteritidis* infections in humans have increased world-wide. Investigations in individual countries suggest it is related to consumption of eggs and poultry which harbour the organism[16]. Salmonellosis is also of growing concern to the chocolate industry. Although the risk of acquiring salmonellosis from chocolate is comparatively low, several

outbreaks have been reported in which chocolate products were incriminated as the source of infection[17].

G. Kapperud et. al. 1990 described an outbreak of *Salmonella typhimurium* infection caused by contaminated chocolate produced by a Norwegian company which occurred in Norway and Finland in 1987. A total of 349 bacteriologically verified cases were recorded in Norway and 12 cases were recorded in Finland. Up to the time it was probably the highest number of patients involved in a chocolate-related outbreak of salmonellosis[17].

Since the 1960's the problem of multiple resistance to antimicrobial agents has been observed in gastroenteric strains of *Salmonella* isolated throughout the world[15].

In April 1990 M.C. Georges-Courbot et. al. reported a cluster of antibiotic-resistant *Salmonella enteritidis* infections in the Central African Republic. These strains were isolated from the blood of 12 hospitalized patients[18].

Seasonal patterns may play a role with the infection of *Salmonella sp.* in certain parts of the country. In Cape Town *Salmonella sp.* were isolated throughout the year, but appeared less commonly in summer[11].

In a study done in Johannesburg[19] *Salmonella sp.* occurred more frequently in the summer months.

1.2. SHIGELLA SPECIES

The genus *Shigella* is subdivided into 4 subgenera or subgroups according to their biochemical reactions: *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*. There are 10 serotypes of *Shigella dysenteriae*, 8 of *Shigella flexneri* and 15 of *Shigella boydii*. Man is both the reservoir and natural host of *Shigella*[20].

Shigella sp. cause classical bacillary dysentery characterized by severe cramping, abdominal pain and diarrhoea with blood and mucus. The organisms invade mucosal cells, causing death and sloughing of the cells into the bowel lumen. However, they seldom invade beyond the mucosa[14].

Shigella sp. has long been recognized as an important agent of enteric infection, especially in the developing world and travellers to these areas. Sporadic outbreaks of enteric disease in the United States have been reported. In August 1988, an estimated 3 175 people who attended an outdoor music festival became ill with shigellosis. Food-borne outbreaks of shigellosis are often encountered[21].

It is generally accepted that effective antimicrobial therapy for shigellosis, especially in the pediatric population, shortens the duration of illness. In children from developing countries treatment has a significant positive impact on growth and nutritional status of the affected children. Multiple resistance to the commonly used antibiotics for treatment of shigellosis, that is, ampicillin, trimethoprim-sulfamethoxazole and tetracycline has been reported from many countries and resistance to nalidixic acid has been similarly encountered[22]. The first report of trimethoprim-resistant shigellae in Ontaria, Canada, appeared in 1980[23].

Infections with *Shigella* are generally considered to be confined to the gastrointestinal tract. Invasion of the bloodstream has been thought to be a rare and usually self-limiting event. A few studies suggest that *Shigella* bacteremia may occur more frequently and may bear a high case fatality rate in the malnourished child. One study suggests that patients who were less than one year old, non-breast fed, malnourished and afebrile were most likely to die from *Shigella* bacteraemia [24]. *Shigella sp.* have also been encountered in patients with acquired immunodeficiency syndrome. A report described a seropositive patient with recurrent and relapsing symptomatic infection due to *Shigella flexneri* who subsequently developed acquired immunodeficiency syndrome[25].

Outbreaks of diarrhoea in day care centres due to *Shigella sp.* have been reported where the younger children have not yet learned adequate personal hygiene[26].

1.3. YERSINIA ENTEROCOLITICA

Yersinia enterocolitica is a facultatively anaerobic bacterium and is a member of the family Enterobacteriaceae as are *Salmonella, Escherichia coli* and *Shigella*. These bacteria all share the potential to cause diarrhoea in humans[27].

Yersinia enterocolitica is ubiquitous in the environment and found both in soil and in aquatic habitats, but only certain biovars and serovars are consistently shown to be pathogenic to man and animals. Yersinosis in humans is more common in children and affects mainly the terminal ileum and mesenteric lymph nodes, often resulting in diarrhoea, fever and abdominal pain[28].

The first description of *Yersinia enterocolitica* was rendered in the United States in 1939 by Schleifstein and Coleman who described five isolates[29]. By 1957 only nine new cases, of the then called *Bacterium enterocoliticum* by Schleifstein and Coleman, were collected and reported as part of the Annual report of the New York State Department of Health[29]. In Europe

additional case reports may have appeared that were most likely due to *Yersinia enterocolitica*, but as the species was as yet unclassified, the isolates were probably described as resembling *Yersinia pseudotuberculosis* or *Actinobacillus lignieresi*. They also discovered various animal hosts were important reservoirs[29]. In South Africa only the pig has regularly been shown to carry the same serotype and phage type as that found in man. A case has been recorded where a labourer on a pig farm developed generalized yersinosis following close contact with infected animals[30].

In 1964 Frederiksen[27] proposed the name *Yersinia enterocolitica* to describe the then unclassified species. In South Africa the first isolates of *Yersinia enterocolitica* from human cases were described in 1972 by Rabson and Koornhof[31]. This study described 15 cases. Ten patients presented with diarrhoea, four with generalised infections and one with mesenteric adenitis. In 1973 Finlayson described cases in the Western Cape[32]. Studies have been carried out in Belgium[33] and in Italy 1,4% of the 2 500 faecal samples collected from children with diarrhoea were found to be positive for *Yersinia enterocolitica*[34]. In the Eastern Cape a study was made during May 1982 to December 1984. *Yersinia enterocolitica* was isolated in 1% of the 1 634 faecal samples examined[35]. A number of outbreaks due to the consumption of food contaminated with *Yersinia enterocolitica* have been reported in

different countries[36]. A report where two episodes of *Yersinia* infection occurred in a single ward of a district general hospital is described by Greenwood and Hooper[37]. *Yersinia enterocolitica* biotype 1 serotype O.10K was isolated from 19 patients in the paediatric wards of a district general hospital over a period of 3 months. Fifteen cases were patients in the medical ward. Shortly afterwards, *Yersinia enterocolitica* biotype 1 serotype O.6,30 was isolated from a further 17 patients in this ward in 1 month. The same serotypes of *Yersinia enterocolitica* were isolated from the pasteurized milk supplied to the ward.

In certain places the infection rate of *Yersinia enterocolitica* may show a seasonal pattern. In Europe the peak incidence has been recorded in late autumn and winter, while in Johannesburg the majority of cases occurred in late summer and autumn with an early peak in spring[38].

1.4. CAMPYLOBACTER SPECIES

The genus *Campylobacter* consists of a well-defined group of bacteria. They are spirally curved rods, 0,2 to 0,5 /um wide and 0,5 to 5 /um long.
Campylobacter may be comma, "S" or gull wing in shape and may occur in short or occasionally long chains[14].

Campylobacter fetus (formerly known as *Vibrio fetus*) has long been recognized as an animal pathogen[39]. In 1947 the first human infection due to the then called *V. fetus* was recognised[40]. In the next 20 years about 100 cases were reported, mostly from blood cultures. In 1972 the first report of so-called "related vibrios" were isolated from the stools of patients with diarrhoea[41].

Due to the special growth requirements the subspecies of *Campylobacter* namely *C. fetus*, *C. intestinalis* and *C. jejuni* were routinely differentiated by 1973[42]. In 1974 Skirrow developed an improved and simplified method for the isolation of *Campylobacter jejuni*[43]. Following many studies carried out in developed and developing countries *Campylobacter jejuni* is now recognized as a diarrhoea causing pathogen[44]. Another *Campylobacter, C. coli* also causes diarrhoeal disease in humans and is often found in healthy pigs[14].

The source of human infections is usually food of animal origin. It is estimated that as many as 2 million cases of *Campylobacter* infections occur in the U.S.A. each year[14]. The disease appears to be especially prevalent among college students. During the first quarter of 1982 through to the summer quarter of 1985 a study was done on students from a university in the United Kingdom. *C. jejuni* was isolated from 24,1% of the stool specimens submitted. The infection was mainly from eating chicken and association with cats[45]. *C. jejuni* enteritis is a well-recognised consequence of raw milk consumption[46,47]. In 1988 the first milk-borne outbreak was reported in which the same two serotypes of *C. jejuni* were isolated both from the affected people and from the implicated dairy cows[48].

Raw milk was implicated in 14 (61%) of 23 *Campylobacter* enteritis outbreaks reported to the Centres for Disease Control between 1980 and 1982[46,47].

Campylobacter can also be isolated from water[15]. Large community outbreaks in Sweden and the U.S.A. were attributed to water, but the microbiological confirmation was lacking[49]. In 1983 an outbreak of *Campylobacter* enteritis in a boarding school was reported in which there was both epidemiological and microbiological evidence implicating the water supply as source of infections[50]. The organisms are excreted in the faeces of healthy domestic animals[14]. In a study

done in 1979 in Soweto, Johannesburg, *C. jejuni* was isolated from 12 out of 33 dogs tested. The dogs had no diarrhoea, which suggests that the dogs are possible carriers of *C. jejuni*[51]. Other studies elsewhere on chicken, turkey and cattle have shown that as many as 50-100% of a flock or herd of these animals excrete *C. jejuni*[14].

Campylobacter is more prevalent in developing countries than in developed countries[52,53,55,56,57]. Several researchers have shown the existence of large numbers of healthy carriers[54,55,56,57]. In a study conducted at the Baragwanath hospital, Johannesburg, C. fetus was recovered from 35% of the children with diarrhoea and from 16% of asymptomatic children. It was found that in the 0-8 month age group the carrier rate was much lower[56]. This was confirmed by other studies done in the Johannesburg area[55,57]. Carriers of enteric *Campylobacter sp.* are rare or non-existent in developed countries, where the prevalence is low. In a study in central Africa in 1978 *Campylobacter* was isolated in 11% of those with diarrhoea. The highest number was in children aged 12 - 24 months suggesting probably self-infection from contaminated soil[58].

1.5. AEROMONAS HYDROPHILA

The genera *Aeromonas* belongs to the family *Vibrionaceae*[59]. They are flagellated Gram-negative rods which give a positive oxidase reaction[60]. Three species have been recognized namely *Aeromonas caviae, Aeromonas hydrophila, Aeromonas sobria* and one non-motile species *Aeromonas salmonicida*. *A. salmonicida* is not considered pathogenic for humans[61]. *A. hydrophila* is readily recovered from a wide variety of aquatic and terrestrial environments[62]. Many aeromonads are pathogenic for cold-blooded creatures such as fish, frogs and salamanders[60] and all species have been isolated from water sources including tap water[63]. The organism was originally called *Bacillus hydrophila* but in 1923 was renamed *Proteus hydrophila*. After careful study the organism was finally placed in the genus *Aeromonas*[64].

Authors differed in their opinion as to whether it was a normal inhabitant of the gastrointestinal tract or not.

In 1961 *A. hydrophila* was implicated in cases of severe gastroenteritis in Colombia. It was found to be the predominant organism in the aerobic stool flora of patients with diarrhoea and was isolated in pure culture in a fatal case of enteritis in a newborn[65].

In 1963 Rosner described a case of a 10 year old White girl in the U.S.A. who complained of severe abdominal pain, fever and bloody stools. The organism isolated from her stool specimens proved to be ***A. hydrophila***[64]. In December 1979 a case study described a 35 year old Indian doctor who developed abdominal cramps and diarrhoea with blood and mucus while on an extensive tour in India, which included a visit to Calcutta. Seven days later he returned to the United Kingdom. ***A. hydrophila*** was isolated and was considered not significant. He was treated for the presumptive diagnosis of amoebic dysentery. Various laboratory tests revealed nothing and subsequent stool again yielded only a growth of ***A. hydrophila***. He was treated successfully[66]. The increasing frequency of reports from throughout the world now confirms that ***A. hydrophila*** can be pathogenic for humans, producing focal or systemic infections of varying severity[67].

It was also considered that serious infections in humans usually occurred when host defences were compromised, but an acute diarrhoeal syndrome involving enterotoxigenic strains occurred in previously healthy individuals. The enterotoxigenic strains were first reported from India in 1975[68]. Strains isolated from children and adults with diarrhoea were tested for pathogenicity in adult rabbits. Since then, enterotoxin-producing strains have been isolated world-wide[69].

The number of isolations of *Aeromonas sp.* from patients may be related to climatic conditions. In Australia *Aeromonas sp.* were most often isolated towards the end of summer when the maximum temperature often exceeds 35°C[69]. A study in France has shown that *Aeromonas sp.* were found throughout the year but predominantly in late summer[70].

1.6. ENTEROPATHOGENIC ESCHERICHIA COLI

Escherichia coli has long been known as a harmless bacteria, living inside the intestine and probably playing a useful role in providing a regular supply of vitamins and growth factors for the body[71].

The role of *E. coli* as a diarrhoea-producing organism became obvious in the late 1940's with the identification of enteropathogenic *E. coli* associated with nursery infections in children[71]. Since then other pathogenic strains of *E. coli* have been identified namely enterotoxigenic *E. coli*, enteroinvasive *E. coli* and haemorrhagic colitis *E. coli* (serotype 0157:H7)[72]. The isolation of the *E. coli* other than the enteropathogenic *E. coli* requires special techniques which are not available in most clinical microbiology laboratories so that the diagnostic testing is usually reserved for research studies. The enterotoxigenic *E. coli* produce LT and/or ST toxins and also affect adults. The enteroinvasive *E. coli*

produce a shigella-like toxin and affect older children and adults whereas another pathogenic *E. coli*, enteroadherent *E. coli* is associated with travellers' diarrhoea. In laboratory studies enteroadherent *E. coli* is found to adhere to Hep-2 cells. Haemorrhagic colitis *E. coli* is associated with epidemic haemorrhagic colitis in adults and older children. Once enteropathogenic *E. coli* could be typed by Kauffman's serotyping scheme workers around the world confirmed the association of *E. coli* serogroups 055, 0111 and other serogroups with infantile gastroenteritis[73].

Several controlled studies in South Africa have shown enteropathogenic *E. coli* to be the pathogen most frequently identified in the stools of infants[10,19,73]. Koornhof et. al. (1986) also reported high isolation rates during the winter of 1981. The study was conducted at three hospitals in Johannesburg and the frequency of isolation in the patient and control groups were similar[55]. The isolation rates reported in the developed countries have been low. This might indicate that enteropathogenic *E. coli* are more common in the developing countries and high percentages have been confirmed[13,75].

In Johannesburg[19] and Cape Town (D. Coltman, Personal Communication) enteropathogenic *E. coli* was isolated more frequently in the summer months. In other countries no discernable pattern was detected[3].

1.7. ROTAVIRUS

Rotaviruses are double-stranded RNA viruses[76].
They have a wheel-like appearance and are approximately 70 nm in diameter when viewed by electron microscopy in preparations of stool suspensions[77]. Complete virus particles contain a double-shelled outer capsid. In a natural infection, both single-shelled and double-shelled forms of particles are observed in faeces. The surface of the complete virus particle is composed of 32 capsomeres that radiate from a central core. The name of the virus is derived from the Latin "rota", meaning wheel[78].

Before the discovery of ***Rotaviruses*** there was little evidence that viruses were an important cause of diarrhoeal disease. Many large surveys in different countries showed that as many as two-thirds of all diarrhoeal episodes were of unknown aetiology[79].

Australian scientists discovered human ***Rotavirus*** in 1973 when they visualized the agents by electron microscopy in duodenal-biopsy tissue obtained from acutely ill infants and young children with diarrhoea[80].

Early reports noted that the "new" human ***Rotavirus*** was similar in morphology to gastroenteritis viruses which had been known to veterinary scientists[81].

Since then the human *Rotavirus* was identified as possibly the most important aetiological agent in acute non-bacterial gastroenteritis among infants and young children world wide[82,83,84,85,86,87].

After the discovery of the virus similarly sized agents were observed and reported from various parts of the world. They were referred to as orbivirus, duovirus, rotavirus, infantile gastroenteritis virus and human reovirus-like agents, all terms which were used before finally being called *Rotavirus*[78].

At a children's hospital in the U.S.A., a human reovirus-like agent, was detected in 47% of the 152 infants and children hospitalized with acute gastroenteritis[85] and in Australia, the newly described virus, was detected in more than 50% of the patients[89].

Several investigators have proved that *Rotavirus* is also a cause of diarrhoea in adults[76,90,91,92,93]. *Rotavirus* is associated with travellers' diarrhoea[93] and in 1979 another study was done on students from the U.S.A. attending a summer school at an urban Mexican university. *Rotavirus* was found in 24% of the students with diarrhoea[94].

Rotaviruses have also been isolated from soil[95] and water[96]. A water-borne outbreak of *Rotavirus* in China in 1982/1983 affected more than 12 000 adults in two

coal mining districts[97]. The investigators designated the virus Adult Diarrhoea *Rotavirus* (ADRV) to distinguish it from the *Rotavirus* causing infantile diarrhoea. Antigenically it lacked the group antigen shared by known *Rotaviruses* and in 1986 it was typed as Group B *Rotavirus* whereas Group A *Rotavirus* are those known to cause infantile diarrhoea[98].

Person-to-person spread of *Rotavirus* has been described in an adult cardiology ward where patients and members of the staff were affected[91].

Several reports have described a particular seasonal distribution of *Rotavirus* incidence with a peak during winter months[99,100,101,102]. In South African studies conducted in and around Johannesburg, the incidence in the White population has a peak incidence in the colder months of the year[104] and the Black population have a low threshold constant throughout the year[105].

1.8. PROTOZOA

The parasites of humans in the kingdom protozoa are now classified under 3 phyla:

Sarcomastigophora (containing the flagellates and amoebas)
Apicocomplexa (containing the sporozoans)
Ciliophora (containing the ciliates)

Within these large assemblages are found the important parasites of humans causing diarrhoea[106].

Samples were screened for the following parasites:-

1.8.1.***Mastigophora*** - the flagellates, which include the intestinal flagellate ***Giardia lamblia***. ***Giardia lamblia*** is the only common protozoan found in the duodenum and jejunum of humans. ***Giardia lamblia*** occurs worldwide. Humans are infected by ingestion of faecally contaminated water or food containing ***Giardia*** cysts or by direct faecal contamination, as may occur in day care centres[107,108].

1.8.2.***Sarcodina***. These are typically amoeboid and are represented in humans by species of ***Entamoeba histolytica***. ***Entamoeba histolytica*** is a common

parasite in the large intestine of humans, certain other primates and some other animals[103]. Many cases are asymptomatic except in humans or among animals living under stress. Cysts are usually ingested through contaminated water. In the tropics contaminated vegetables and food are also important sources of cysts. Flies have been implicated for spreading cysts in areas where faecal pollution exists. Asymptomatic carriers are the main source of contamination[109]. In Nigeria ***Entamoeba histolytica*** were detected in 23% of the cases. In a survey in the Central African Republic ***Entamoeba histolytica*** were found in 20,3% of the samples[109,110]. In other countries like Egypt findings were very low namely 1,4%[111].

1.8.3.***Sporozoa.*** ***Isospora belli, Sarcocystis species*** and ***Cryptosporidium***, although not exactly typical have been included in ***Sporozoa.*** Most of the intestinal protozoa, except ***Sarcocystis***, are transmitted through faecally contaminated food. ***Sarcocystic species*** are required by the ingestion of the infective stages in raw or poorly cooked beef or pork. ***Sarcocystic*** infections occurs in a variety of hosts, rarely including humans[112].

Isosporiasis caused by ***Isospora belli*** can produce severe intestinal disease. Death from overwhelming infections in immunocompromised patients have been reported [112]. ***Cryptosporidium*** has been known as a cases of

diarrhoea in animals[113]. It was in 1976 that *Cryptosporidium* was first associated with causes of human diarrhoea[114,115]. These first cases were associated with animal contact. In the early 1980's more cases were reported in patients with acquired immunodeficiency syndrome[114]. The increased awareness of the organism as a pathogen and simplified methods of stool diagnosis have led to further studies in different countries. These studies have also shown that *Cryptosporidium* species are a frequent cause of diarrhoea in immunocompetent patients[114,116,117].

1.8.4. *Ciliophora*. These are complex protozoa bearing cilia distributed in rows or paths, with 2 kinds of nuclei in each individual. *Balantidium coli*, a giant intestinal ciliate of humans and pigs, is the only human parasite representative of this group. *B. coli* is found throughout the world, particularly in the tropics, but is a rare infection[106].

1.9. HELMINTHS

The parasitic worms, or helminths, of human beings belong to 2 phyla:

1.9.1. *Platyhelminthes*. All medically important species belong to the classes *Cestoda* and *Trematoda*. *Trematoda* are not included in this study.

The *Cestoda* or tapeworm are usually found in the intestine, whereas larvae develop in the tissues of various intermediate hosts, either vertebrate or invertebrate. The exception is, *Hymenolepis nana*, where the eggs can short-circuit the usual development phase in an insectand infect humans directly from eggs passed in faeces of other humans[106].

Three groups of tapeworms infect humans:

1.9.1.1.The *Taenia* group but including *Echinococcus*

1.9.1.2.The *Hymenolepis* group

1.9.1.3.The *Diphyllobothrium latum* (not yet recorded in Southern Africa or the Republic of South Africa).

1.9.2. ***Nemathelminthes***. They include many parasitic species that infect humans. Members of the phylum ***Nemathelminthes***, class ***Nematoda***, are a richly varied and highly successful group, consisting of enormous numbers of species which occupy essentially every habitat in which multicellular organisms can survive. They infect an enormous variety of hosts. More than 1 billion persons are hosts of *Ascaris lumbricoides*. Infection patterns vary widely. Human intestinal nematodes infect via food-borne, water-borne and soil-borne

routes. *Ascaris* and *Trichuris trichiura* infect by eggs that are strongly resistant to desiccation and other adverse environmental factors[106].

1.10. RESEARCH OBJECTIVES

This study was undertaken to try to clarify the situation in respect of diarrhoeal diseases in the Eastern Cape. Aetiological aspects of the occurrence of the selected organisms were considered. Where possible seasonal and geographical differences are emphasised.

The investigator will deal with the following questions.

1.10.1.What are the aetiological factors leading to diarrhoea?

1.10.2.How do these factors compare in the different population groups? Are certain enteropathogens more prevalent in one population group than another?

1.10.3.What differences in the occurrences of the various enteropathogens are found in the same age groups of the different population groups and what differences are there between the different age groups of the same population group?

1.10.4.What role does climate play in the seasonal incidence of the selected pathogens in the Eastern Cape?

1.10.5.Are certain enteropathogens more prevalent in the rural areas than in the urban areas?

1.10.6.Does the need exist to screen for other pathogens namely *Rotavirus*?

1.10.7.What are the carrier rates of the various enteropathogens in infant day care centres in the area?

1.10.8.How do these findings compare to studies undertaken in other parts of South Africa and other countries in the world.

CHAPTER 2

2. MATERIALS AND METHODS

In the 2 year study period, 1 November 1988 to 30 October 1990, reports on a total of 7 278 faecal samples examined in six different laboratories in the Eastern Cape, were received for analyses. Routine investigations for *Salmonella species* and *Shigella species* on foodhandlers or contacts of *Salmonella typhi* were excluded. These patients were not considered as having a problem of diarrhoeal disease and were therefore left out. Three of the laboratories are situated in Port Elizabeth and one each in Uitenhage, Grahamstown and Cradock.

The laboratories situated in Port Elizabeth process specimens received from Port Elizabeth and surrounding areas. The one is a private laboratory, the other two are laboratories of the The South African Institute for Medical Research (S.A.I.M.R). The one S.A.I.M.R. laboratory is situated in the Livingstone Hospital and mainly serves the Black and Coloured population admitted to Livingstone and Dora Nginza Hospitals.

The Livingstone Hospital provides beds for about 1 200 patients and the Dora Nginza hospital mainly provides an outpatient service. A total of 179 810 patients was

treated at Dora Nginza Hospital during the period November 1988 to November 1990 and 228 851 patients were treated during the same period during 1989 to 1990. The number of patients with gastrointestinal problems seen during November 1988 to November 1989 was 692. A total of 755 were seen during November 1989 to November 1990.

The provincial hospital is served by a second S.A.I.M.R. laboratory and patients are mainly drawn from the White population. The private laboratory receives specimens from patients of general practitioners and patients hospitalized in the provincial hospital or in one of the three private hospitals. The Grahamstown, Uitenhage and Cradock laboratories render services to the local hospitals and also surrounding areas.

Faecal samples received in the laboratories are routinely tested for the presence of ***Salmonella sp., Shigella sp., Yersinia enterocolitica, Aeromonas hydrophila*** and ***Campylobacter sp.*** using standard bacteriological techniques. Additional testing for enteropathogenic ***E. coli*** was done when the patient was under two years of age.

The following media were used for the isolation of the bacteria:

Desoxycholate citrate agar(Des) (Merck)
Salmonella Shigella agar(SS) (Merck)
Selenite F enrichment broth (BDH) for isolation of

Salmonella and Shigella.

The Des and SS plates were incubated at 36°C for 18 - 24 hours. The Selenite F enrichment broth was plated out onto the above mentioned plates after 18 hours incubation. All non-lactose fermenting colonies were tested with the API z (API Systems, BioMerieux) and then subjected to the API 10S identification system where indicated. Isolates of ***Salmonella*** and ***Shigella species*** were serologically confirmed with antisera (Wellcome). The serotyping is confirmed by the S.A.I.M.R. laboratory in Port Elizabeth.

Cellobiose-arginine-lysine agar plates(Oxoid) were used for the isolation of ***Yersinia enterocolitica***. Plates were incubated at 30°c for 48 hours. Colonies resembling ***Yersinia enterocolitica*** were subjected to the API 20E identification system. Where possible the cultures were confirmed serologically.

Campylobacter media (Merck), were used for the isolation of ***Campylobacter species***. The plates were incubated at 42°C for 48 hours in an atmosphere containing 5% oxygen. This was obtained by incubating the plates in an anaerobic jar (BBL Gaspak Anaerobic Systems) without the catalyst in the lid and creating the correct atmosphere. MacConkey agar plates (Merck) were used for the isolation of enteropathogenic ***E. coli***. Six lactose-fermenting colonies suggestive of ***E. coli*** were inoculated into

six peptone waters and DST media(Mast). After incubation overnight at 36°C the colonies producing a positive indol test were tested against the antisera for enteropathogenic *E. coli*. (Wellcome). MacConkey, Des and SS medium were used for the isolation of *Aeromonas hydrophila*. As these plates were not selective media for *Aeromonas hydrophila* optimal isolation could not be expected.

When requested, stool samples were examined microscopically for the presence of helminths and protozoa. Screening for *Cryptosporidium* oocysts was performed at most laboratories on stools from children under the age of two years.

A concentration technique, using formalin and ether to separate the faecal matter from the ova and cysts,[118] was used for the detection of the following parasites:

Ova of *Ascaris lumbricoides, Trichuris trichiura, Hymenolepis nana* and *Taenia species*. Cysts of *Giardia lamblia, Entamoeba histolytica* and *Entamoeba coli*.

A modified Ziehl Neelsen staining technique was used for the detection of *Cryptosporidium* oocysts[114].

Testing for *Rotavirus* is not being done routinely, but the Livingstone laboratory were requested to submit

every second stool sample received from children under two years of age for testing for *Rotavirus*. The samples were submitted to the investigator from February 1989 to April 1990 for the purpose of this survey. The stool samples received were stored at -40°C and an Elisa test (IDL) was performed monthly on the batched stool samples.

Additional studies at the day care centres were made during May 1989 and February 1991. Stool samples were collected from 52 Black children, 65 Coloured and 47 White children during the first survey and from 49 Black, 55 Coloured and 36 White children during the second survey. This study was undertaken to determine the carrier rate of enteropathogens in day care centres, as many of the children act as reservoirs for some of the enteropathogens. All samples were examined microscopically for helminths, protozoa and *Cryptosporidium* by the same methods as mentioned above. Bacterial cultures were examined by standard bacteriological techniques on all the specimens. The information on all the reports of the stool samples examined were processed by means of a Xpert computor (IBM compatible) using the Lotus 123 program.

CHAPTER 3

3. RESULTS

A total of all the samples results submitted from the various laboratories amounted to 7 278. These samples consisted of 7 144 faecal samples and 134 rectal swabs. The different population groups were as follows: White 1 775, Asian 18, Coloured 1 294, Blacks 4 189 and 2 unknown. The number of males tested was 3 841, females 3 376 and 61 were unknown.

The number of samples examined and the enteropathogens found are shown in table I and figure 1.

No pathogens were detected in 5 446 of the 7 278 samples examined.

Multiple infections occurred in 258/7 278 (3,5%) of the cases representing an incidence of 233/258 (90,3%) double, 24/258 (9,3%) triple and 1/258 (0,4%) quadruple infections. Considering the double infections, in 70 of the cases, double infestations occurred with parasites only, 51 double infections were with bacteria and 97 cases had a combination of parasitic and bacterial infections. In 3 cases *Rotavirus* was detected in combination with a parasite and in 12 cases *Rotavirus* was found together with a bacterial infection.

TABLE I - ENTEROPATHOGENS DETECTED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990

ALL POPULATION AND AGE GROUPS			
PATHOGENS	NO TESTED	POS	% POS
SALMONELLA SPECIES	7227	331	4.58
SHIGELLA SPECIES	7227	617	8.54
Y. ENTEROCOLITICA	7189	8	0.11
CAMPYLOBACTER SPECIES	7189	23	0.32
A. HYDROPHILA	7190	6	0.08
ESCHERICHIA COLI	3930	356	9.06
ROTAVIRUS	803	106	13.20
CRYPTOSPORIDIUM	2991	229	7.66
PROTOZOA	7085	109	1.54
HELMINTHS	7085	366	5.17

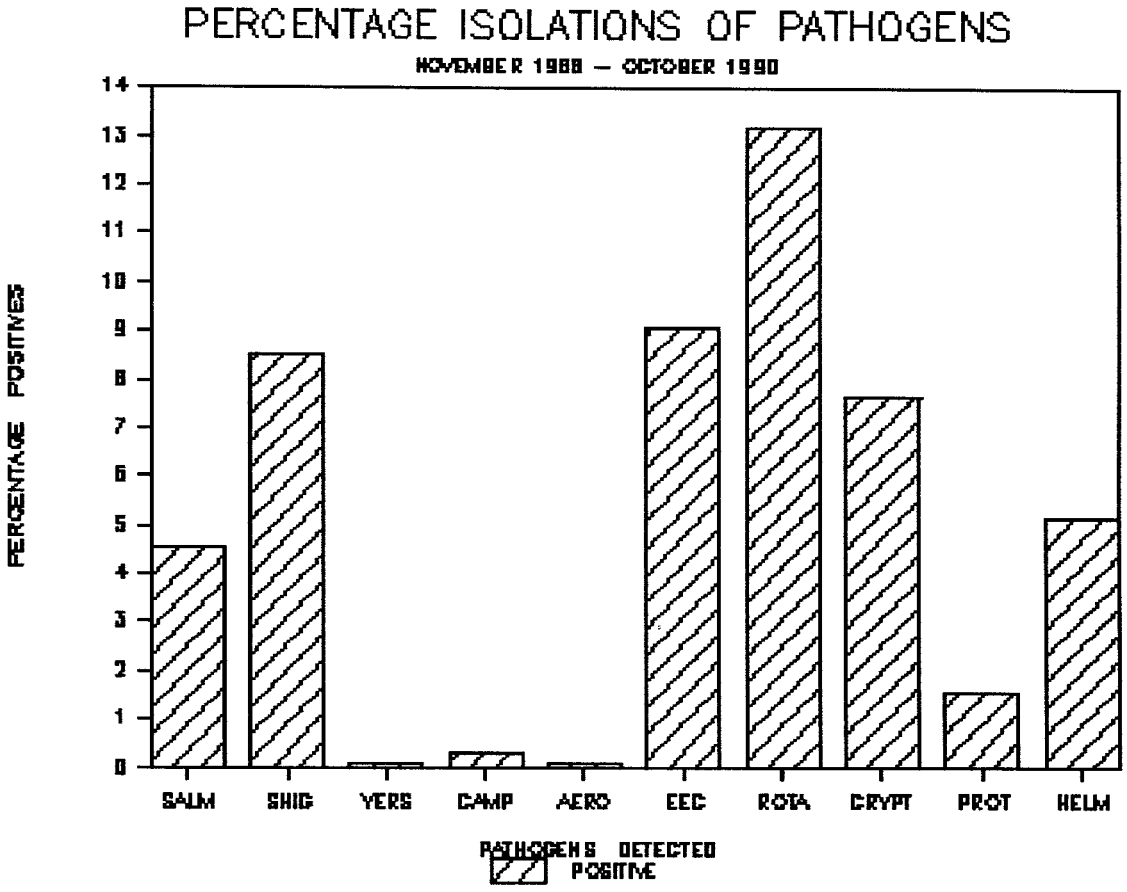

Figure 1 - Enteropathogens detected during the period 1 November 1988 to 30 October 1990 from all populations and age groups.

Two cases had triple infestations with parasites; one had a triple infection with bacteria, 13 cases had a combination of parasites and bacteria, and 8 cases were found with *Rotavirus*, parasites and bacteria. The quadruple infection was caused by parasites.

Of the 70 double parasitic infestations 55 cases had infestations with more than one helminth, 3 of these were found in the White population group, 37 in the Coloured population group and 15 in the Black population group.

Infestations with helminths and protozoa were found in 4 cases, one in each of the White and Coloured population group and two from the Black population group. Five cases from the Black population group and one of the Coloured population group had infestations with helminths and *Cryptosporidium*. Five infestations with a protozoa and *Cryptosporidium* were found in the Black population group. The 2 triple infections with parasites appeared in the Coloured population group.

3.1. SALMONELLA SPECIES

Salmonella species were isolated from 331/7 227 (4,58%) faecal samples examined. The isolation rate in the different population groups was as follows: White 23/1 766 (1,3%), Asians 0/18, Coloured 41/1 282 (3,20%) and Blacks 267/4 159 (6,4%). The two faecal samples from unknown race groups were negative.

The highest percentage of *Salmonella sp.* isolated from the White population group occurred in the >2 to 12 year old age group 6/354 (1,7%) and the Black population group of the same age group also had the highest incidence. 31/337 (9,2%). In the Coloured population the highest percentage of isolates occurred in the age group <=2 years (table II and figure 2).

The higher frequency of diarrhoea occurred during August, September and December in 1989. In 1990, however, the higher percentage isolates were found in July and the lowest in February, May and June (figure 3).

The seasonal pattern for the White population group showed a marked increase during January 1989 and no isolates during June and July 1989. During 1990, however, there was an increase in the month of June (figure 4).

In the Coloured population a peak was noted in the summer months with February 1989 and October 1990 having the

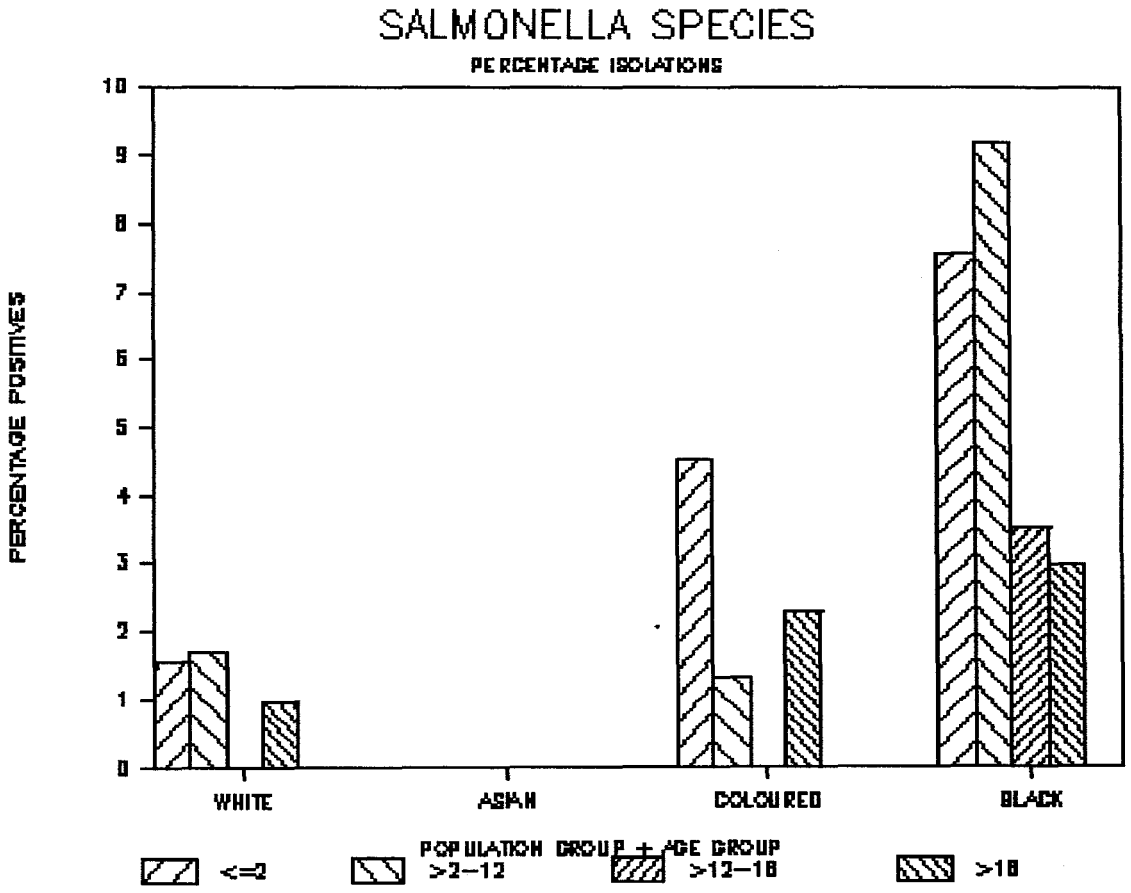

Figure 2 - Salmonella species isolated during the period 1 November 1988 to 30 October 1990 from all population and age groups.

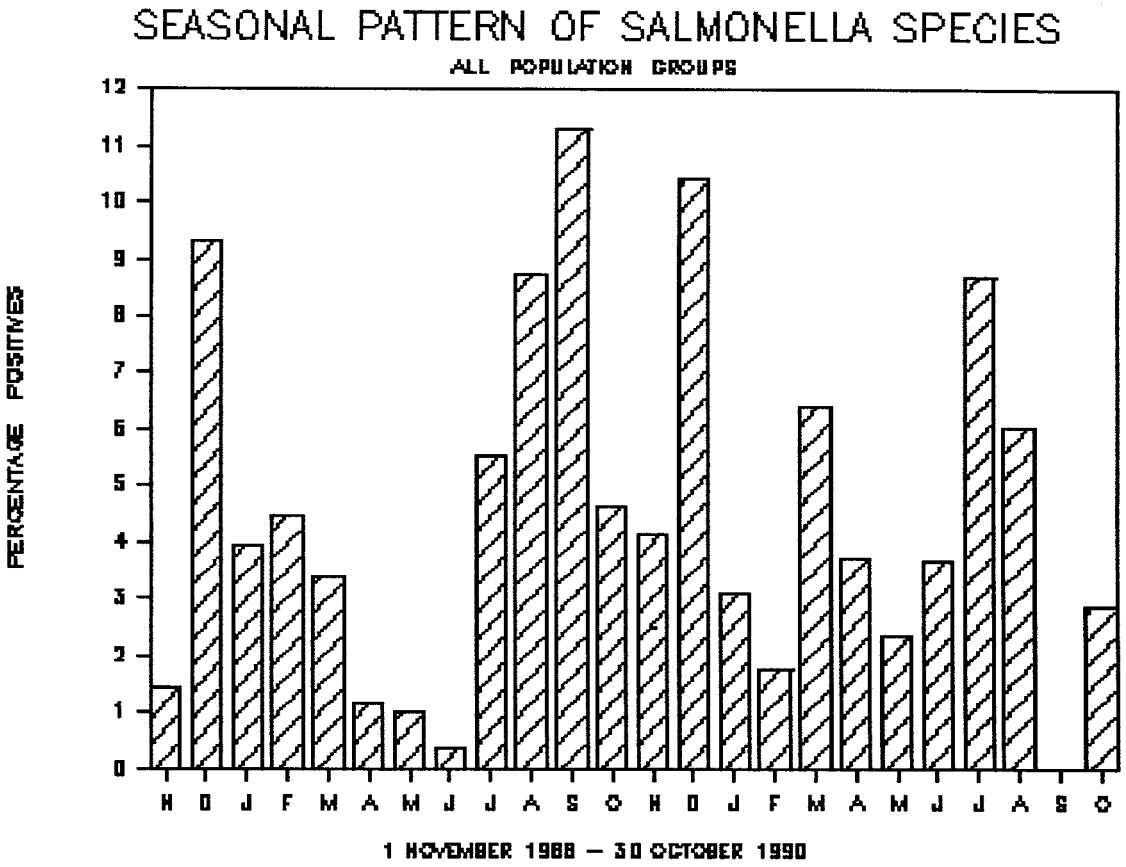

Figure 3 - Seasonal pattern of Salmonella species in the Eastern Cape for the period 1 November 1988 to 30 October 1991.(See appendix 1)

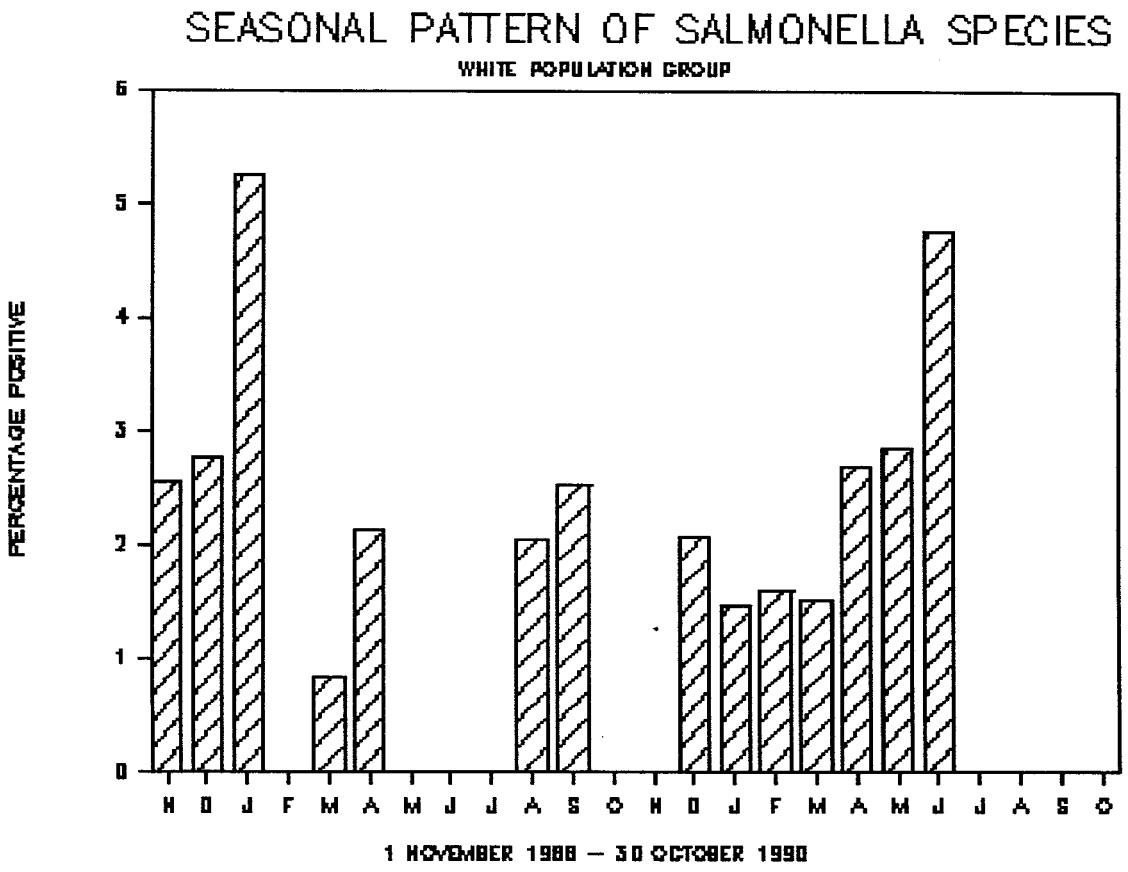

Figure 4 - Seasonal pattern of Salmonella species in the White population group for the period 1 November 1988 to 30 October 1991.(See appendix 2)

TABLE II - SALMONELLA SPECIES ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990 FROM ALL POPULATION AND AGE GROUPS.

	POPULATION GROUPS TESTED															
	WHITE				ASIAN				COLOURED				BLACK			
AGE	NUMBER TESTED	POS	NEG	% POS	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS
<=2	657	10	647	1.52	4	0	4	0	618	28	590	4.53	2656	201	2455	7.57
>2-12	354	6	348	1.69	3	0	3	0	153	2	151	1.31	337	31	306	9.20
>12-18	30	0	30	0.00	1	0	1	0	26	0	26	0.00	57	2	55	3.51
>18	725	7	718	0.97	10	0	10	0	485	11	474	2.27	1109	33	1076	2.98
TOTAL	1766	23	1743	1.30	18	0	18	0	1282	41	1241	3.20	4159	267	3892	6.42

higher percentage of *Salmonella sp.* isolated (figure 5).

The seasonal pattern in the Black population group was similar to the overall pattern with September and December 1989 having the higher percentage of positives and July and August in 1990 (figure 6).

The *Salmonella sp.* were serotyped where possible. Complete typing of *Salmonella sp.* was not carried out at the smaller laboratories due to the high cost in carrying the full range of antisera when the number of isolates are limited. Of the 331 *Salmonella sp.* isolated 210 were typed (table III). *Salmonella typhimurium* was the predominant isolate in all the population groups.

TABLE III - SEROTYPING RESULTS OF 210 SALMONELLA SPECIES ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

TYPING RESULTS	
SALMONELLA SPECIES	NUMBER (n=210)
S. TYPHIMURIUM	131
S. TYPHI	1
S. ENTERITIDIS	19
S. ENTERITIDIS gr var overchurch	2
gr var SII heilbron	1
gr var SI	2
gr var III arizona	1
gr var SII mobeni	1
gr var infantis	1
var norwich	1
var SII bloemfontein	1
var isanga	1
var othmarshen	1
S. DUBLIN	4
S. STANLEY	1
S. STERRENBOS	1
S. JOHANNESBURG	8
S. BLOCKLEY	20
S. AGAMA	2
S. HADAR	1
S. KISII	3
S. IRUMU	1
S. MEUNCHEN	2
S. SOFIA	1
S. BOVIS-MORBIFICANS	2
S. ROSTOCK	1

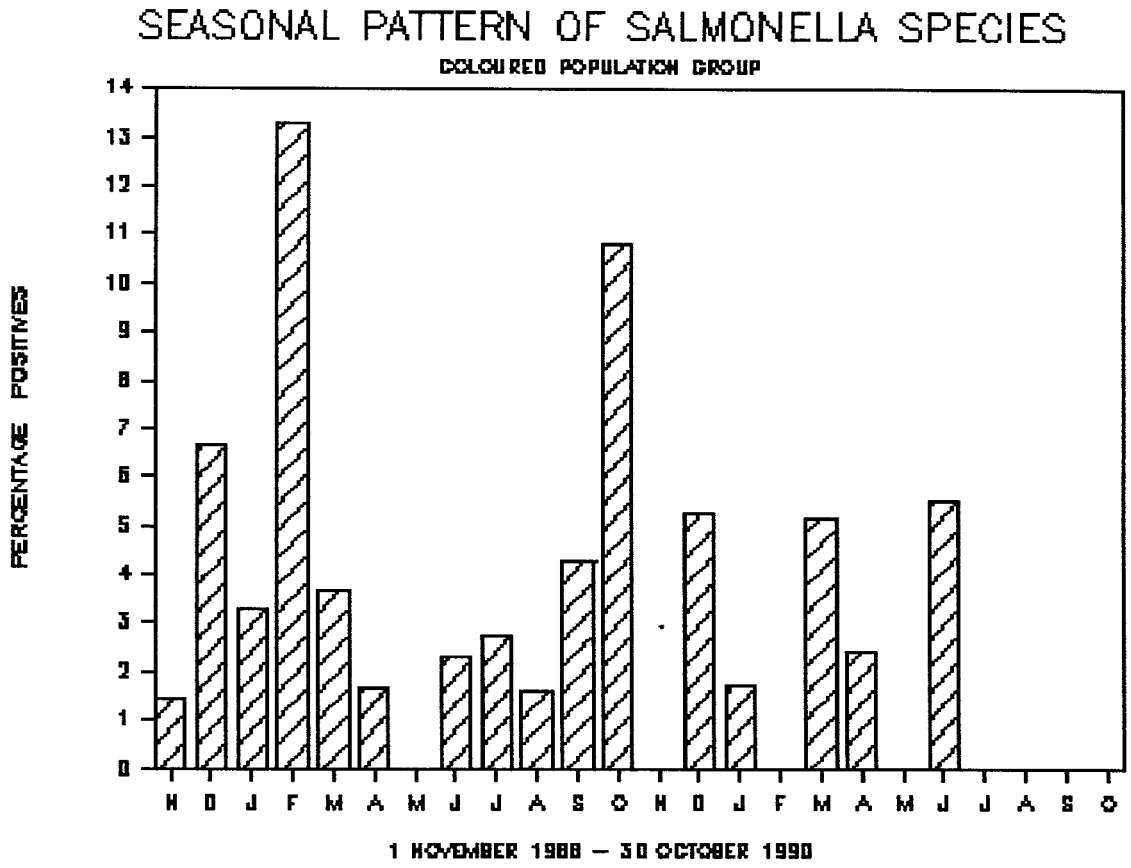

Figure 5 - Seasonal pattern of Salmonella species in the Coloured population group for the period 1 November 1988 to 30 October 1991.(See appendix 3)

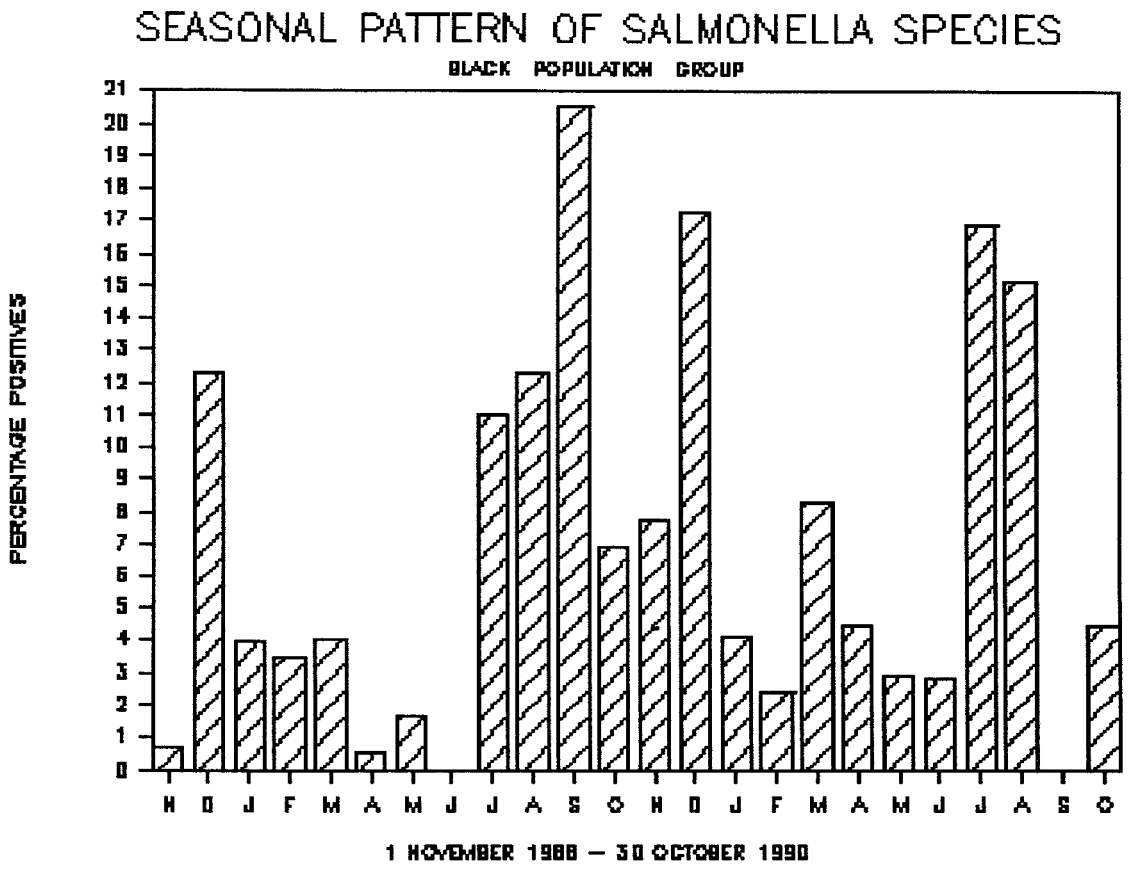

Figure 6 - Seasonal pattern of Salmonella species in the Black population group during the period 1 November 1988 to 30 October 1990.(See appendix 4)

3.2. SHIGELLA SPECIES

Shigella sp. were isolated in 617/7 227 (8,5%) of faecal samples examined. The isolation rate of the different population groups was as follows: White 47/1 766 (2,7%), Asian 3/18 (16,6%), Coloured 123/1 282 (9,6%) and Black 444/4 159 (10,6%). The two faecal samples from unknown population groups were negative.

In the White population group the higher percentage positives occurred in the >2 -12 year old group 11/354 (3,1%) and the over 18 year olds 22/725 (3,0%). The three *Shigella sp.* isolated from Asians were in the over 18 year old group. In the Coloured population group the higher percentage isolates were detected in the >12 - 18 year old group 7/26 (26,9%) and in the Black population the over 18 year old group had the higher percentage positives 219/1 109 (19,7%) (table IV and figure 7).

The highest percentage of *Shigella sp.* was isolated during November 1988 to May 1989 and November 1989 to April 1990 (figure 8). June 1990 is the month with the highest number of *Shigella sp.* isolated.

A seasonal pattern for *Shigella sp.* could be detected in the White population group as the majority of isolates appeared in the summer months (figure 9).

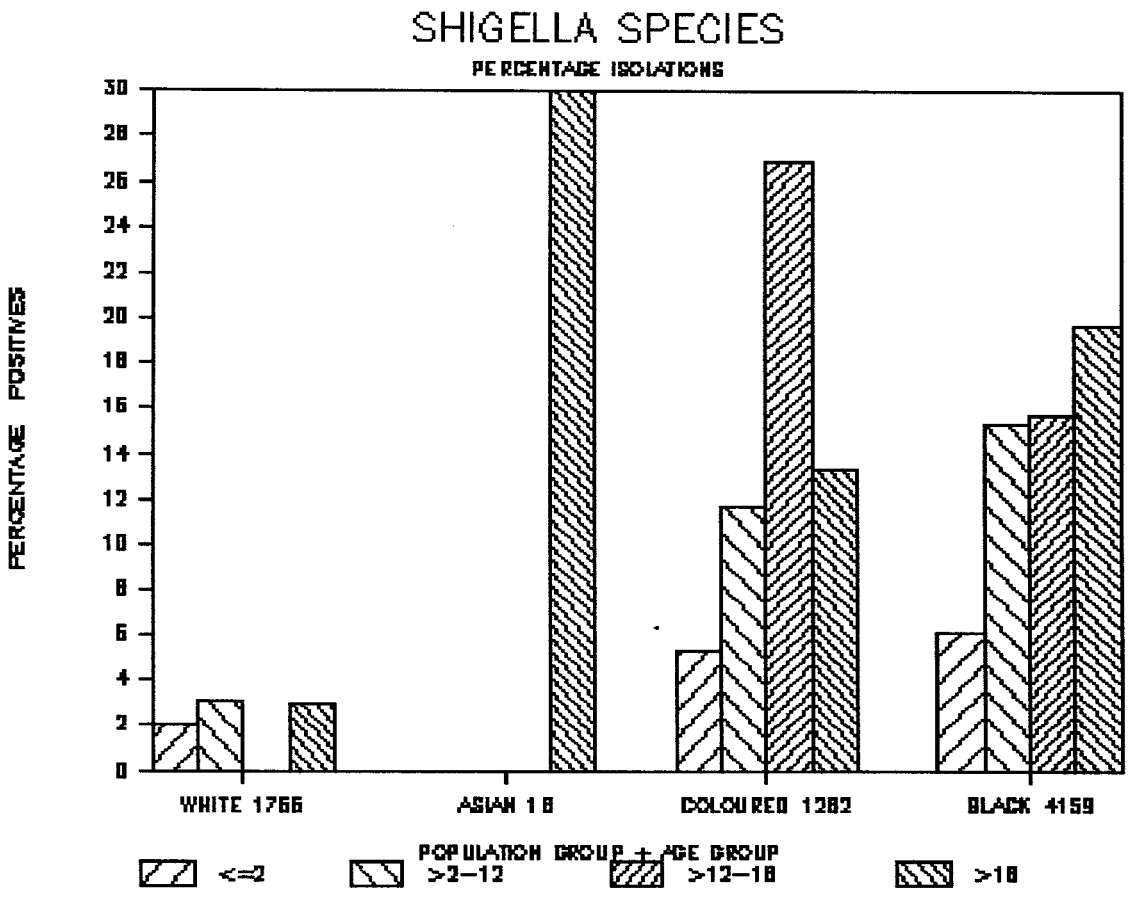

Figure 7 - Shigella species isolated during the period 1 November 1988 to 30 October 1990 from all population and age groups.

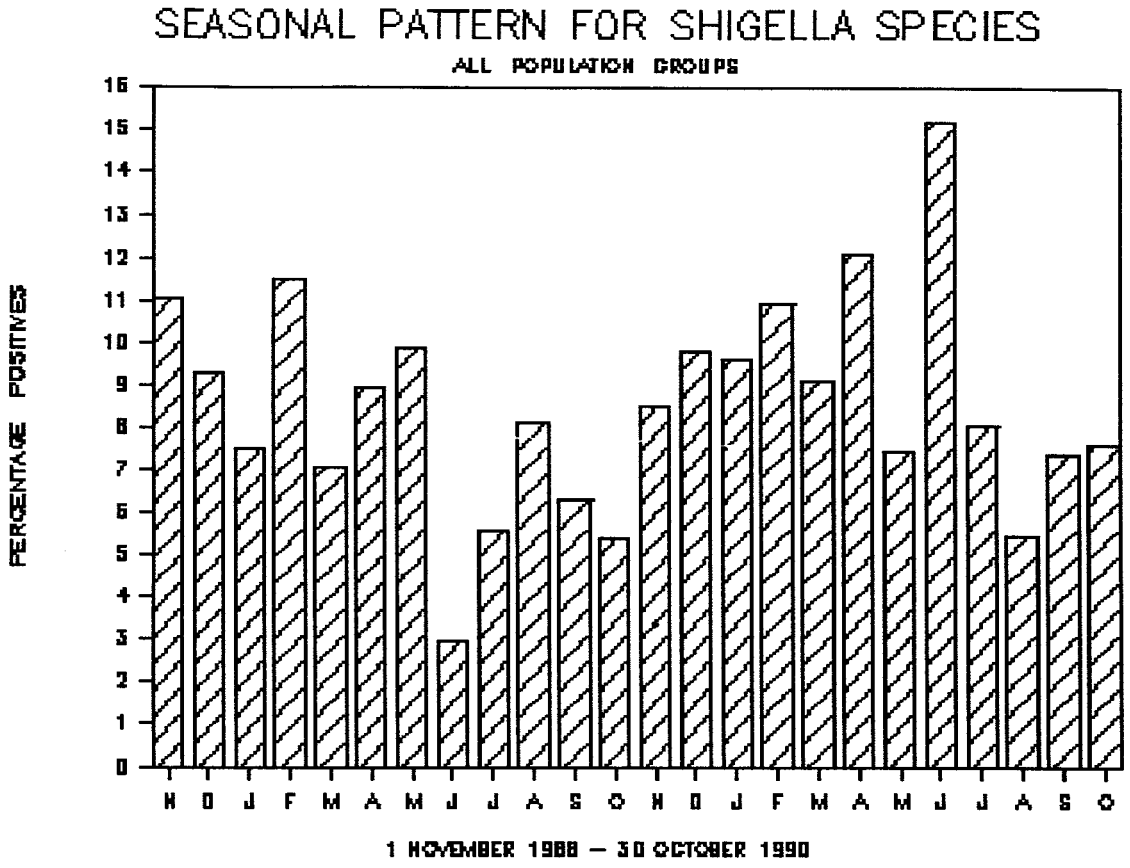

Figure 8 - Seasonal pattern of Shigella species in the Eastern Cape during the period 1 November 1988 to 30 October 1990.(See appendix 5)

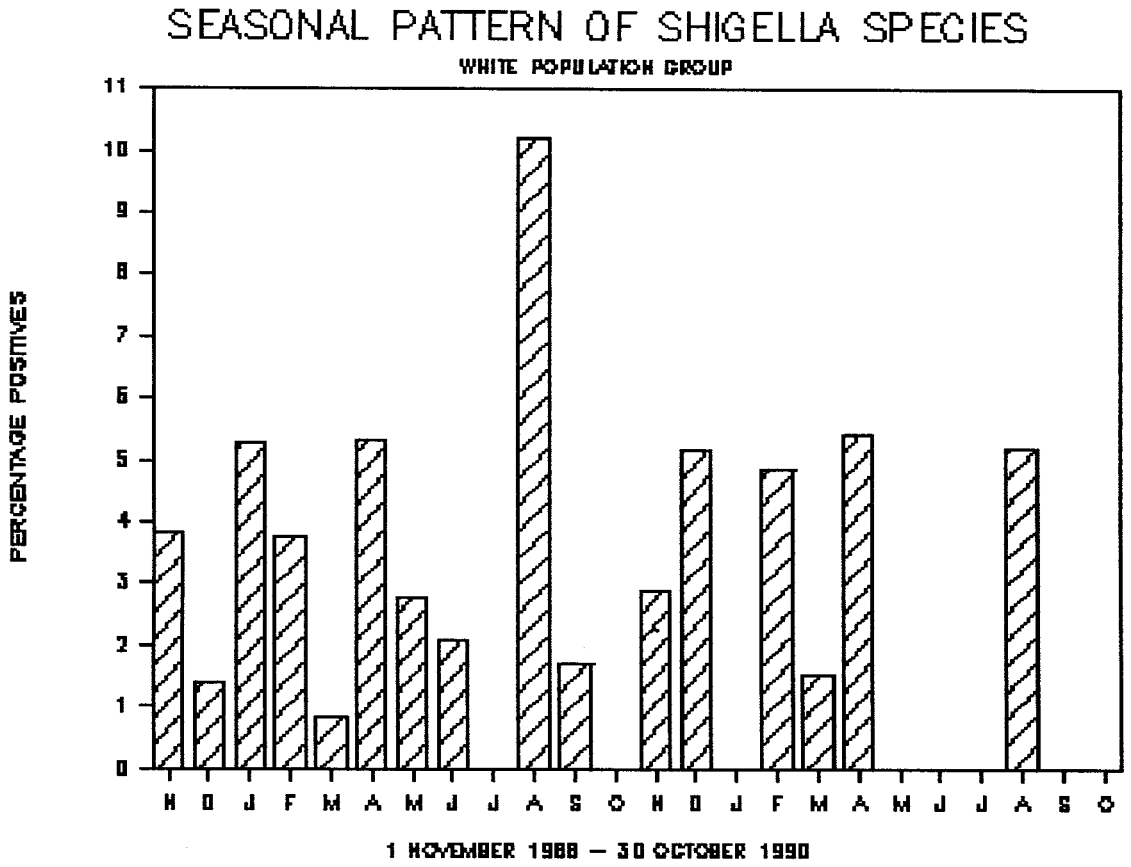

Figure 9 - Seasonal pattern of Shigella species in the White population group during the period 1 November 1988 to 30 October 1990.(See appendix 6)

TABLE IV - SHIGELLA SPECIES ISOLATED DURING THE PERIOD 1 NOVEMBER 1989 TO 30 OCTOBER 1991 FROM ALL POPULATION AND AGE GROUPS.

	POPULATION GROUPS TESTED															
	WHITE				ASIAN				COLOURED				BLACK			
AGE	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS
<=2	657	14	643	2.13	4	0	4	0	618	33	585	5.34	2656	164	2492	6.17
>2 - 12	354	11	343	3.11	3	0	3	0	153	18	135	11.76	337	52	285	15.43
>12 -18	20	0	20	0.00	1	0	1	0	26	7	19	26.92	57	9	48	15.79
>18	725	22	703	3.03	10	3	7	30	485	65	420	13.40	1109	219	890	19.75
TOTAL	1766	47	1719	2.66	18	3	15	16.66	1282	123	1159	9.59	4159	444	3715	10.68

In the Coloured population the lowest isolation rates were found in December 1988, January and March 1989. In 1990 the lowest percentage of ***Shigella sp.*** isolated was also noted in January, February, March and May. The highest percentage isolates were detected in June and July 1990 (figure 10).

The seasonal pattern in the Black population group compared with the overall pattern (figure 11).

Serotyping was done on the ***Shigella sp.***.
Shigella flexneri was most frequently isolated in all the population groups (table V).

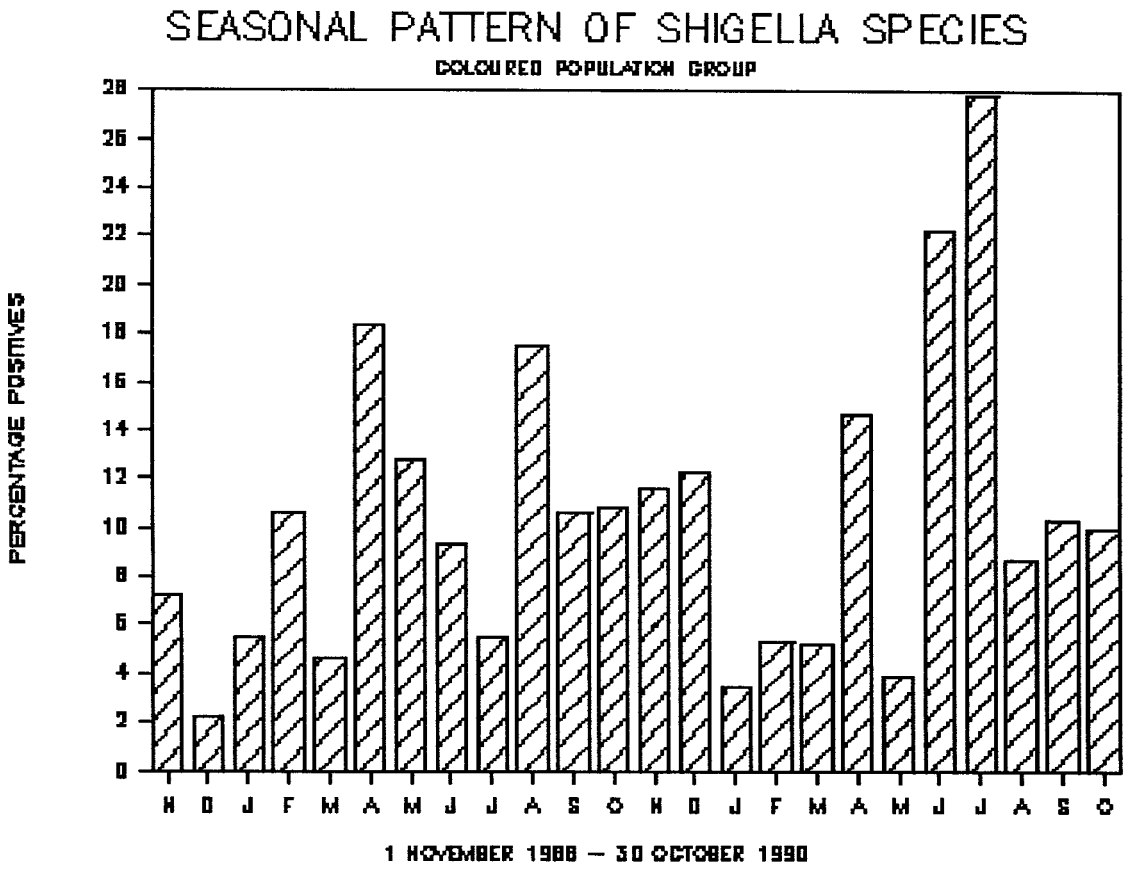

Figure 10 - Seasonal pattern of Shigella species in the Coloured population group during the period 1 November 1988 to 30 October 1990.(See appendix 7)

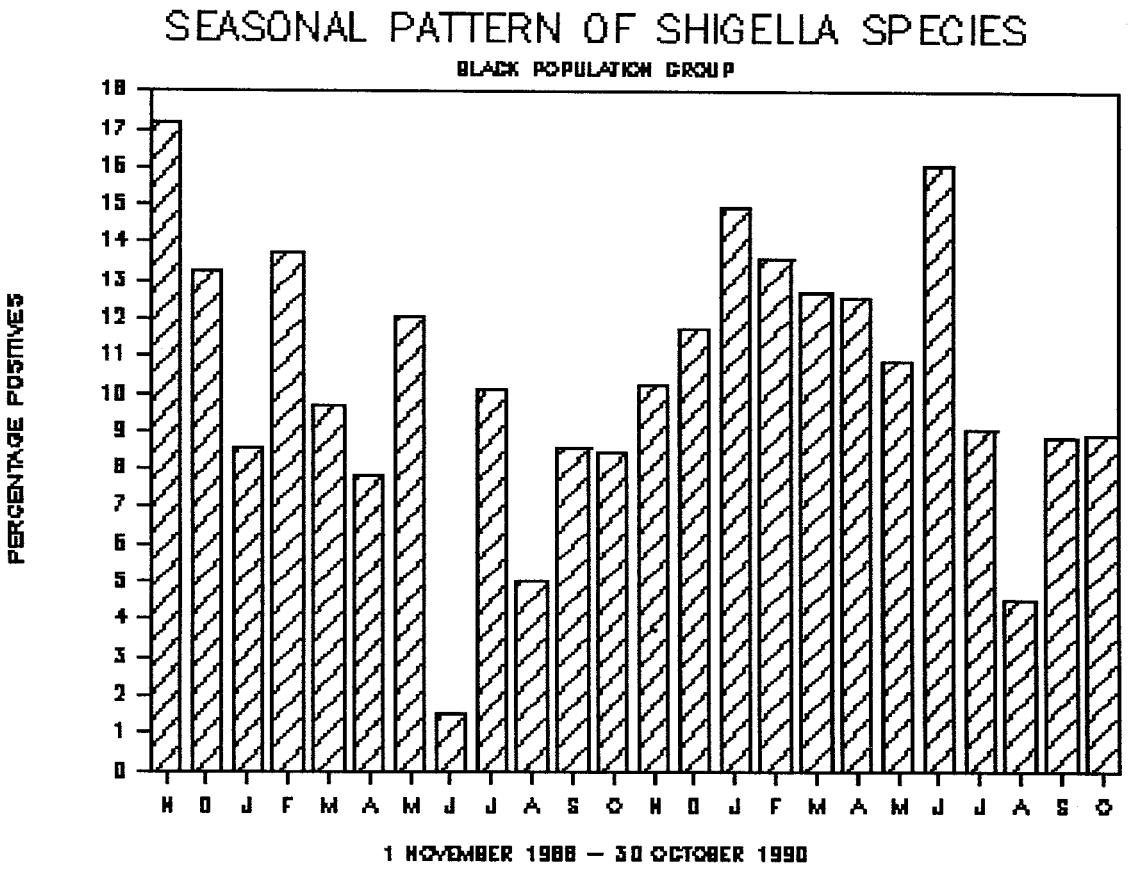

Figure 11 - Seasonal pattern of Shigella species in the Black population group during the period 1 November 1988 to 30 October 1990.(See appendix 8)

TABLE V - SEROTYPING RESULTS OF 617 SHIGELLA SPECIES ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

		TYPING RESULTS				
AGE	POPULATION GROUP	S. FLEX	S. SON	S. DYS	S. BOY	OTHERS*
<=2	WHITE	3	9	2	0	0
	ASIAN	0	0	0	0	0
	COLOURED	21	4	5	2	1
	BLACK	107	37	14	6	0
	UNKNOWN	0	0	0	0	0
>2-12	WHITE	5	6	0	0	0
	ASIAN	0	0	0	0	0
	COLOURED	12	3	1	2	0
	BLACK	29	12	10	1	0
	UNKNOWN	0	0	0	0	0
>12-18	WHITE	0	0	0	0	0
	ASIAN	0	0	0	0	0
	COLOURED	5	1	0	1	0
	BLACK	9	0	0	0	0
	UNKNOWN	0	0	0	0	0
>18	WHITE	14	5	2	0	1
	ASIAN	2	1	0	0	0
	COLOURED	51	7	7	0	0
	BLACK	176	15	6	19	3
	UNKNOWN	0	0	0	0	0

*Specific typing result not recorded.

3.3. YERSINIA ENTEROCOLITICA

Yersinia enterocolitica was isolated in 8/7 189 (0,11%) of the faecal samples examined. In the White population group 4/1 761 (0,23%) were isolated, the Coloured group 1/1 268 (0,07%) and in the Black population group 3/4 140 (0,07%) (table VI and figure 12).

The majority of isolates were detected in the <=2 year old age group (table VII).

No seasonal pattern was detected due to the limited number of isolations.

3.4. CAMPYLOBACTER SPECIES

Campylobacter sp. was isolated in 23/7 189 (0,32%) of faecal samples examined (figure 13).

The highest percentage positive *Campylobacter sp.* was isolated in the White population group 12/1 761 (0,67%). This was followed by the Coloured population group with 5/1 268 (0,38%) and the minority of isolates were detected in the Black population group 3/4 140 (0,14%). In the 18 Asians screened no *Campylobacter sp.* were found (table VIII and IX). No seasonal patterns were detected due to the limited number of *Campylobacter sp.* isolated. The majority of species were *Campylobacter jejuni*.

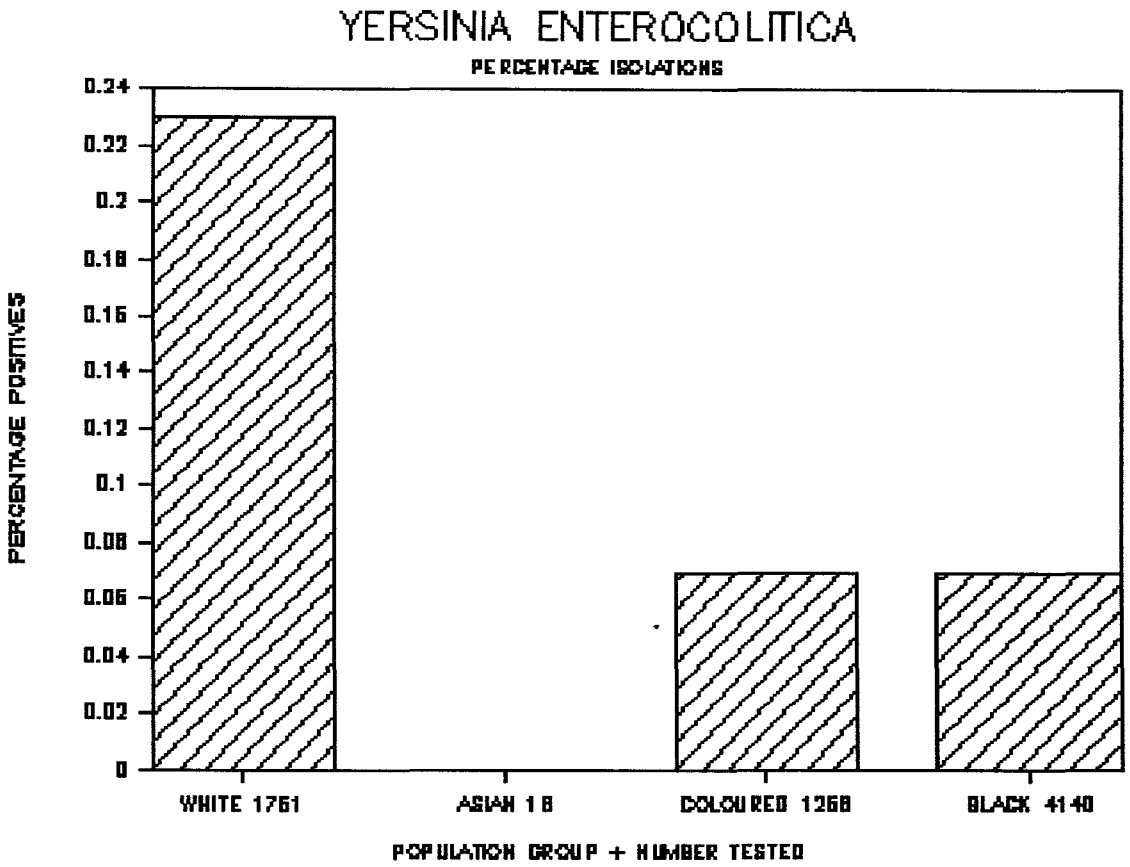

Figure 12 - Yersinia enterocolitica isolated in all population and age groups during 1 November 1988 to 30 October 1990.

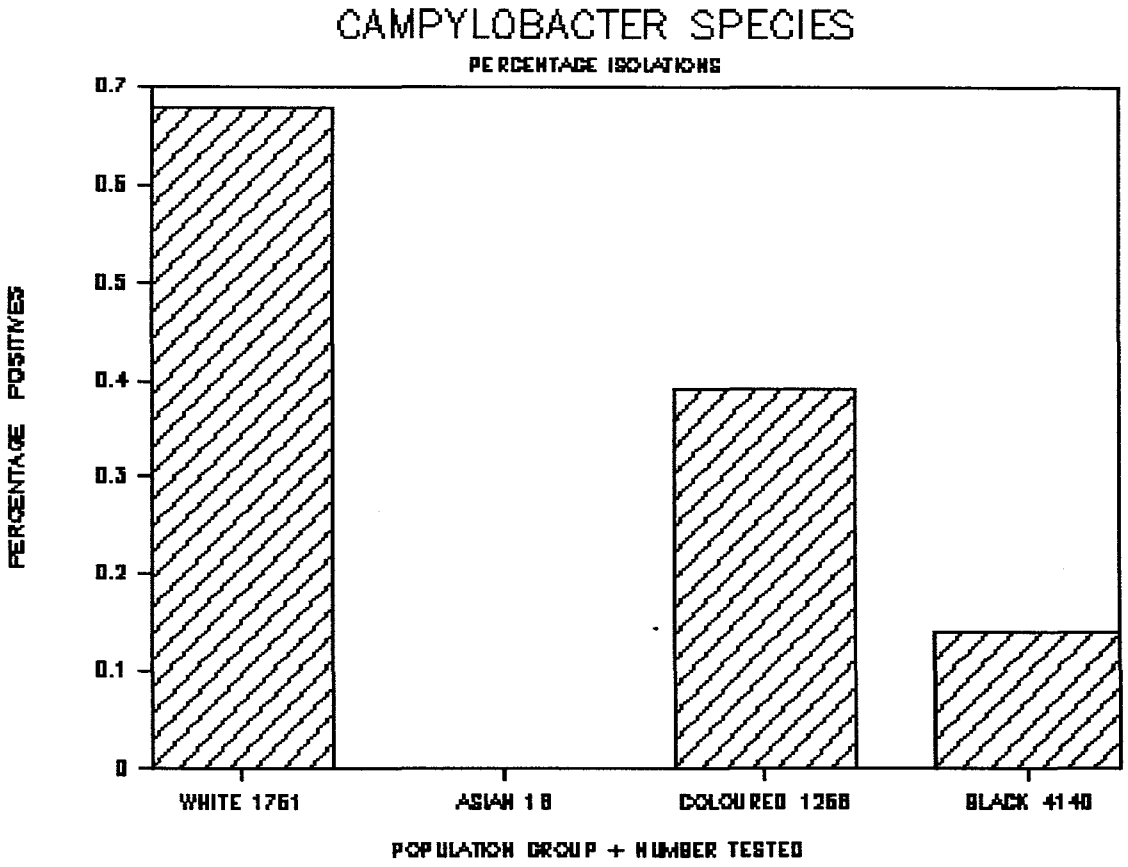

Figure 13 - Campylobacter species isolated during the period 1 November 1988 to 30 October 1990.

TABLE VI - YERSINIA ENTEROCOLITICA ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990 FROM ALL POPULATION GROUPS.

POPULATION GROUP	NUMBER TESTED	POS	NEG	%POS
WHITE	1761	4	1757	0.23
ASIAN	18	0	18	0
COLOURED	1268	1	1267	0.07
BLACK	4140	3	4137	0.07
UNKNOWN	2	0	2	0

TABLE VII - YERSINIA ENTEROCOLITICA ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990 FROM DIFFERENT POPULATION AND AGE GROUPS.

	POPULATION GROUPS			
AGE GROUPS	WHITE (n=1761)	ASIAN n=(18)	COLOURED (n=1268)	BLACK (n=4140)
<=2	1	0	1	2
>2-12	1	0	0	0
>12-18	0	0	0	0
>18	2	0	0	1
TOTAL	4	0	1	3

TABLE VIII - CAMPYLOBACTER SPECIES ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990 FROM ALL POPULATION GROUPS

POPULATION GROUPS	NUMBER TESTED	POS	NEG	%POS
WHITE	1761	12	1749	0.68
ASIAN	18	0	18	0.00
COLOURED	1268	5	1263	0.39
BLACK	4140	6	4134	0.14
UNKNOWN	2	0	2	0

TABLE IX - CAMPYLOBACTER SPECIES ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990 FROM ALL POPULATION AND AGE GROUPS.

	POPULATION GROUPS			
AGE GROUPS	WHITE (n=1761)	ASIAN (n=18)	COLOURED (n=1268)	BLACK (n=4140)
<=2	4	0	2	6
>2-12	1	0	0	0
>12-18	0	0	0	0
>18	7	0	3	0
TOTAL	12	0	5	6

3.5. AEROMONAS HYDROPHILA

Aeromonas hydrophila was isolated in 6/7 189 (0,08%) of the faecal samples examined. Five of the isolates were detected in the Black population group 5/4 140 (0,12%). In the Coloured population group only 1/1 268 (0,07%) was isolated and none in the White and Asian population groups (figure 14). Four of the isolates were detected in the >18 year old group (table X and XI).

No seasonal pattern was detected due to the limited number of *Aeromonas hydrophila* isolated.

3.6. ENTEROPATHOGENIC ESCHERICHIA COLI

Enteropathogenic *E. coli* were isolated in 356/3930(9.0%) of the faecal samples examined.

The highest percentage of enteropathogenic *E. coli* was isolated in the Black population group 273/2 648 (10,3%) followed by the White population group 53/657 (8,0%). In the Coloured population group 30/619 (4,85%) were positive for enteropathogenic *E. coli*. The four Asians tested were negative as well as the two of unknown population group (table XII and figure 15).

In all the population groups more faecal samples from males than females were screened.

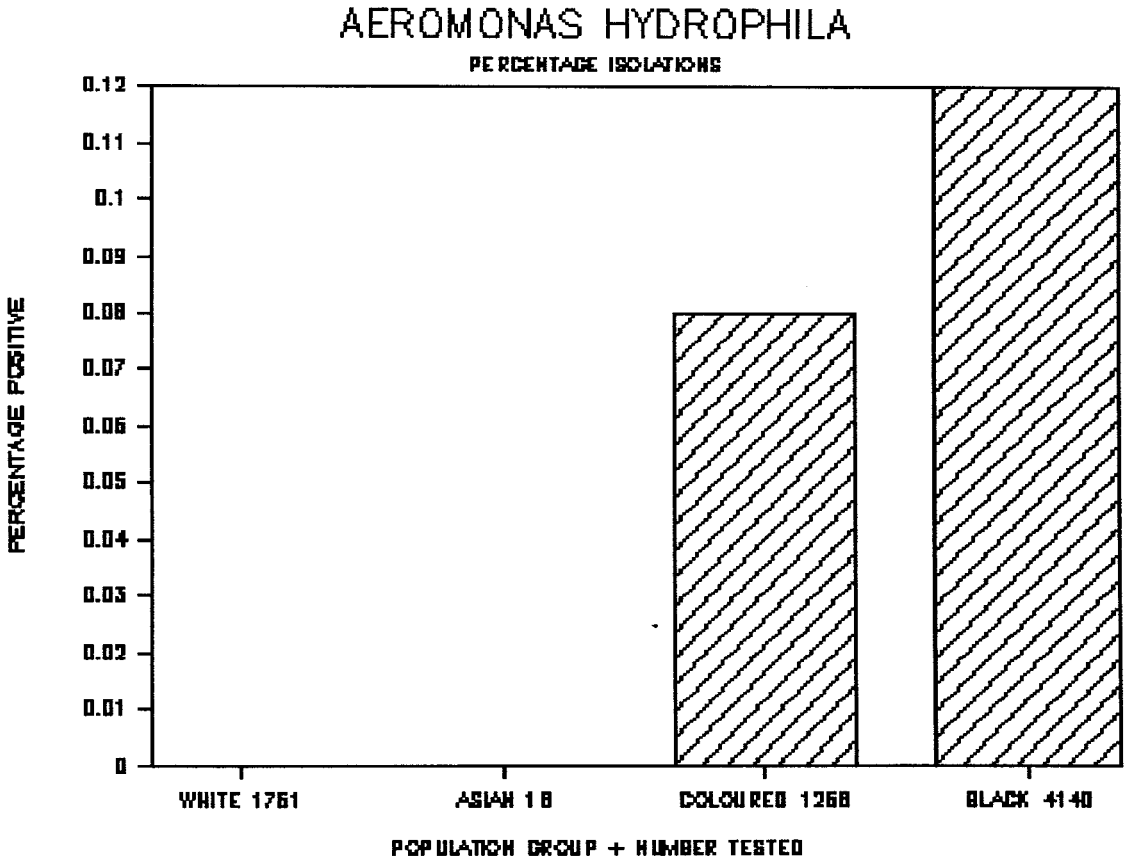

Figure 14 - Aeromonas hydrophila isolated during the period 1 November 1988 to 30 October 1990 from all population and age groups.

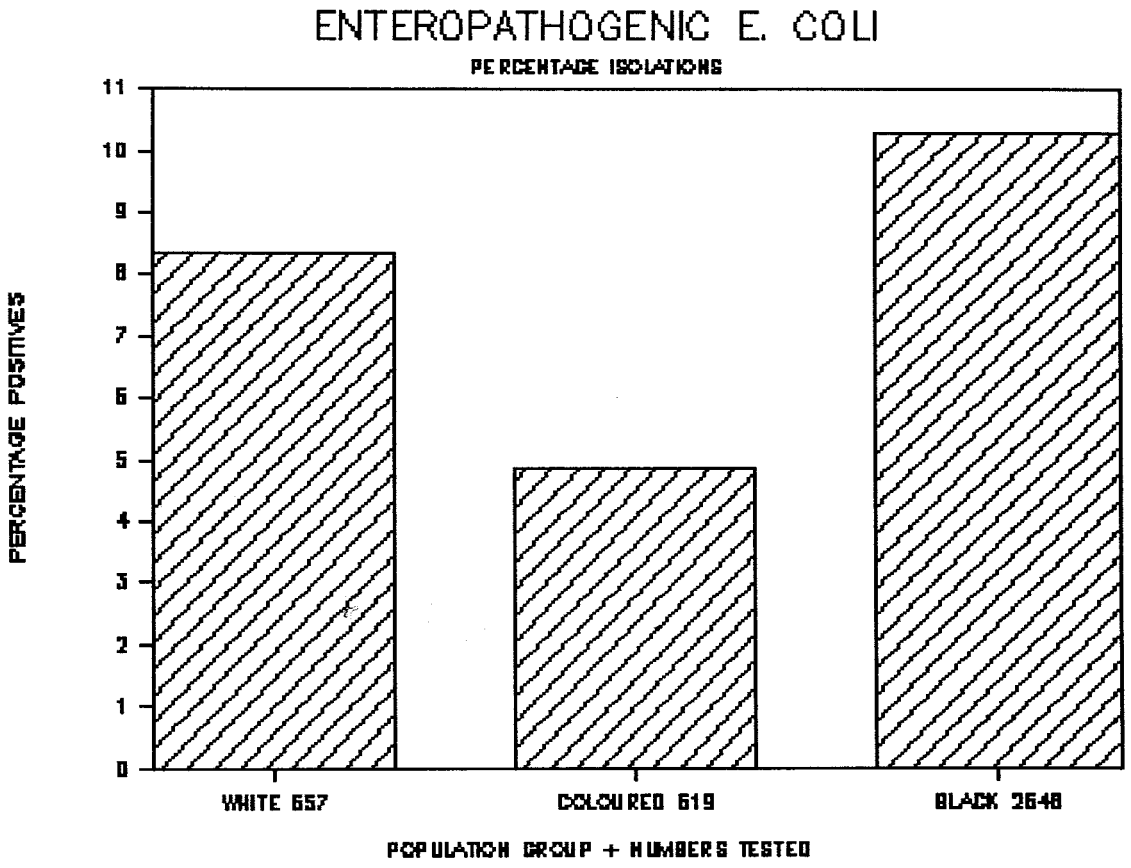

Figure 15 - Enteropathogenic Escherichia Coli isolated during the period 1 November 1988 to 30 October 1990 from all population and age groups.

TABLE X - AEROMONAS HYDROPHILA ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990 FROM ALL POPULATION GROUPS.

POPULATION GROUPS	NUMBER TESTED	POS	NEG	%POS
WHITE	1761	0	1761	0
ASIAN	18	0	18	0
COLOURED	1268	1	1267	0.08
BLACK	4140	5	4135	0.1
UNKNOWN	2	0	2	0

TABLE XI - AEROMONAS HYDROPHILA ISOLATED DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990 FROM ALL POPULATION AND AGE GROUPS.

	POPULATION GROUPS			
AGE GROUPS	WHITE (n=1761)	ASIAN (n=18)	COLOURED (n=1268)	BLACK (n=4140)
<=2	0	0	0	1
>2-12	0	0	0	0
>12-18	0	0	0	1
>18	0	0	1	3
TOTAL	0	0	1	5

TABLE XII - ENTEROPATHOGENIC ESCHERICHIA COLI ISOLATED DURING THE PERIOD 1 NOVEMBER 1989 TO 30 OCTOBER 1990 FROM CHILDREN <=2 YEARS OF AGE.

POPULATION GROUPS	NUMBER TESTED	MALE	FEMALE	UNKNOWN	POS	NEG	%
WHITE	657	343	290	24	53	580	8.37
COLOURED	619	353	263	3	30	586	4.85
BLACK	2648	1562	1082	4	273	2374	10.30
ASIAN	4	4	0	0	0	4	0
UNKNOWN	2	2	0	0	0	2	0

The higher frequency of diarrhoea was detected during August 1989 and April to July in 1990 (figure 16).

In the White population group the percentage positive enteropathogenic *E. coli* dropped in 1990 (figure 17).

The higher percentage positive isolates for the Coloured population group was detected during July and October 1989. For several months during 1990 no enteropathogenic *E. coli* was isolated from this population group (figure 18).

In the Black population group the higher incidence was detected in August 1989 and between April and July 1990 (figure 19).

The majority isolates were only typed with the polyvalent antisera and results have not been recorded.

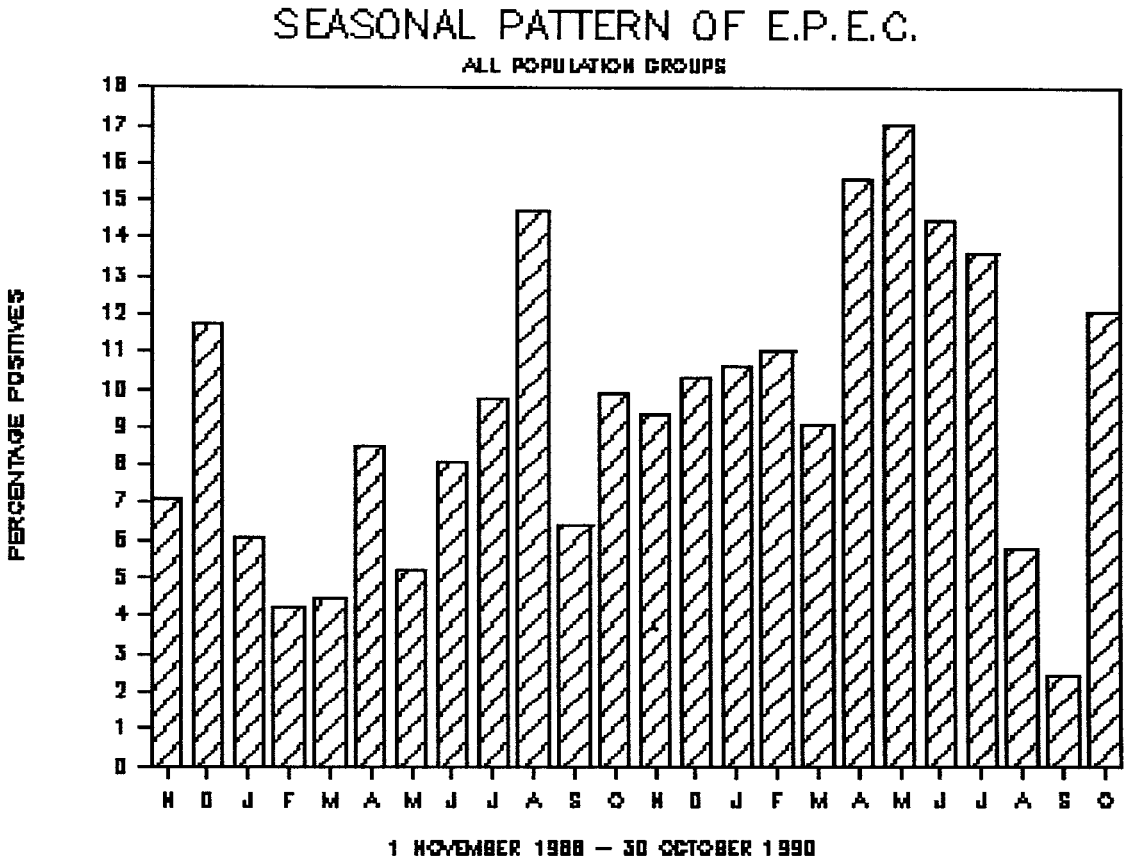

Figure 16 - Seasonal pattern of enteropathogenic Escherichia coli in all population groups during the period 1 November 1988 to 30 October 1990.(See appendix 9)

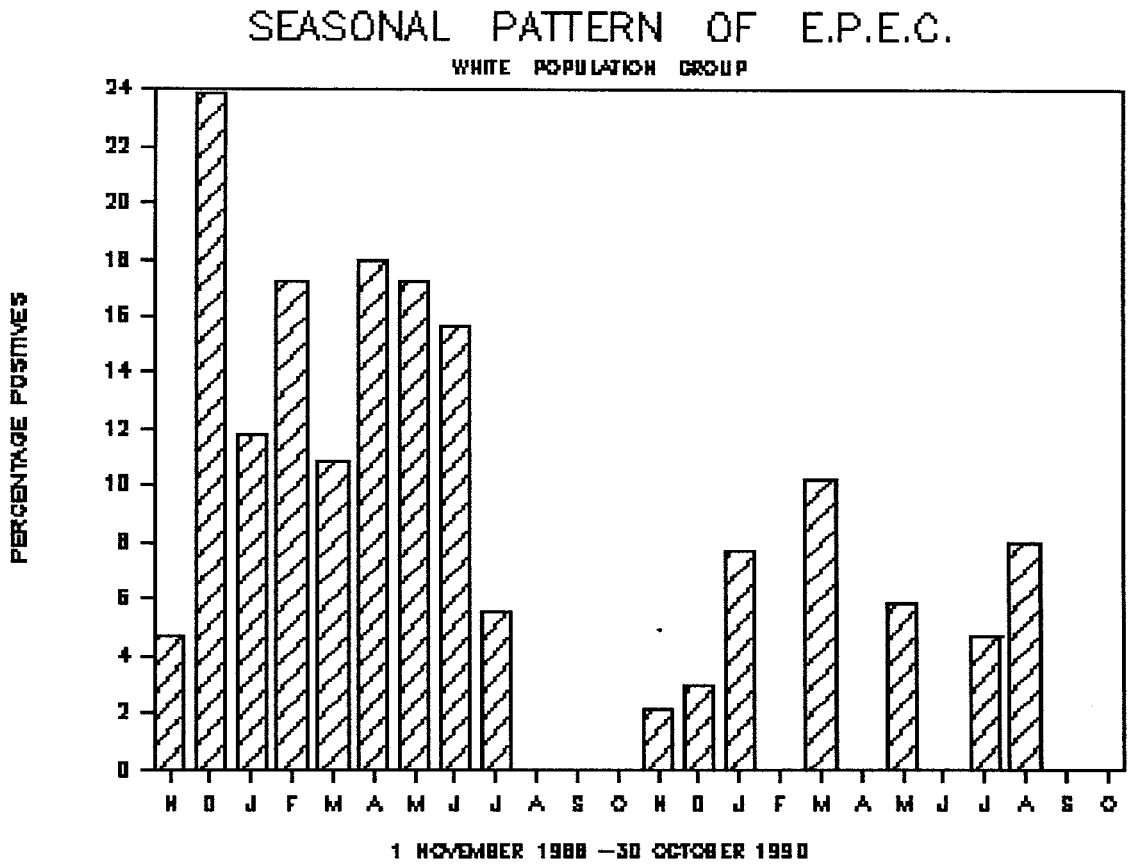

Figure 17 - Seasonal pattern of enteropathogenic Escherichia coli in White population group during the period 1 November 1988 to 30 October 1990.(See appendix 10)

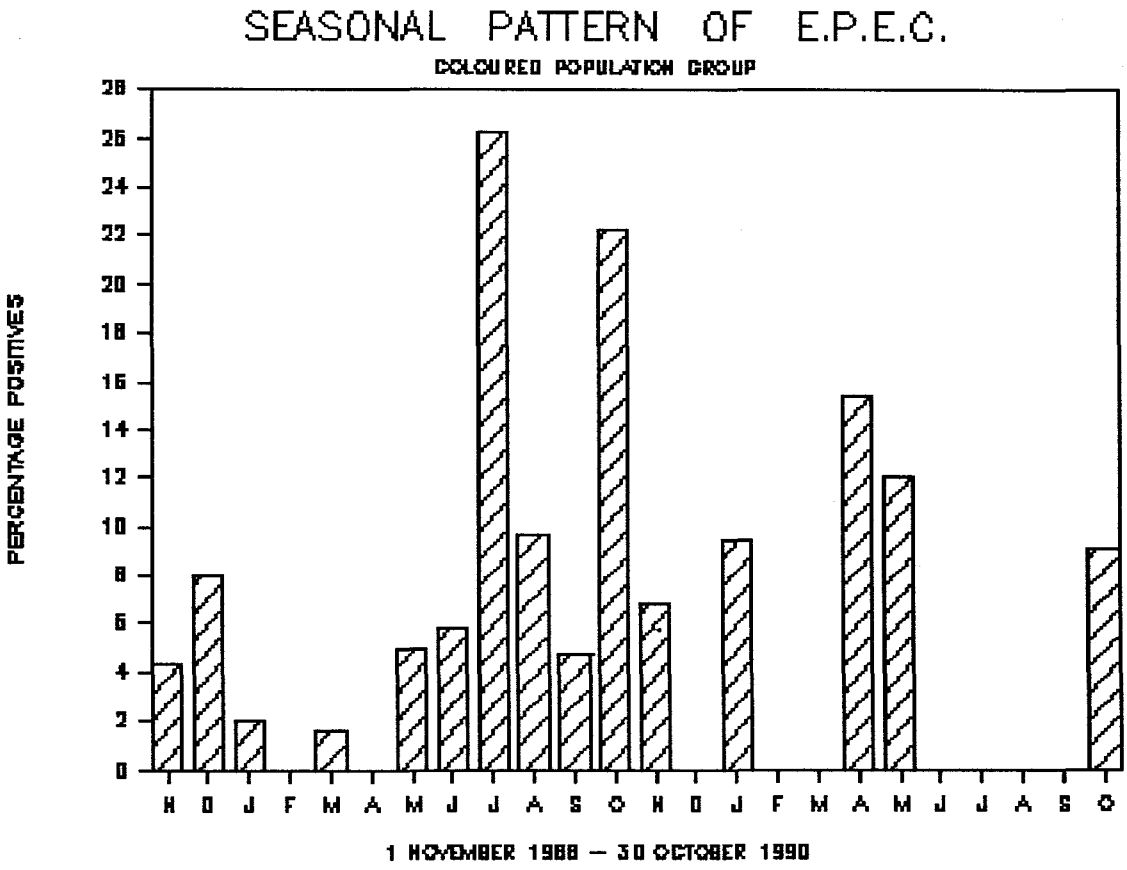

Figure 18 - Seasonal pattern of enteropathogenic Escherichia coli in Coloured population group during the period 1 November 1988 to 30 October 1990.(See appendix 11)

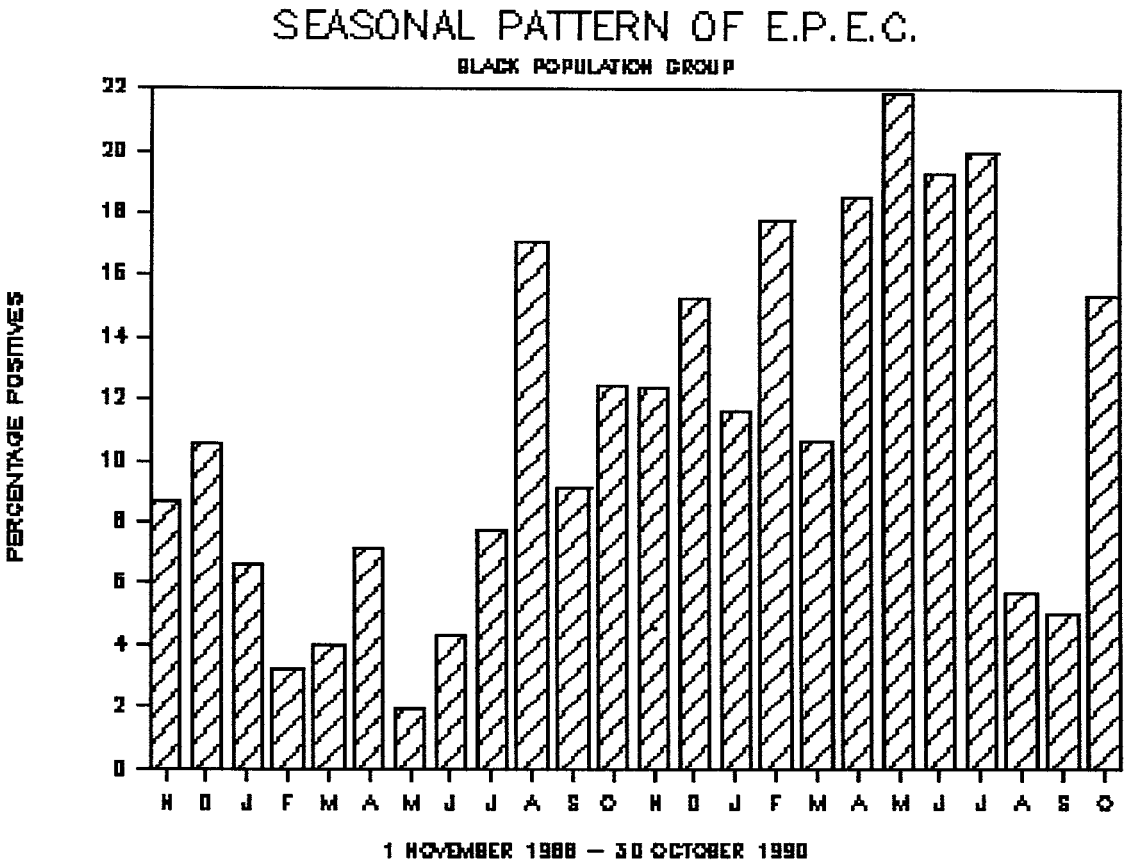

Figure 19 - Seasonal pattern of enteropathogenic Escherichia coli in Black population group during the period 1 November 1988 to 30 October 1990.(See appendix 12)

3.7. ROTAVIRUS

Rotavirus were detected in 106/806 (13,2%) of the specimens examined.

In the White population group only 11 specimens were screened as this was only done on special request.

In the Coloured population 8/98 (8,1%) faecal samples were positive for ***Rotavirus***. The Black population group had the highest percentage positives 96/694 (13,85%) (table XIII).

The higher frequency of isolations from the Black population group occurred in June 1989 and in December of the same year (figure 20).

In the Coloured population group all the samples positive for ***Rotavirus*** were detected in the warmer months (figure 21).

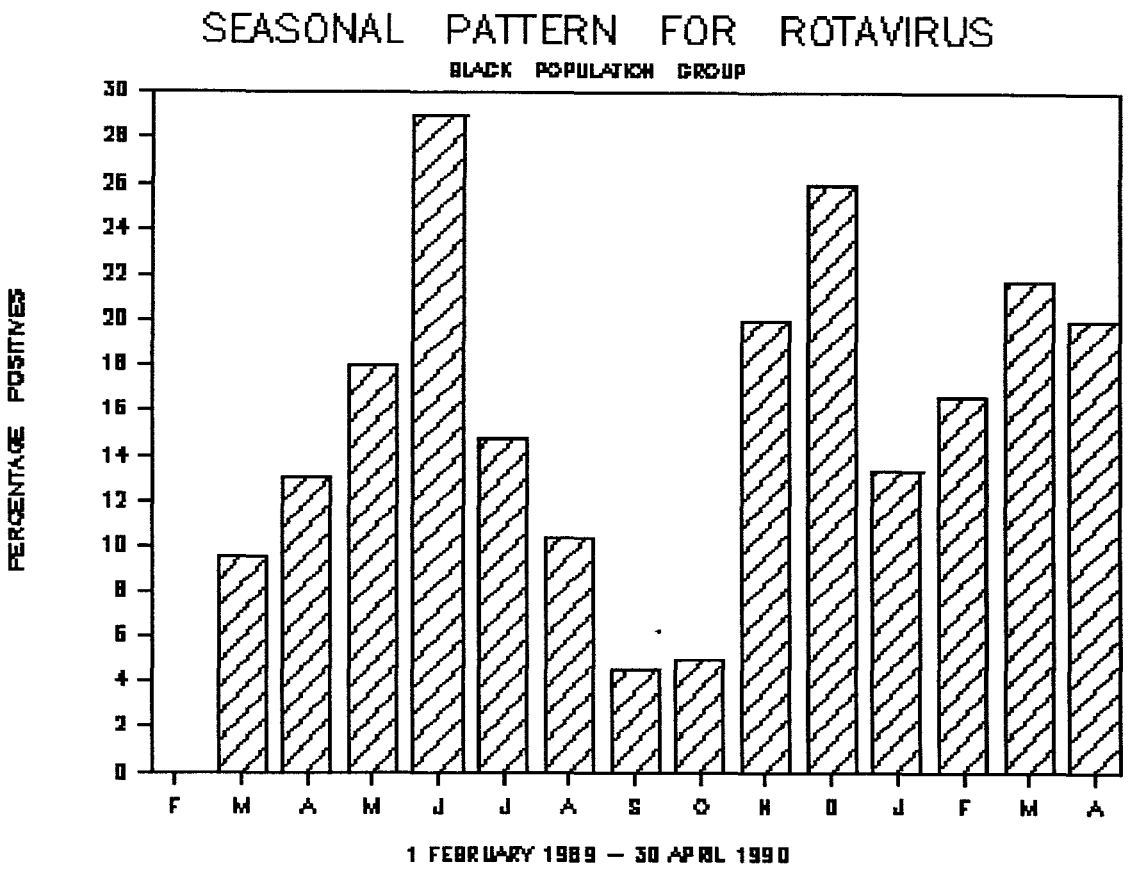

Figure 20 - Seasonal pattern of Rotavirus in Black population group during the period 1 February 1989 to 30 April 1990.(See appendix 13)

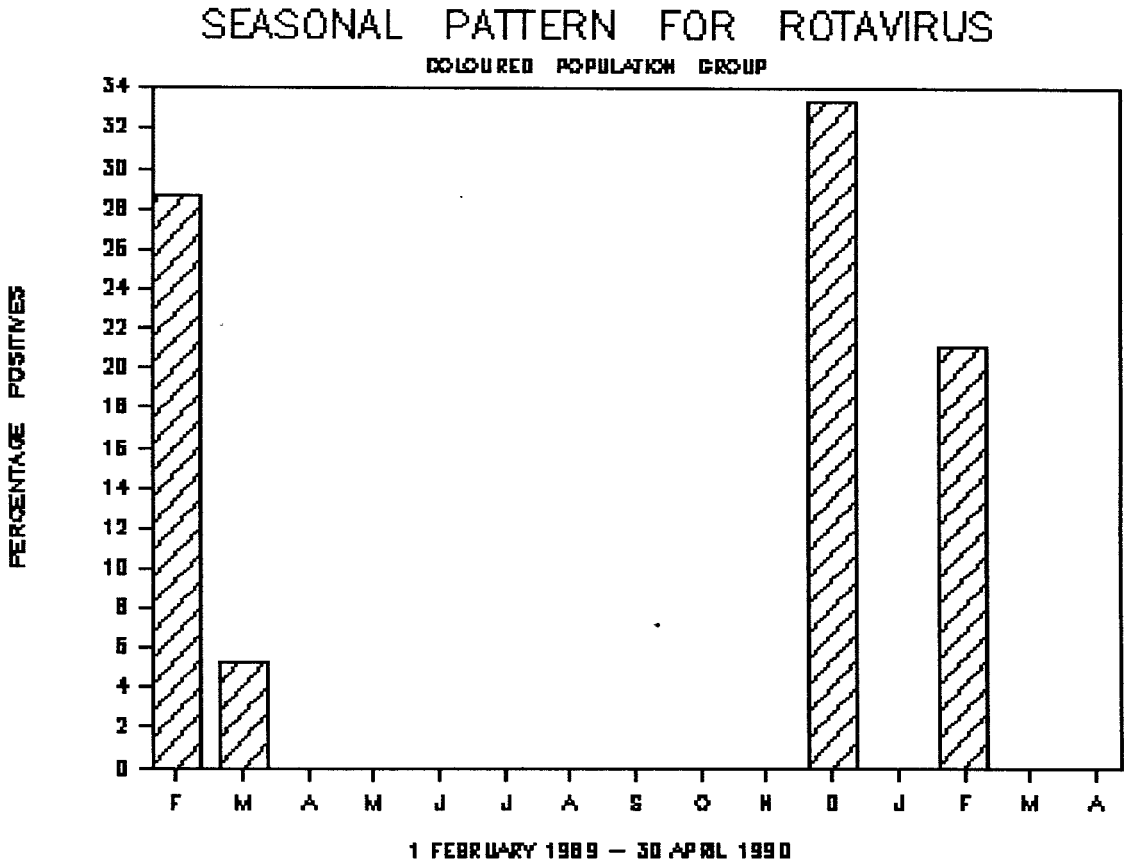

Figure 21 - Seasonal pattern of Rotavirus in Coloured population group during the period 1 February 1989 to 30 April 1990.(See appendix 14)

3.8. PROTOZOA

A total of 109/7 085 (1,5%) different protozoa were detected in all the population groups. The higher percentage of protozoa was detected in the White population group 35/1 719 (2,0%), followed by the Black 58/4 091 (1,4%) and the Coloured population groups 16/1 257 (1,2%). In the Asians no protozoa were detected (table XIV and figure 22).

The highest yearly percentage of protozoa detected was during May in 1989 and June in 1990 (figure 23). In the White population group the highest percentage of protozoa was detected in May 1989 followed by the months of January in 1989 and February in 1990 (figure 24). In the Coloured population group the highest percentage of protozoa was detected in September 1989 and also in May 1989. In 1990 the only protozoa detected was in March and May (figure 25). In the Black population group the month of May in 1989 was dominant followed by January 1989 and February 1990 (figure 26).

In the White population group 14/337 (4,1%) of the >2-12 year age group were found to have *Giardia lamblia* (table XV).

The Coloured population group also had the higher percentage of *Giardia lamblia* detected in the >2-12 year age group 4/156 (2,56%) (table XVI).

TABLE XIII - ROTAVIRUS DETECTED DURING THE PERIOD FEBRUARY 1989 TO APRIL 1990 FROM CHILDREN <=2 YEARS OF AGE IN ALL POPULATION GROUPS.

POPULATION GROUPS	NUMBER TESTED	MALE	FEMALE	POS	NEG	%POS
WHITE	11	6	5	2	9	18
COLOURED	98	56	42	8	90	8.1
BLACK	694	409	284	96	597	13.9

TABLE XIV - PROTOZOA DETECTED IN ALL POPULATION GROUPS FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990

	POPULATION GROUPS TESTED															
	WHITE				ASIAN				COLOURED				BLACK			
AGE	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS	NUMBER TESTED	POS	NEG	%POS
<=2	639	7	632	1.10	4	0	4	0	612	6	606	0.98	2607	39	2568	1.50
>2 - 12	337	14	323	4.15	1	0	1	0	156	6	150	3.85	324	5	319	1.54
>12 -18	28	1	27	3.57	1	0	1	0	24	0	24	0.00	56	2	54	3.57
>18	715	13	702	1.82	10	0	10	0	465	4	461	0.86	1104	12	1092	1.09
TOTAL	1719	35	1684	2.04	16	0	16	0	1257	16	1241	1.27	4091	58	4033	1.42

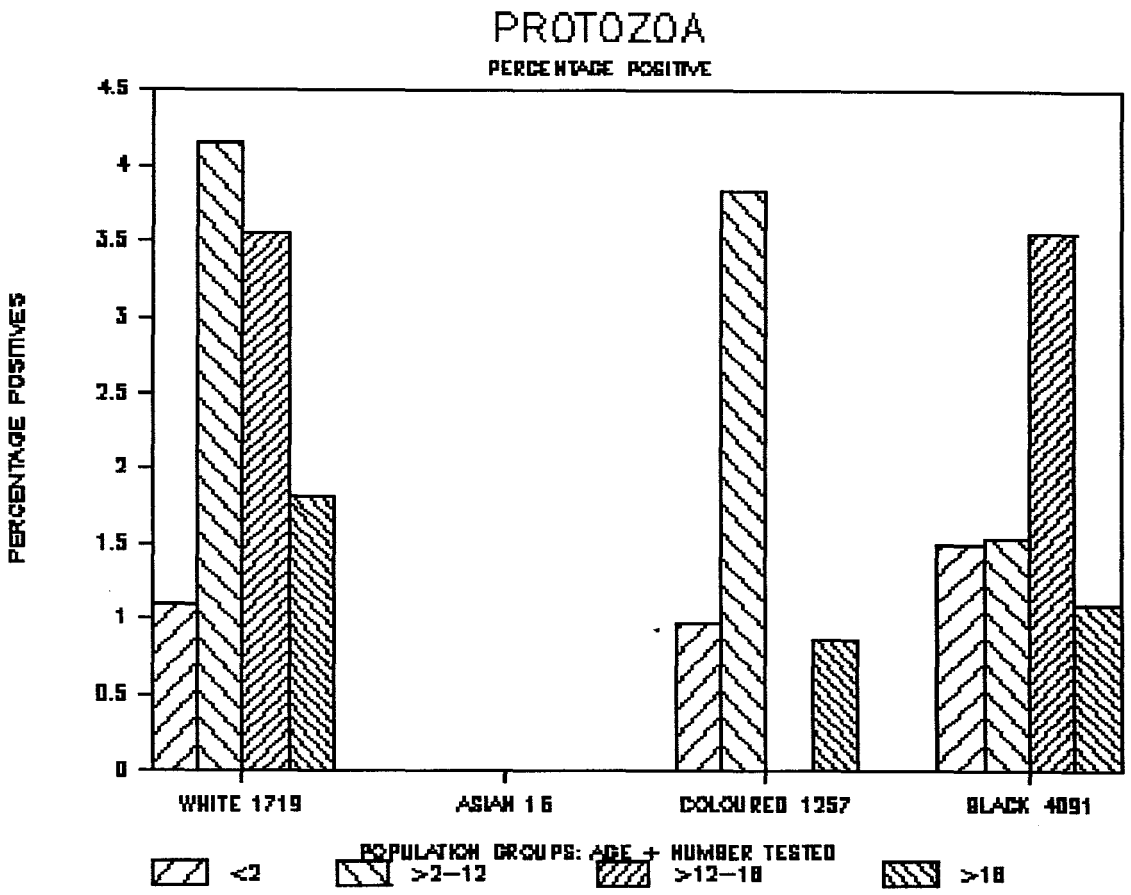

Figure 22 - Protozoa detected in all population groups during the period 1 November 1988 to 30 October 1990.

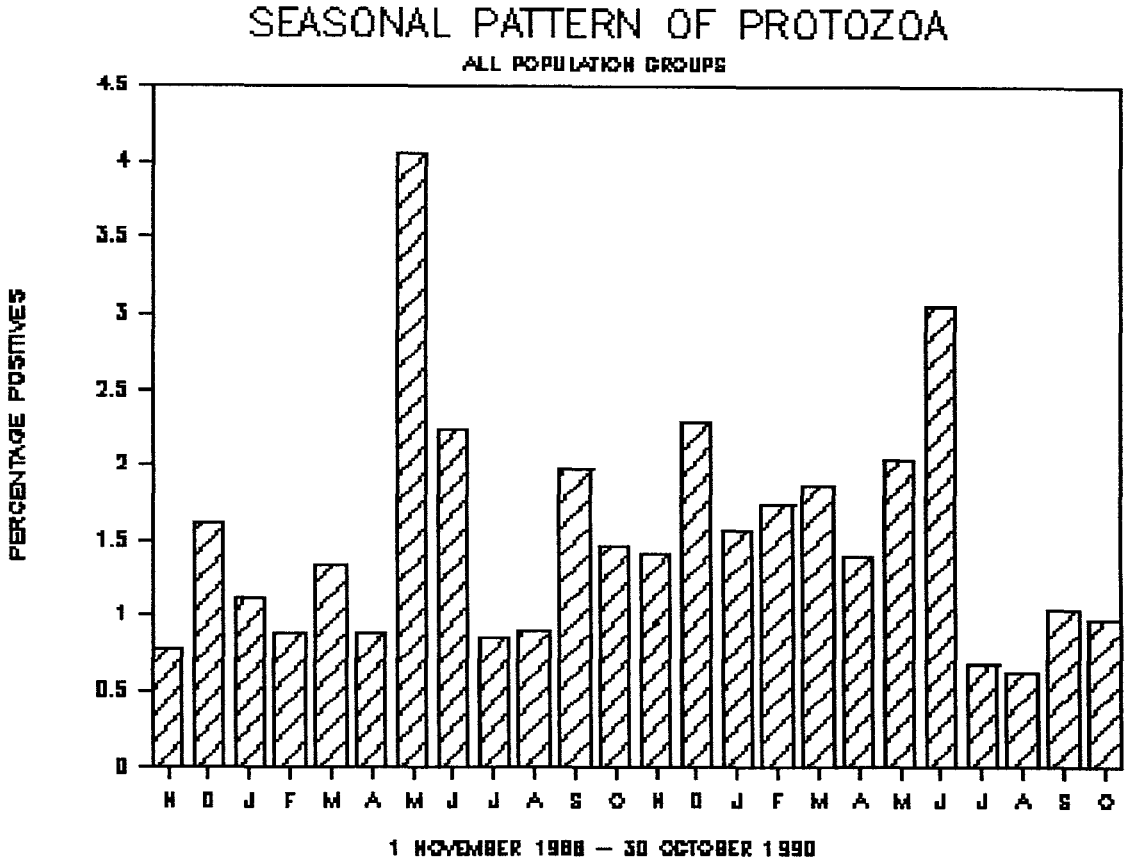

Figure 23 - Seasonal pattern of protozoa in all population groups during the period 1 November 1988 to 30 October 1990.(See appendix 15)

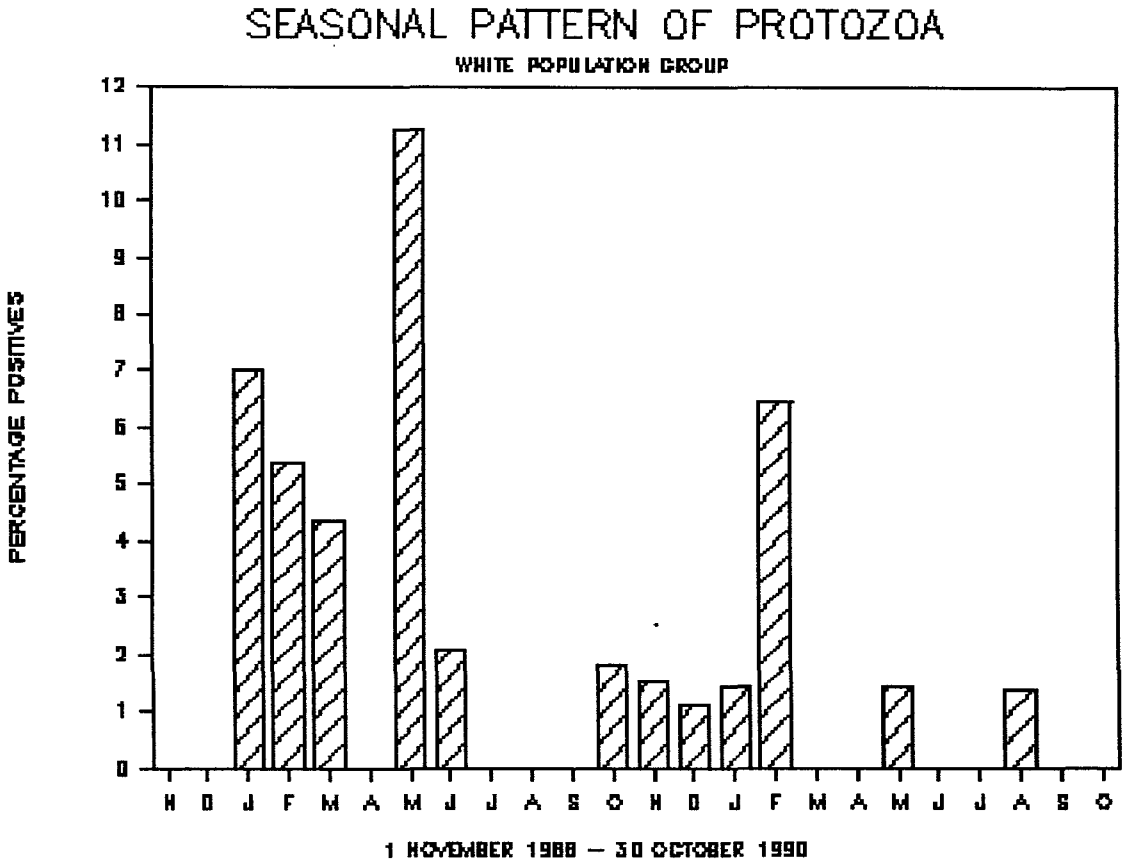

Figure 24 - Seasonal pattern of protozoa in White population group during the period 1 November 1988 to 30 October 1990.(See appendix 16)

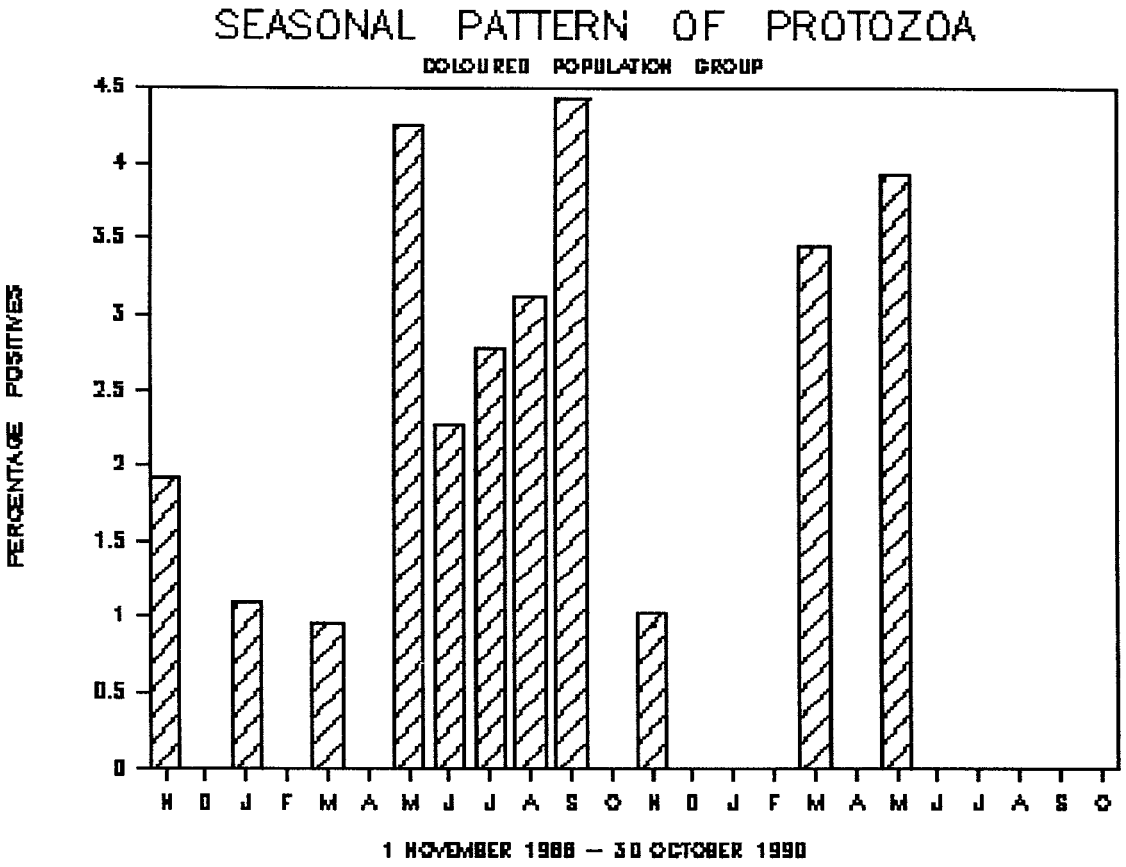

Figure 25 - Seasonal pattern of protozoa in Coloured population group during the period 1 November 1988 to 30 October 1990.(See appendix 17)

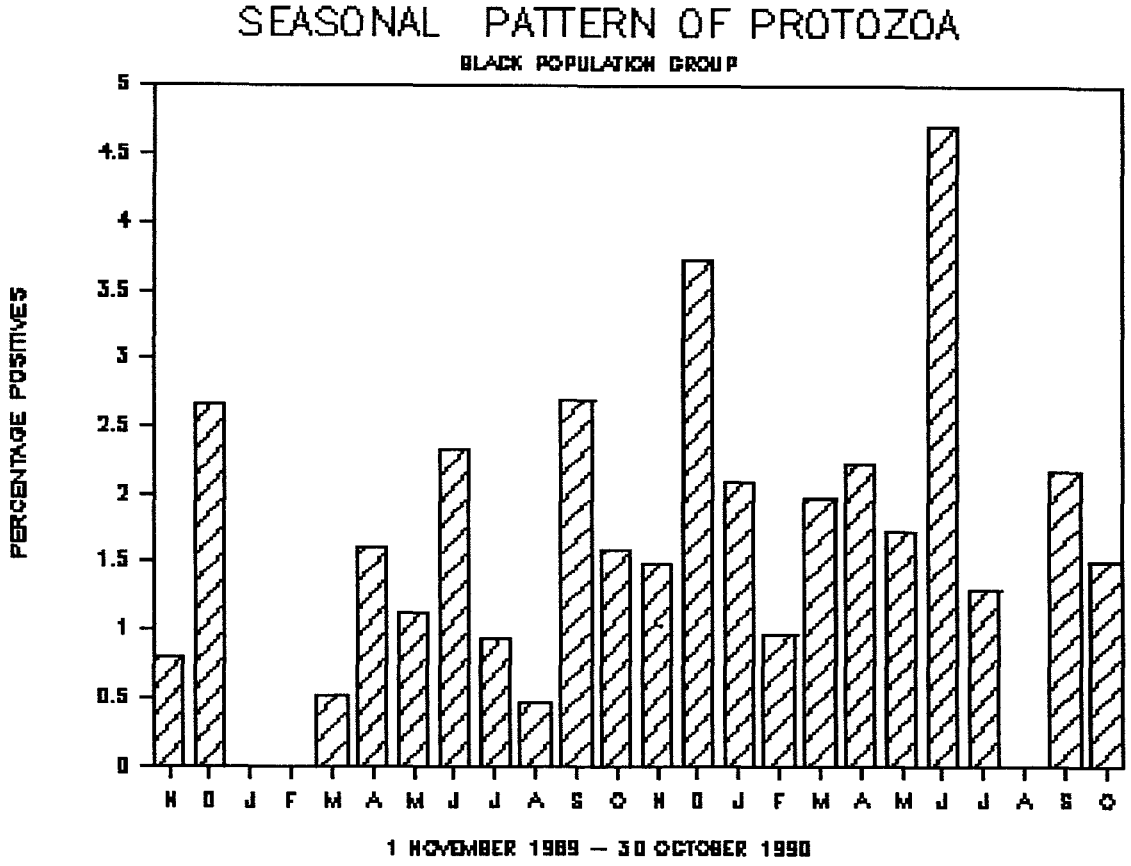

Figure 26 - Seasonal pattern of protozoa in Black population group during the period 1 November 1988 to 30 October 1990.(See appendix 18)

TABLE XV - PROTOZOA DETECTED IN WHITE POPULATION GROUP FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

AGE GROUPS	<=2				>2-12				>12-18				>18			
PROTOZOA	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%
Giardia lamblia	639	7	632	1.10	337	14	323	4.15	28	0	28	0	715	10	705	1.398
Ent. histolytica	639	0	639	0	337	12	325	3.56	28	1	27	3.57	715	2	713	0.279
Other	639	0	639	0	337	2	335	0.59	28	0	28	0	715	1	714	0.139

TABLE XVI - PROTOZOA DETECTED IN COLOURED POPULATION GROUP FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

AGE GROUPS	<=2				>2-12				>12-18				>18			
PROTOZOA	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%
Giardia lamblia	612	5	607	0.82	156	4	152	2.56	24	0	24	0.00	465	2	463	0.43
Ent. histolytica	612	1	611	0.16	156	2	154	1.28	24	0	24	0.00	465	2	463	0.43
Other	612	0	612	0.00	156	0	156	0.00	24	0	24	0.00	465	0	465	0.00

In the Black population group *Giardia lamblia* was the most frequent protozoan detected (table XVII).

3.9. HELMINTHS

Ova of helminths were detected in 366/7 085 (5,2%) of the faecal samples examined. The majority of ova were detected in the Coloured population group 156/1 257 (12,4%) followed by the Black population group 192/4 091 (4,7%). In the White population group only 20/1 719 (1,2%) contained ova (table XVIII and figure 27).

The highest percentage of ova were detected during June 1990 (figure 28). In the White population group very few ova were detected in 1990 (figure 29). In the Coloured population group the most were detected in August 1990 (figure 30) and in the Black population group the months of June and July 1990 had more ova (figure 31).

In the White population group ova of *Ascaris lumbricoides* were detected more frequently in the <=2 year old group (table XIX).

The >2-12 year age group of the Coloured population had the higher percentages of ova for *Ascaris lumbricoides* and *Trichuris trichiura* (table XX).
In the Black population group ova of *Ascaris lumbricoides* were found more frequently than the ova of *Trichuris trichiura* (table XXI).

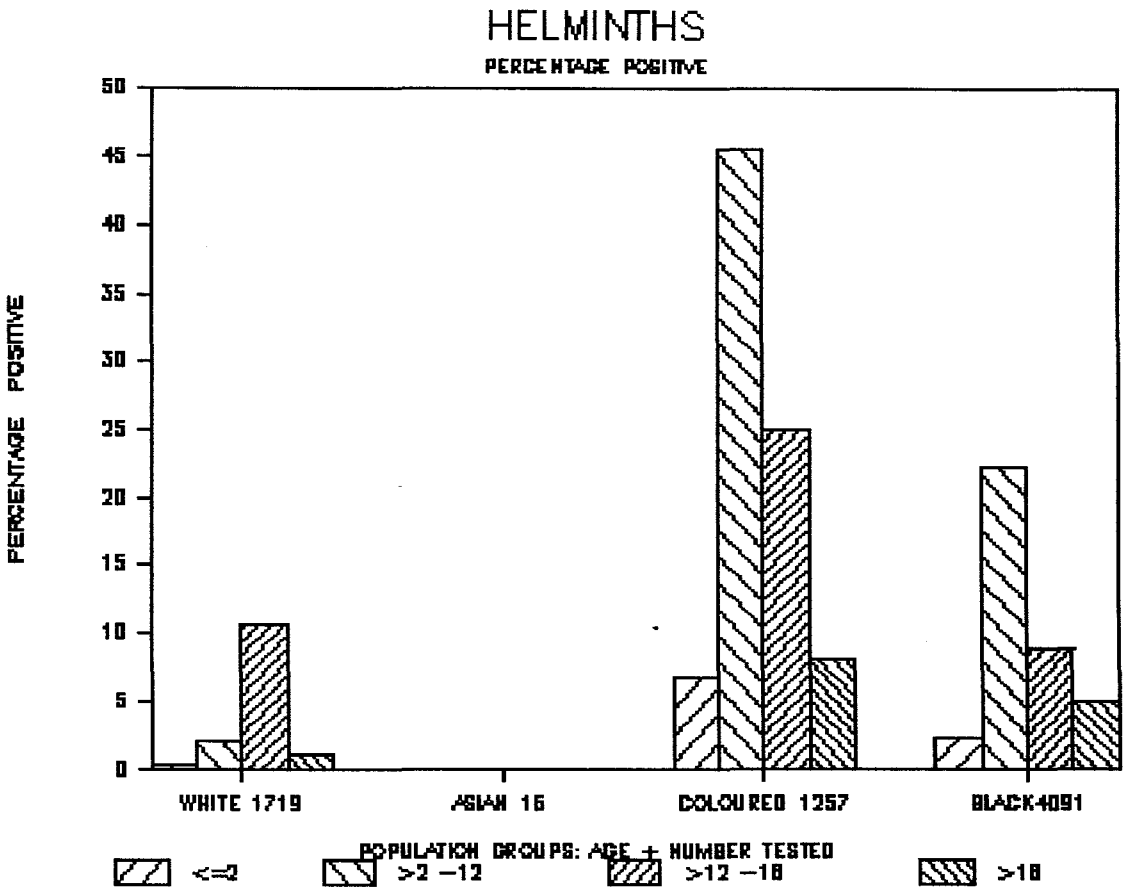

Figure 27 - Helminths detected in all population groups during the period 1 November 1988 to 30 October 1990.

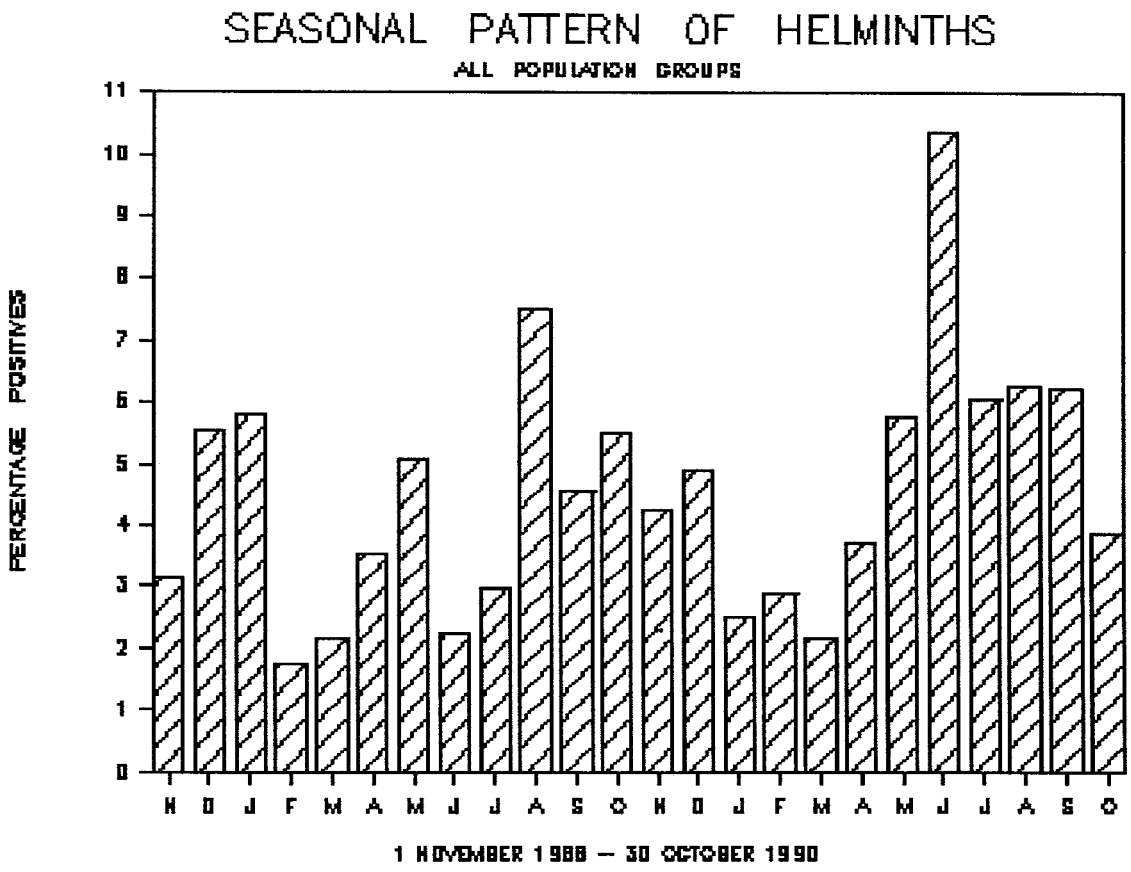

Figure 28 - Seasonal pattern of helminths in all population groups during the period 1 November 1988 to 30 October 1990.(See appendix 19)

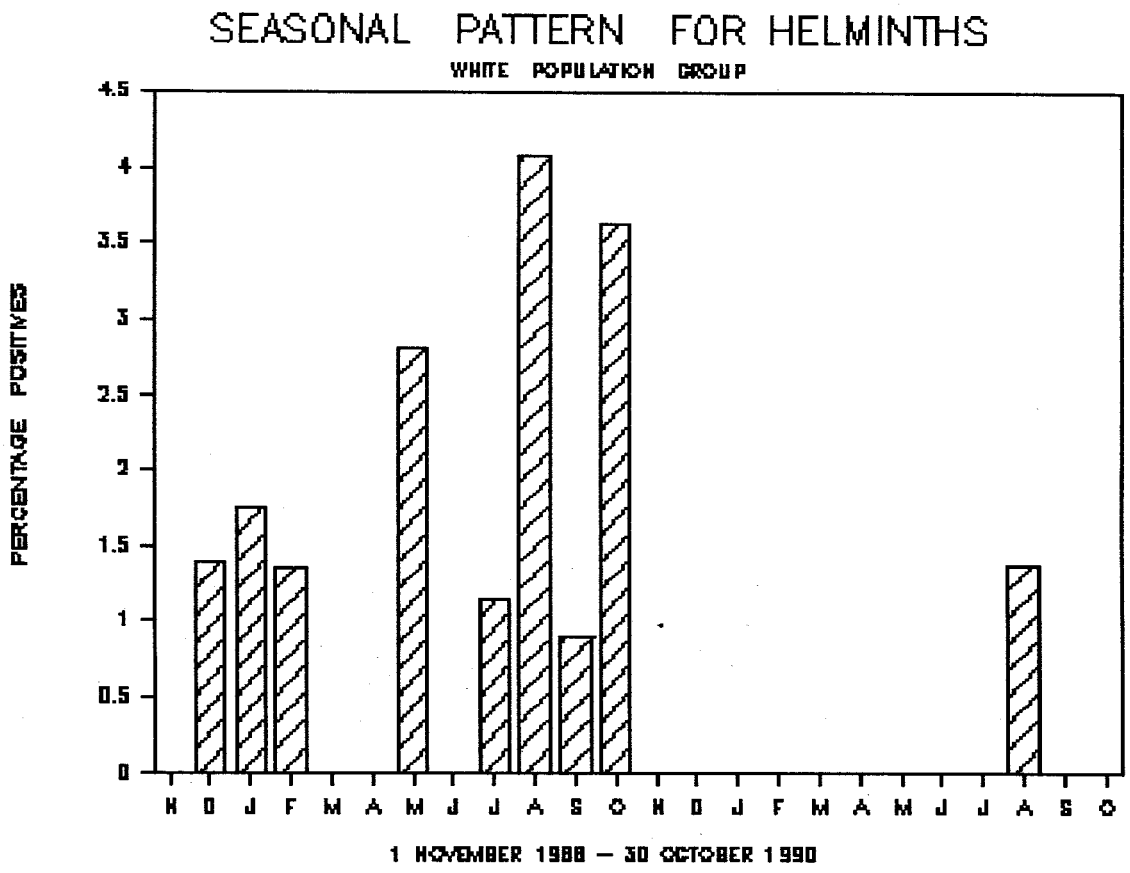

Figure 29 - Seasonal pattern of helminths in White population group during the period 1 November 1988 to 30 October 1990.(See appendix 20)

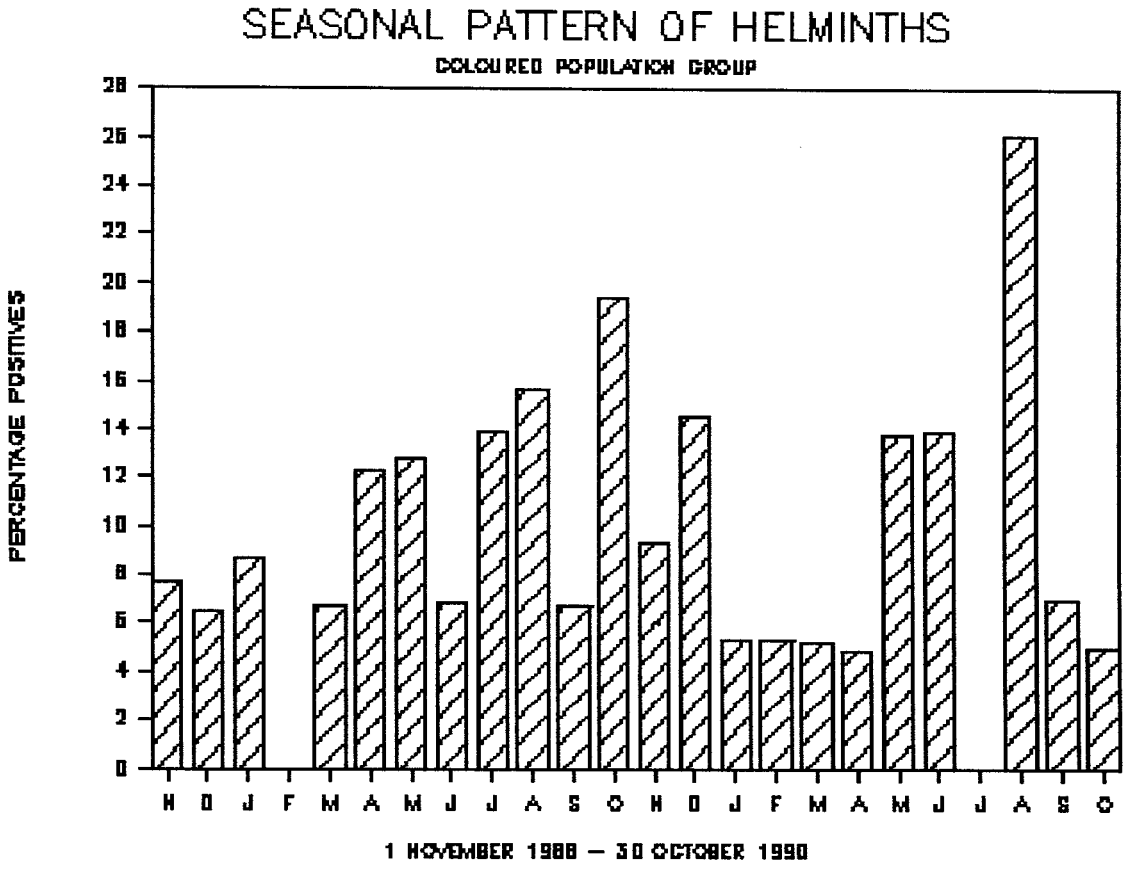

Figure 30 - Seasonal pattern of helminths in Coloured population group during the period 1 November 1988 to 30 October 1990.(See appendix 21)

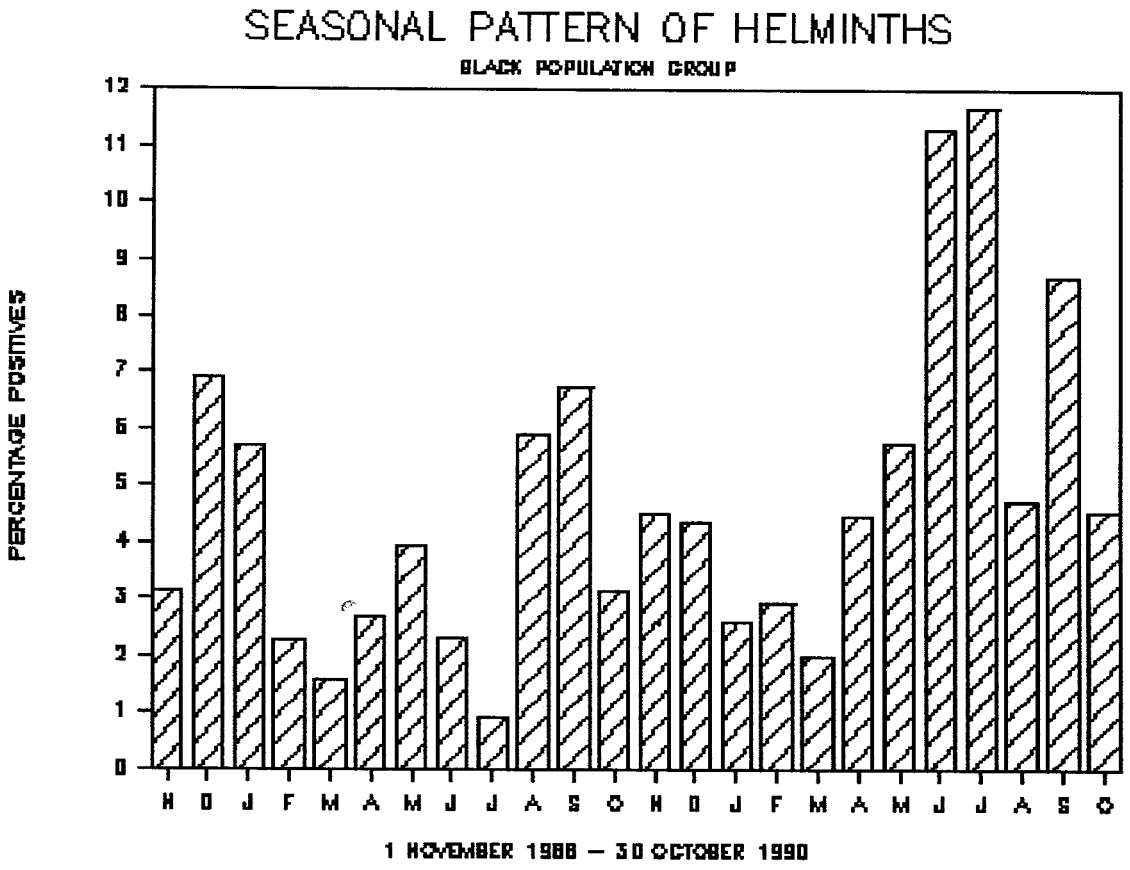

Figure 31 - Seasonal pattern of helminths in Black population group during the period 1 November 1988 to 30 October 1990.(See appendix 22)

TABLE XVII - PROTOZOA DETECTED IN BLACK POPULATION GROUP FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

AGE GROUPS	<=2				>2-12				>12-18				>18			
PROTOZOA	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%
Giardia lamblia	2607	39	2568	1.50	324	4	320	1.23	56	1	55	1.79	1104	5	1099	0.45
Ent. histolytica	2607	0	2607	0.00	324	0	324	0.00	56	1	55	1.79	1104	6	1098	0.54
Other	2607	0	2607	0.00	324	1	323	0.31	56	0	56	0.00	1104	1	1103	0.09

TABLE XVIII - HELMINTHS DETECTED FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

	POPULATION GROUPS TESTED															
	WHITE				ASIAN				COLOURED				BLACK			
AGE	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%
<=2	639	2	637	0.31	4	0	4	0	612	41	571	6.70	2607	60	2547	2.30
>2 - 12	337	7	330	2.08	1	0	1	0	156	71	85	45.51	324	72	252	22.22
>12 - 18	28	3	25	10.71	1	0	1	0	24	6	18	25.00	56	5	51	8.93
>18	715	8	707	1.12	10	0	10	0	465	38	427	8.17	1104	55	1049	4.98
TOTAL	1719	20	1699	1.16	16	0	16	0	1257	156	1101	12.41	4091	192	3899	4.69

TABLE XIX - HELMINTHS DETECTED IN WHITE POPULATION GROUP FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

AGE GROUPS	<=2				>2-12				>12-18				>18			
HELMINTHS	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%
Ascaris lumbricoides	639	2	637	0.31	337	7	330	2.08	28	3	25	10.71	715	8	707	1.12
Trichuris trichiura	639	0	639	0.00	337	1	336	0.30	28	0	28	0.00	715	3	712	0.42
Hymenolepis nana	639	0	639	0.00	337	1	336	0.30	28	1	27	3.57	715	0	715	0.00
Taenia species	639	1	638	0.16	337	1	336	0.30	28	1	27	3.57	715	2	713	0.28

TABLE XX - HELMINTHS DETECTED IN COLOURED POPULATION GROUP FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990

AGE GROUPS	<=2				>2-12				>12-18				>18			
HELMINTHS	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%
Ascaris lumbricoides	612	25	587	4.08	156	32	124	20.51	24	2	22	8.33	465	17	448	3.66
Trichuris trichiura	612	16	596	2.61	156	36	120	23.08	24	2	22	8.33	465	20	445	4.30
Hymenolepis nana	612	0	612	0.00	156	2	154	1.28	24	1	23	4.17	465	0	465	0.00
Taenia species	612	0	612	0.00	156	1	155	0.64	24	1	23	4.17	465	1	464	0.22

3.10. CRYPTOSPORIDIUM OOCYSTS

Cryptosporidium oocysts were detected in 229/2 989 (7,7%) of faecal samples examined.

The highest percentage positives occurred in the Black population group 194/2 260 (9,39%). In the Coloured population 32/495 (6,43%) were found positive and in the White population group 4/231 (1,7%). Only 3 Asians were tested and were found to be negative (table XXII and figure 32).

The highest percentages of *Cryptosporidium* oocysts were detected during August, September and December in 1989 and in April 1990 (figure 33).

As low numbers of *Cryptosporidium* oocysts were detected in the White population group no seasonal pattern can be detected (figure 34).

In the Coloured population a marked increase was detected in October 1989 and April 1990 (figure 35).

The Black population group was similar to the overall pattern with September and December 1990 and April 1991 showing peaks (figure 36).

TABLE XXI - HELMINTHS DETECTED IN BLACK POPULATION GROUP FROM 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

AGE GROUPS	<=2				>2-12				>12-18				>18			
HELMINTHS	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%	NUMBER TESTED	POS	NEG	%
Ascaris lumbricoides	2607	49	2558	1.88	324	53	271	16.36	56	4	52	7.14	1104	29	1075	2.63
Trichuris trichiura	2607	8	2599	0.31	324	14	310	4.32	56	1	55	1.79	1104	16	1088	1.45
Hymenolepis nana	2607	2	2605	0.08	324	4	320	1.23	56	0	56	0.00	1104	4	1100	0.36
Taenia species	2607	1	2606	0.04	324	1	323	0.31	56	0	56	0.00	1104	6	1098	0.54

TABLE XXII - CRYPTOSPORIDIUM OOCYSTS DETECTED IN CHILDREN <=2 YEARS OF AGE DURING THE PERIOD 1 NOVEMBER 1988 TO 30 OCTOBER 1990.

POPULATION GROUPS	NUMBER TESTED	MALE	FEMALE	UNKNOWN	POS	NEG	% POS
WHITE	231	134	93	4	4	227	1.73
COLOURED	497	287	209	1	32	465	6.43
BLACK	2260	1322	938	0	194	2066	9.39

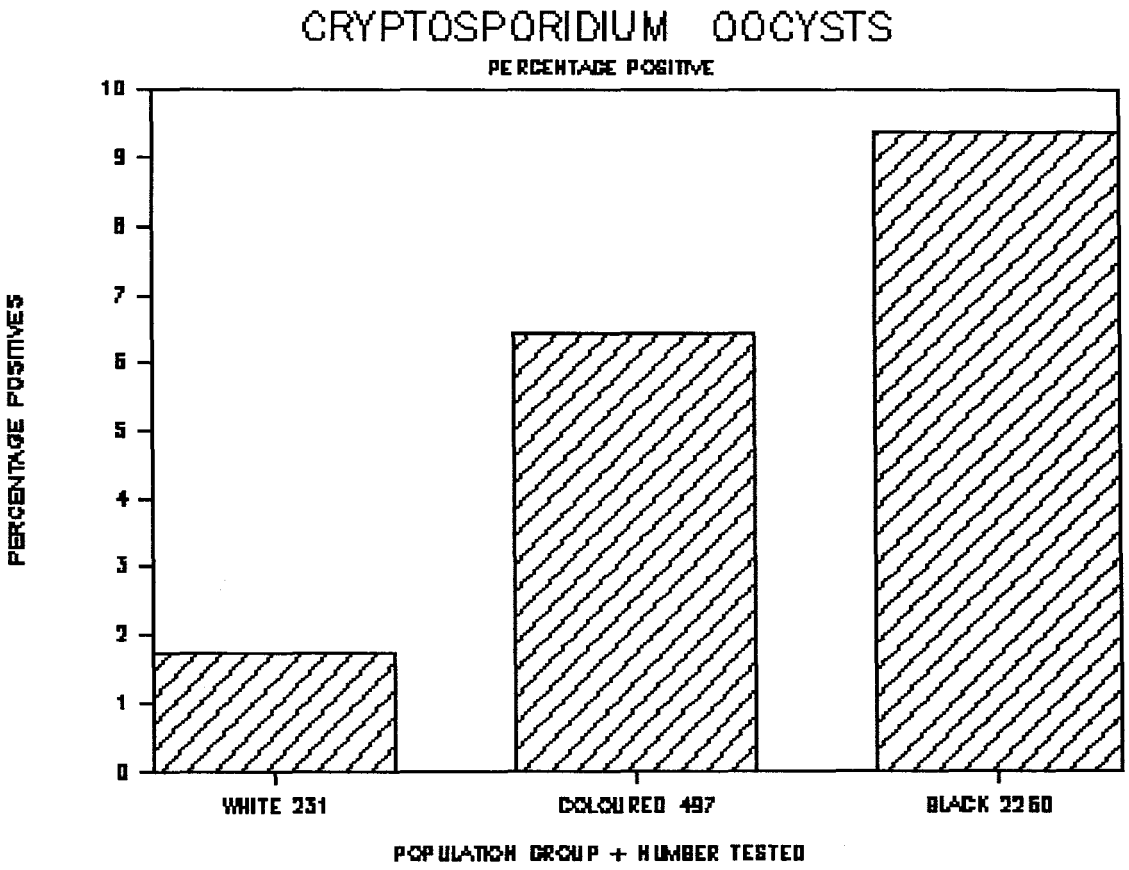

Figure 32 - Cryptosporidium oocysts detected in all population groups during the period 1 November 1988 to 30 October 1990.

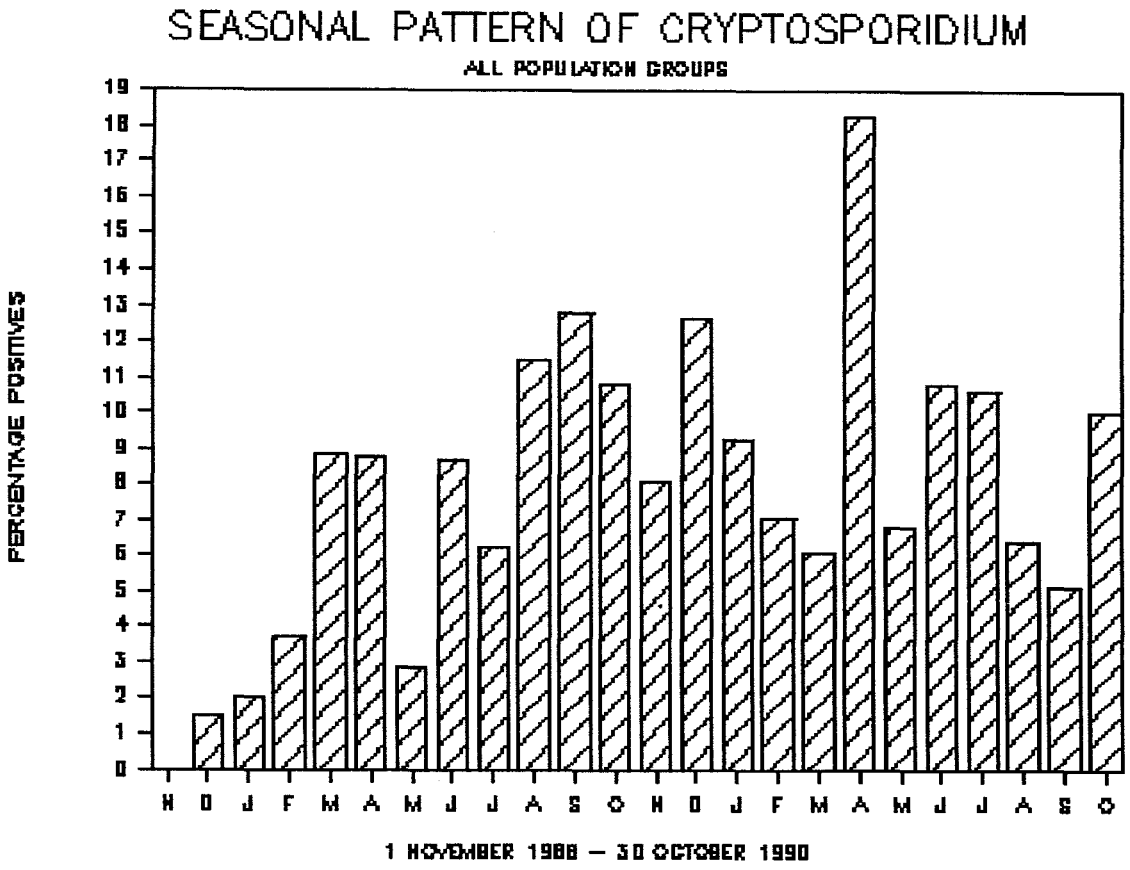

Figure 33 - Seasonal pattern of Cryptosporidium in children <=2 years of age in all population groups during the period 1 November 1988 to 30 October 1990.
(See appendix 23)

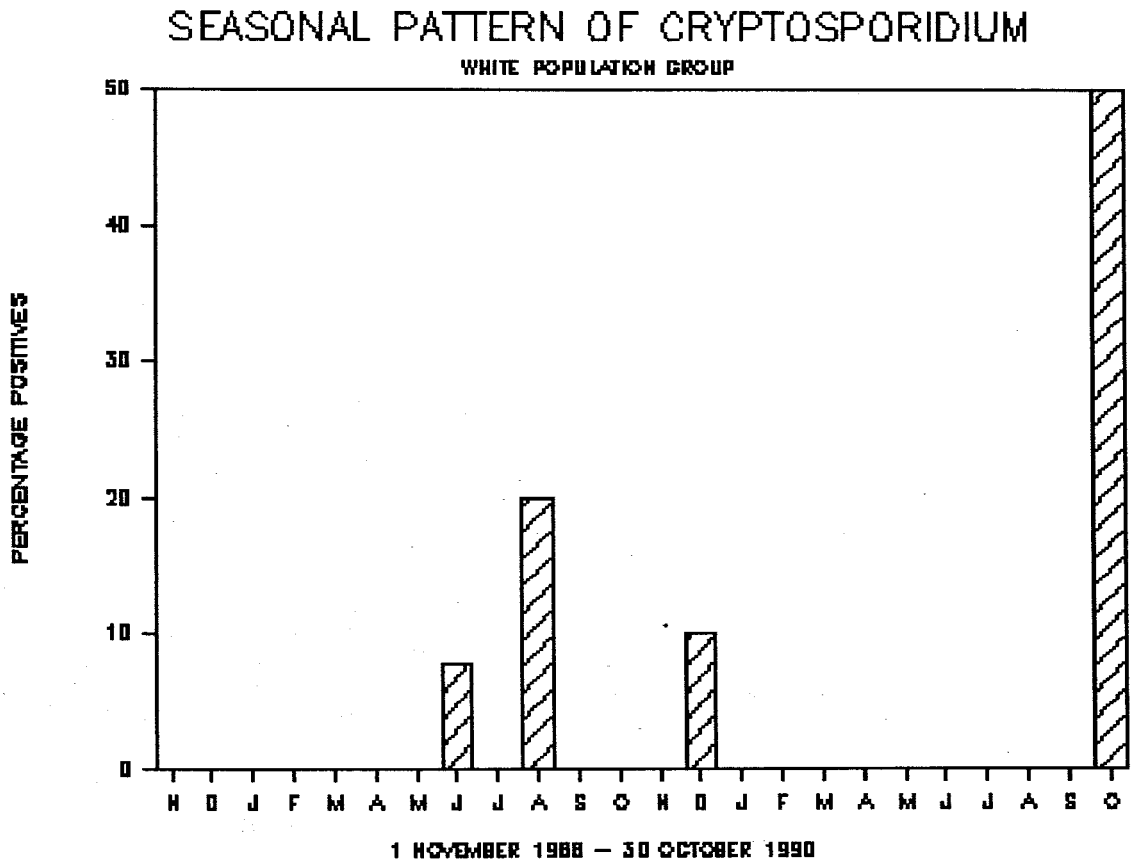

Figure 34 - Seasonal pattern of Cryptosporidium in children <=2 years of age in White population group during the period 1 November 1988 to 30 October 1990.
(See appendix 24)

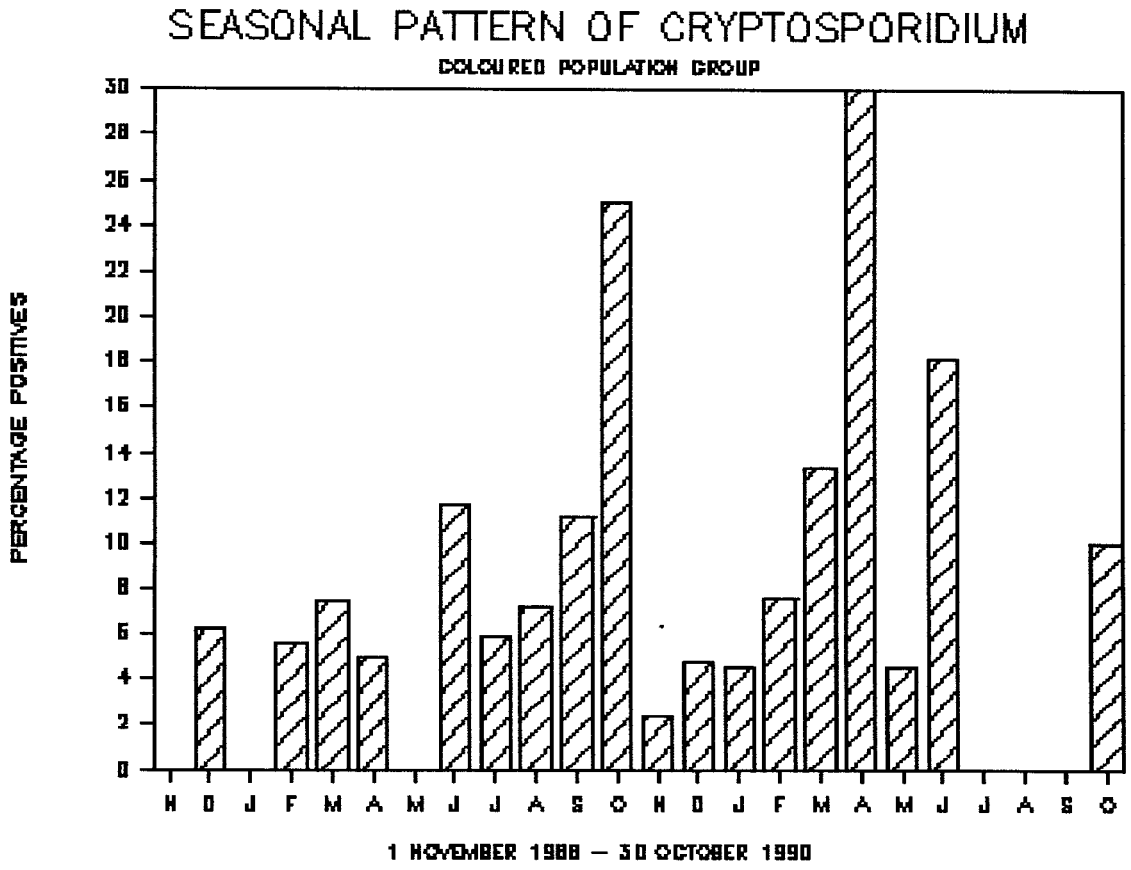

Figure 35 - Seasonal pattern of Cryptosporidium in children <=2 years of age in Coloured population group during the period 1 November 1988 to 30 October 1990. (See appendix 25)

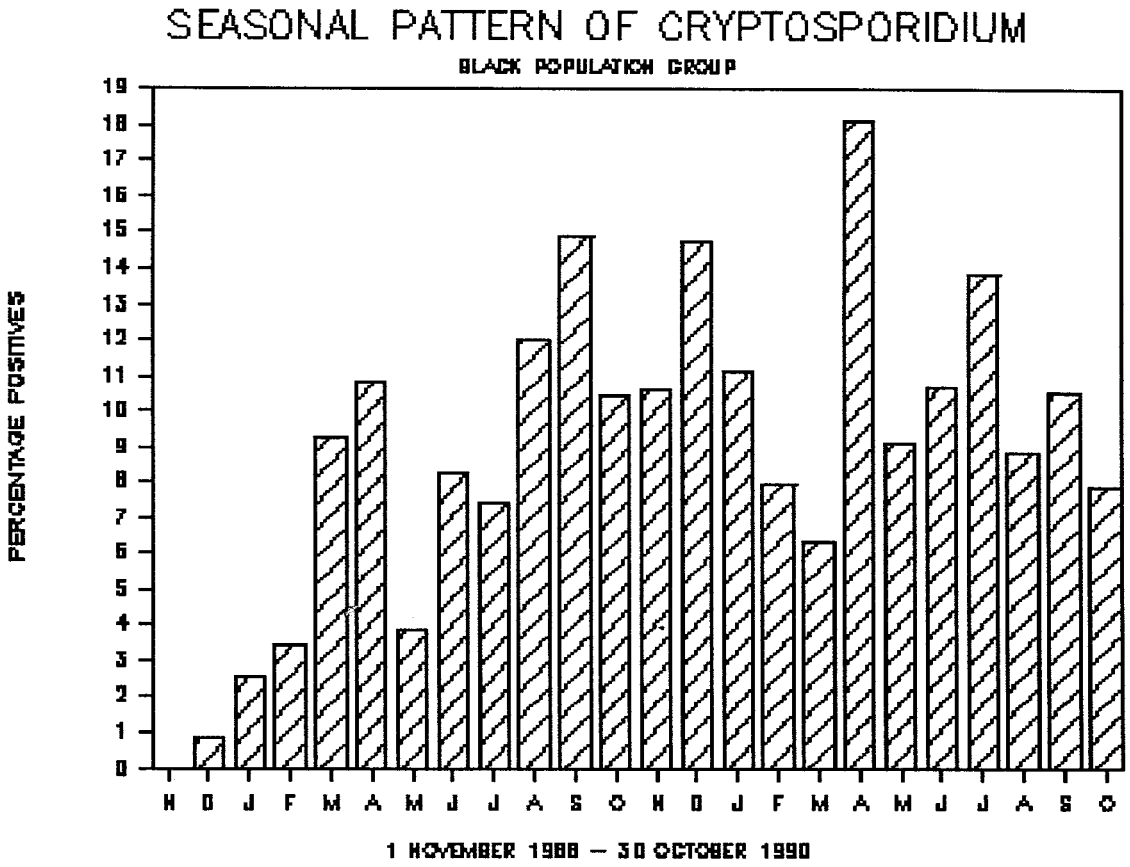

Figure 36 - Seasonal pattern of Cryptosporidium in children <=2 years of age in Black population group during the period 1 November 1988 to 30 October 1990.
(See appendix 26)

3.11. GEOGRAPHICAL DISTRIBUTION

The distribution of enteropathogens detected in the Eastern Cape was problematic as the origin of all the patients was not known. For this reason the enteropathogens detected were listed from the laboratory of origin rather than the specific area. The private laboratories and the S.A.I.M.R. laboratory serving the provincial hospital were combined for this purpose.

In the Cradock area the helminths were the most frequently detected 26/122 (21,31%). *Shigella sp.* were isolated more frequently than any of the other bacteria 30/159 (18,87%) (figure 37).

In the Grahamstown area *Cryptosporidium* oocysts were detected in 10/120 (8,33%), and this is a higher percentage than the helminths 42/606 (6,93%). The bacteria that were the probable cause of the highest incidence of diarrhoea were enteropathogenic *E. coli* 12/295 (4,07%) and *Shigella sp.* 23/606 (3,80%) (figure 38).

Cryptosporidium oocysts were the most frequently detected in the Uitenhage area 13/193 (6,74%). The helminths and protozoa were only detected in a very low percentage 4/532 (0,75%) and 1/532 (0,19%). Enteropathogenic *E. coli* were isolated more often than the other bacteria 14/389 (3,60%) (figure 39).

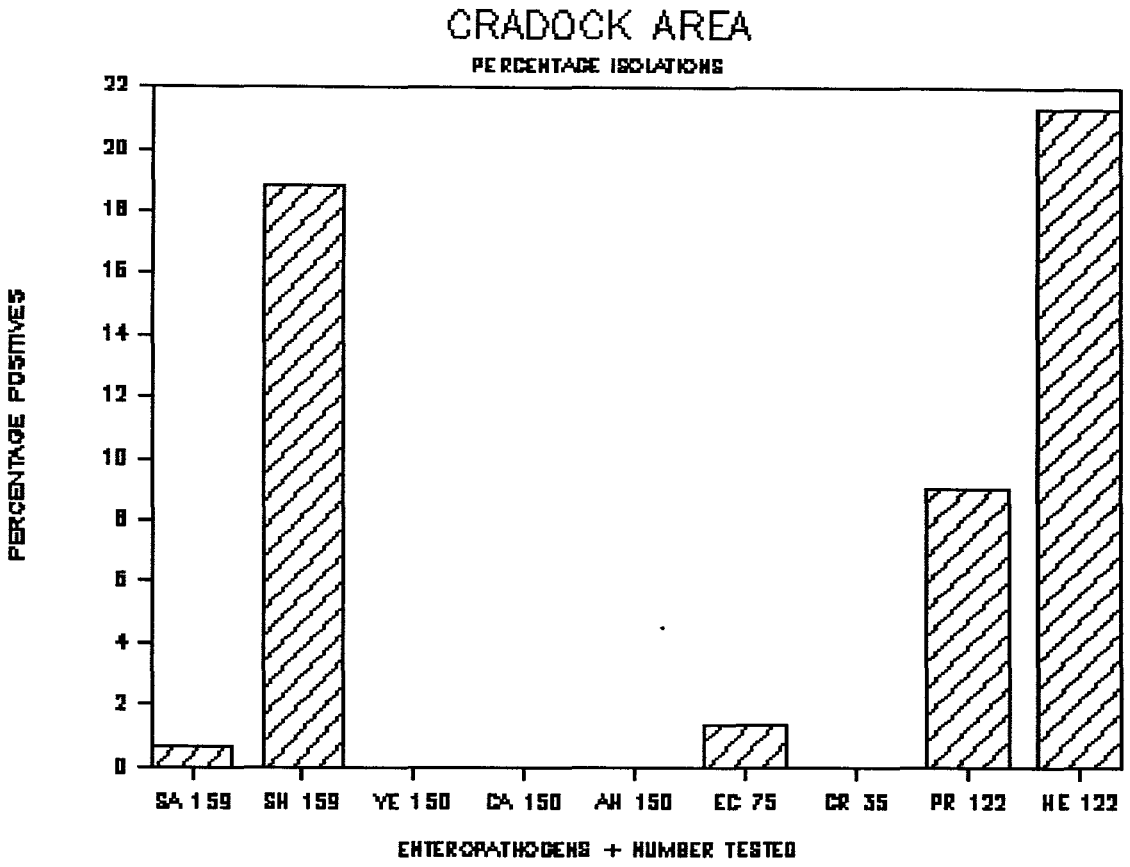

Figure 37 - Enteropathogens detected in the Cradock area in all population groups during the period 1 November 1988 to 30 October 1990. (SA - Salmonella species, SH - Shigella species, YE - Yersinia enterocolitica, CA - Campylobacter species, AH - Aeromonas hydrophila, EC - enteropathogenic E coli, CR - Cryptosporidium oocysts, PR - protozoa, HE - helminths)

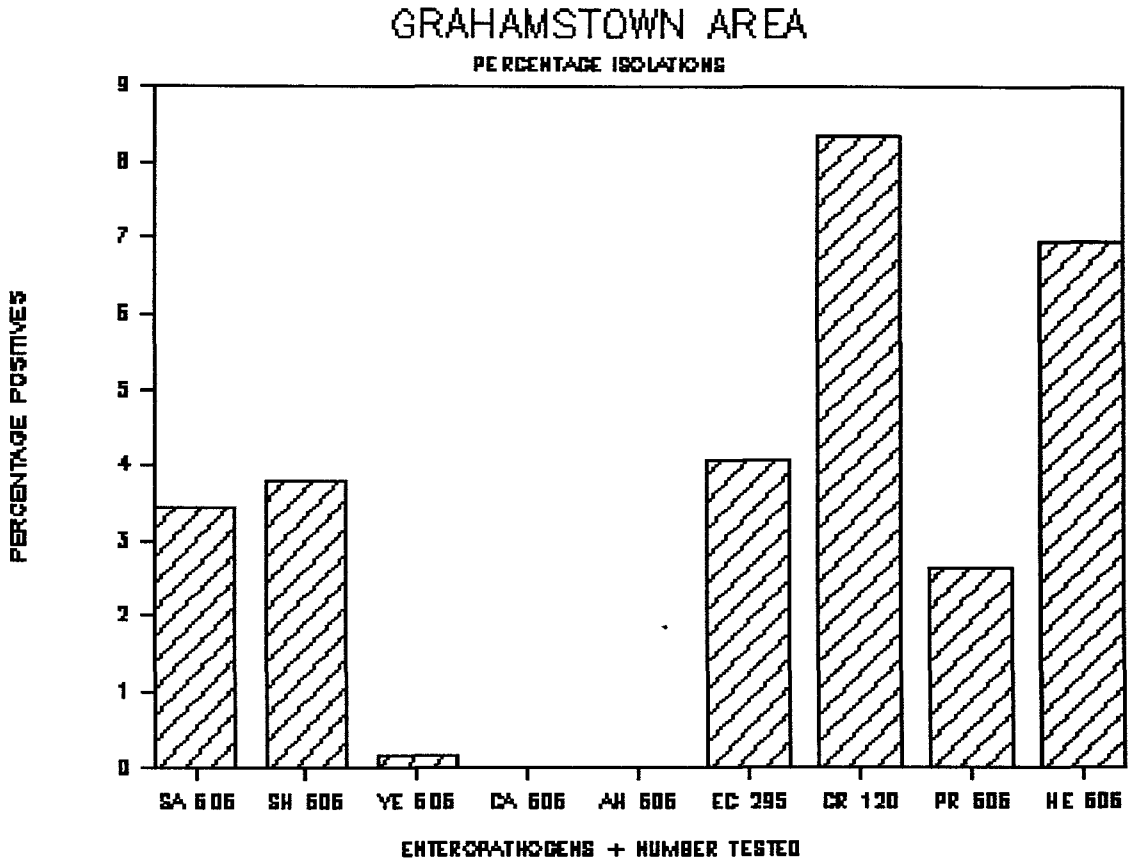

Figure 38 - Enteropathogens detected in the Grahamstown area in all population groups during the period 1 November 1988 to 30 October 1990. (SA - Salmonella species, SH - Shigella species, YE - Yersinia enterocolitica, CA - Campylobacter species, AH - Aeromonas hydrophila, EC - enteropathogenic E coli, CR - Cryptosporidium oocysts, PR - protozoa, HE - helminths)

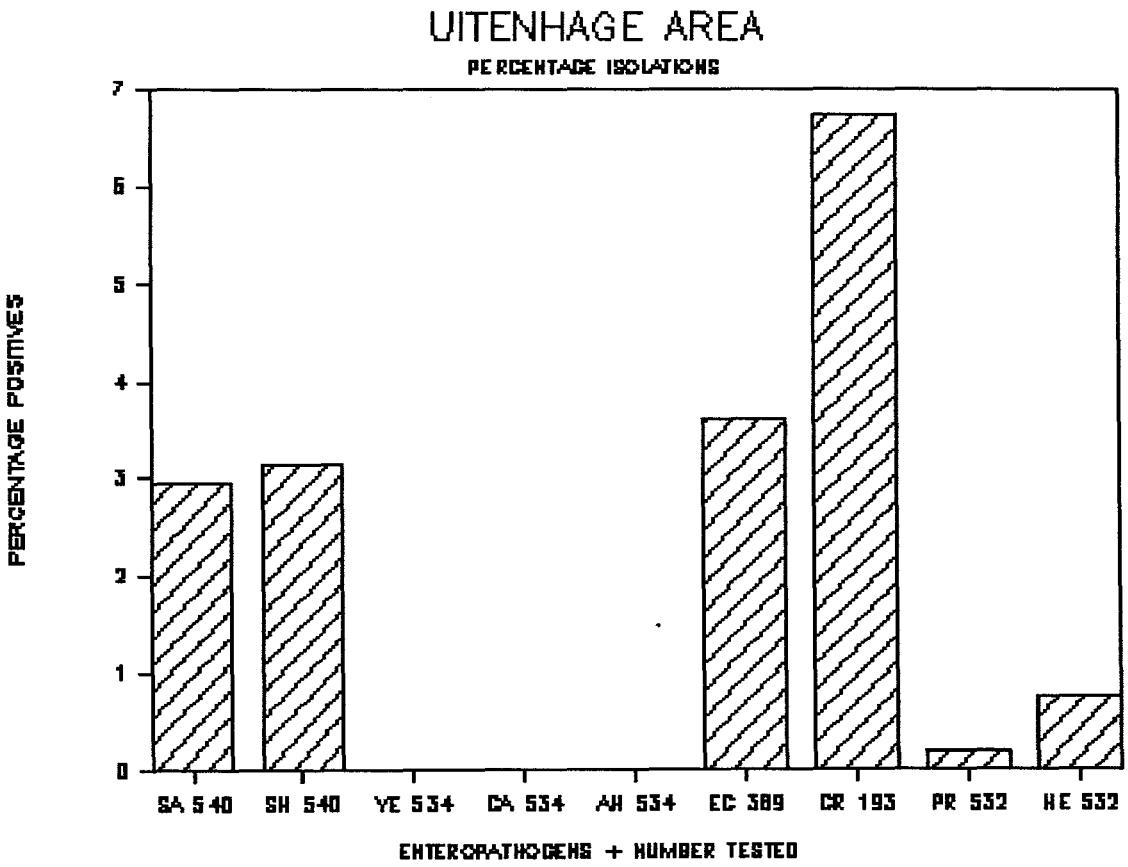

Figure 39 - Enteropathogens detected in the Uitenhage area in all population groups during the period 1 November 1988 to 30 October 1990. (SA - Salmonella species, SH - Shigella species, YE - Yersinia enterocolitica, CA - Campylobacter species, AH - Aeromonas hydrophila, EC - Enteropathogenic E coli, CR - Cryptosporidium oocysts, PR - protozoa, HE - helminths)

In the Port Elizabeth laboratory *Rotavirus* was detected in 4/31 (12,9%) of the faecal samples tested. In these laboratories enteropathogenic *E. coli* were the most often isolated 58/812 (7,14%) (figure 40).

In the Livingstone laboratory *Rotavirus* had the highest percentage of all the enteropathogens 102/76 (13,39%). *Shigella sp.* were isolated more often than enteropathogenic *E. coli* 480/3 926 (12,23%) and 271/2 359 (11,49%) respectively (figure 41).

3.12. SEASONS

TEMPERATURE AND RAINFALL

The information regarding the average monthly rainfall and temperature for Port Elizabeth is noted in figure 42 and 43.

The periods of highest rainfall were recorded during October 1989 and in June 1990. The lowest average monthly temperature was recorded in July 1990 and the highest average temperature in January 1990.

In Cradock the lowest average temperature was recorded in June 1989 and the highest in January 1990. During December 1988 and November 1989 the highest rainfalls were recorded (figure 44 and 45). The information regarding the temperature for Grahamstown is incomplete. The

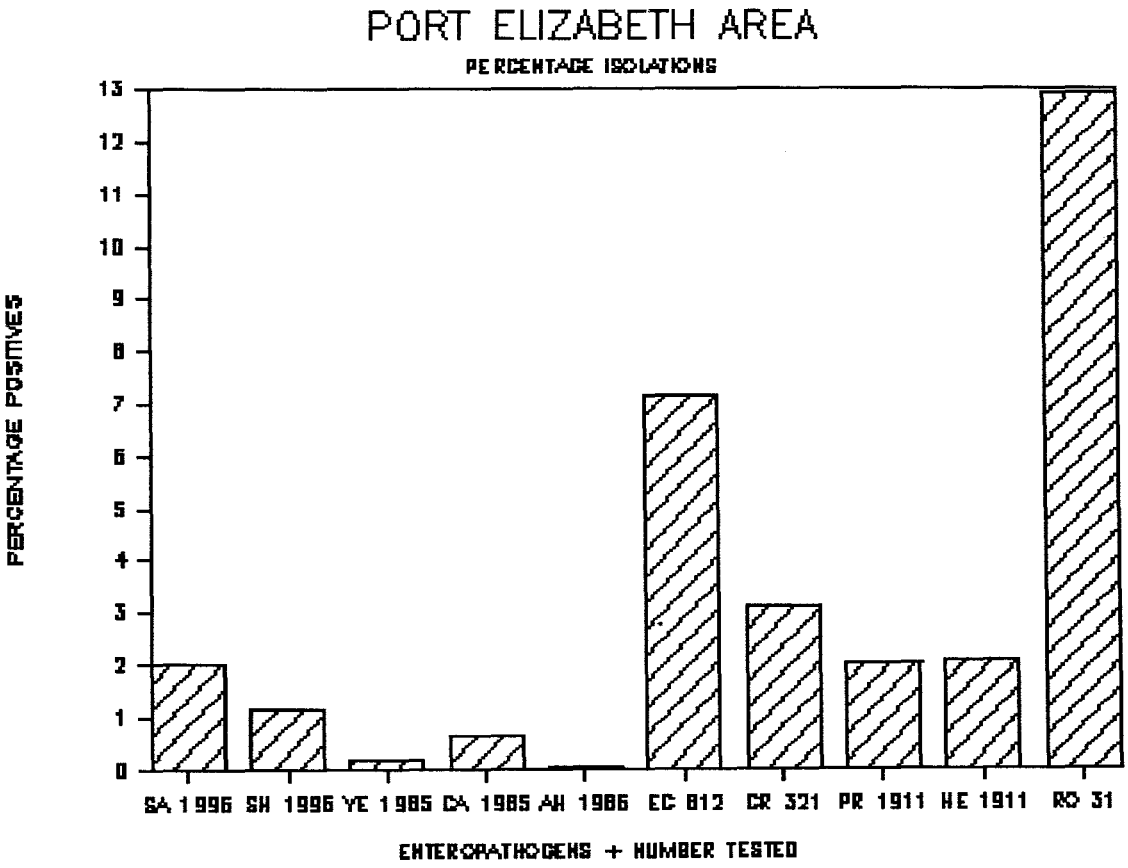

Figure 40 - Enteropathogens detected in the Port Elizabeth area in all population groups during the period 1 November 1988 to 30 October 1990. (SA - Salmonella species, SH - Shigella species, YE - Yersinia enterocolitica, CA - Campylobacter species, AH - Aeromonas hydrophila, EC - Enteropathogenic E coli, CR - Cryptosporidium oocysts, PR - protozoa, HE - helminths, RO - Rotavirus).

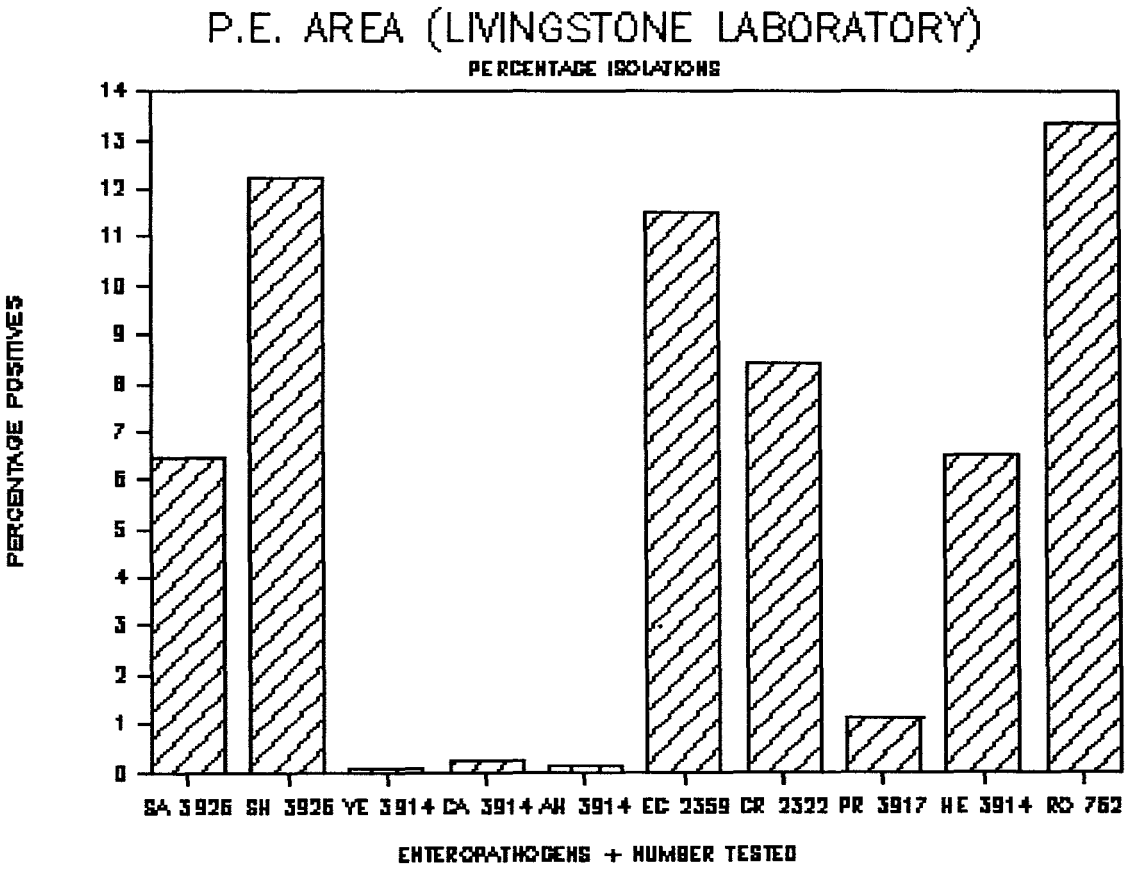

Figure 41 - Enteropathogens detected in the Port Elizabeth area (Livingstone Laboratory) in all population groups during the period 1 November 1988 to 30 October 1990. (SA - Salmonella species, SH - Shigella species, YE - Yersinia enterocolitica, CA - Campylobacter species, AH - Aeromonas hydrophila, EC - Enteropathogenic E coli, CR - Cryptosporidium oocysts, PR - protozoa, HE - helminths, RO - Rotavirus).

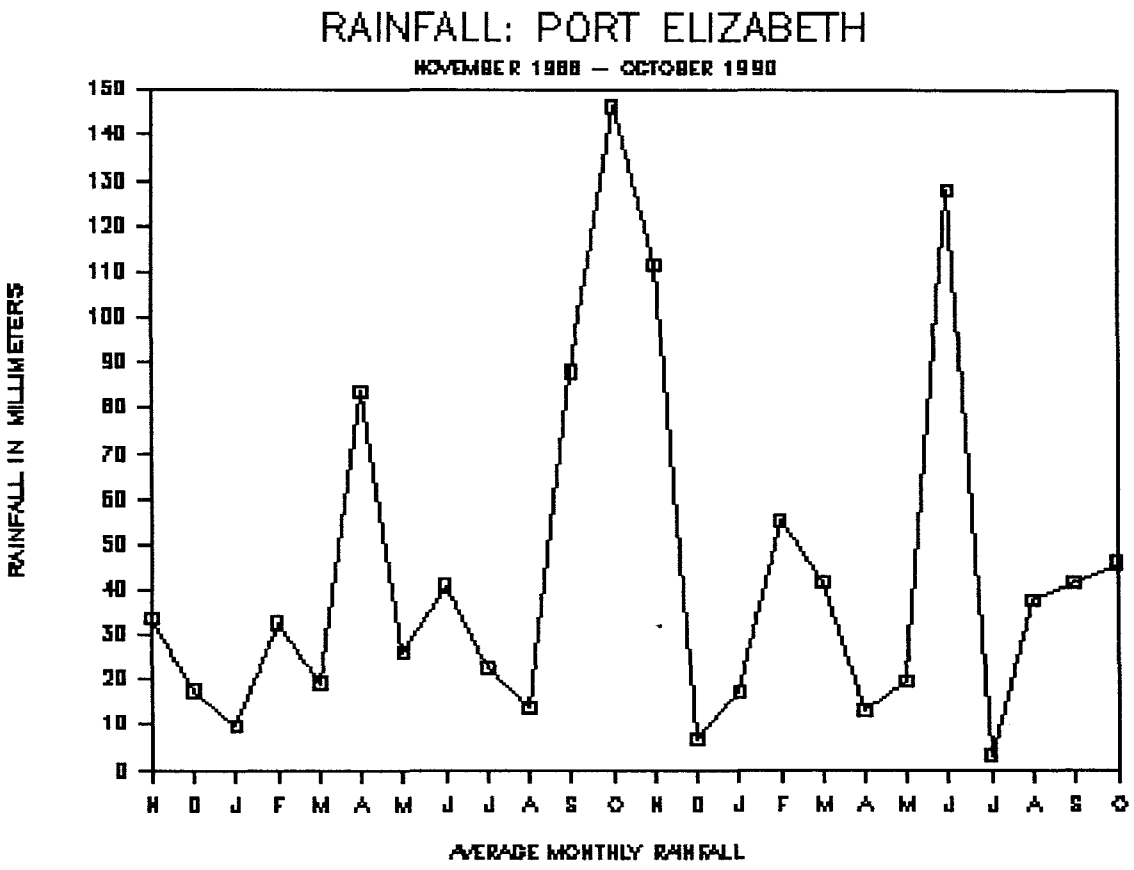

Figure 42 - Rainfall recorded in Port Elizabeth during the period 1 November 1988 to 30 October 1990.

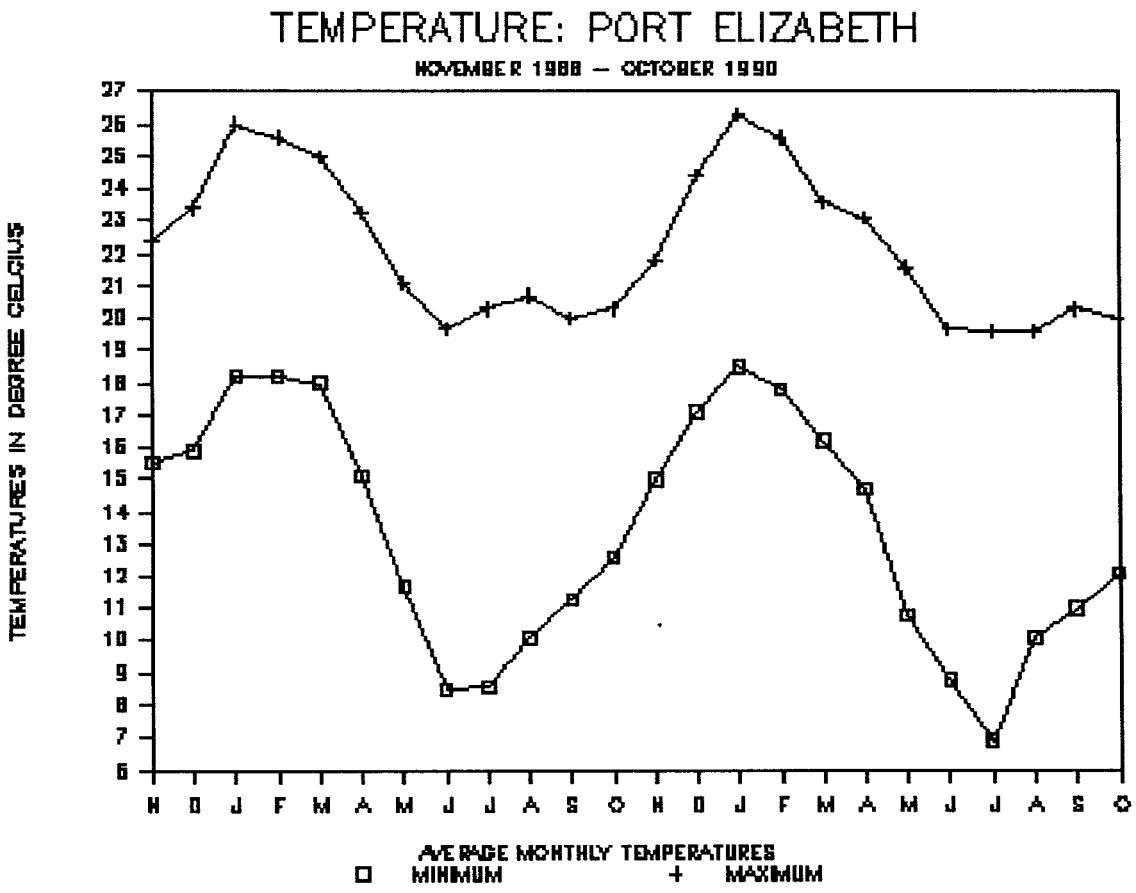

Figure 43 - Average temperature recorded in Port Elizabeth during the period 1 November 1988 to 30 October 1990.

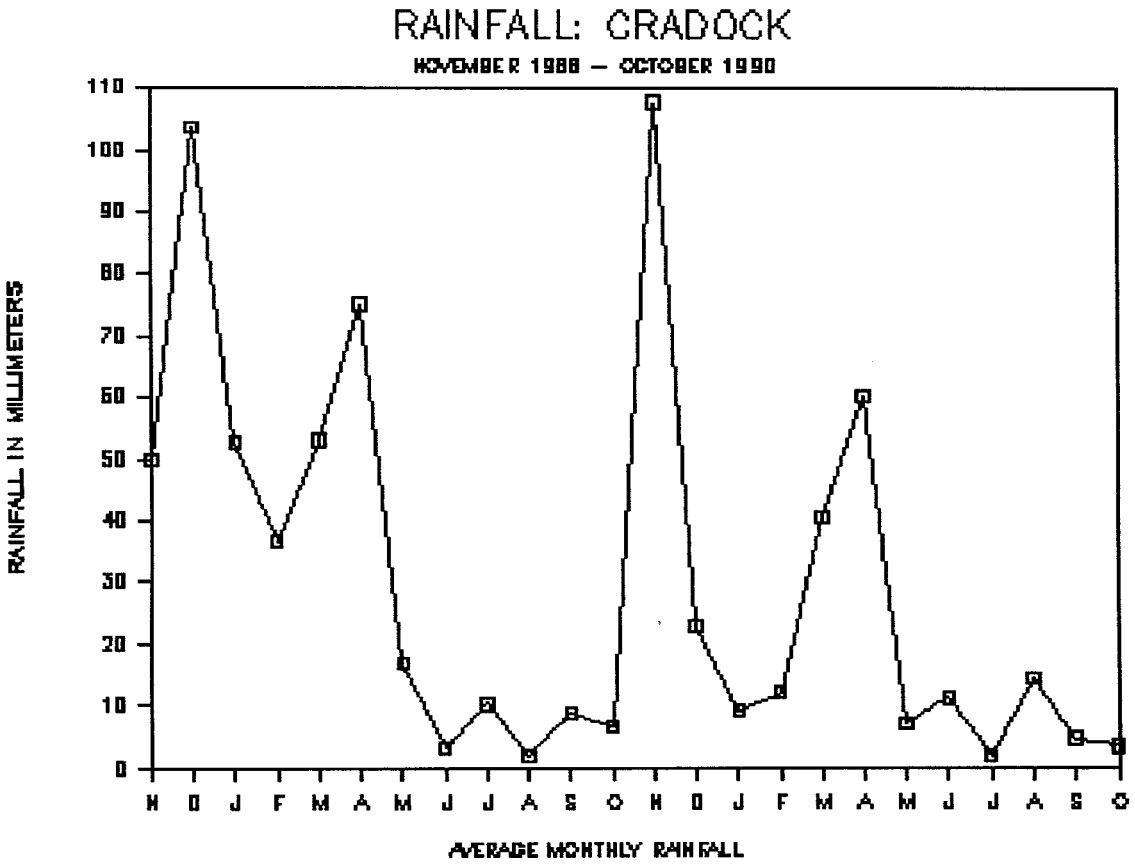

Figure 44 - Rainfall recorded in Cradock during the period 1 November 1988 to 30 October 1990.

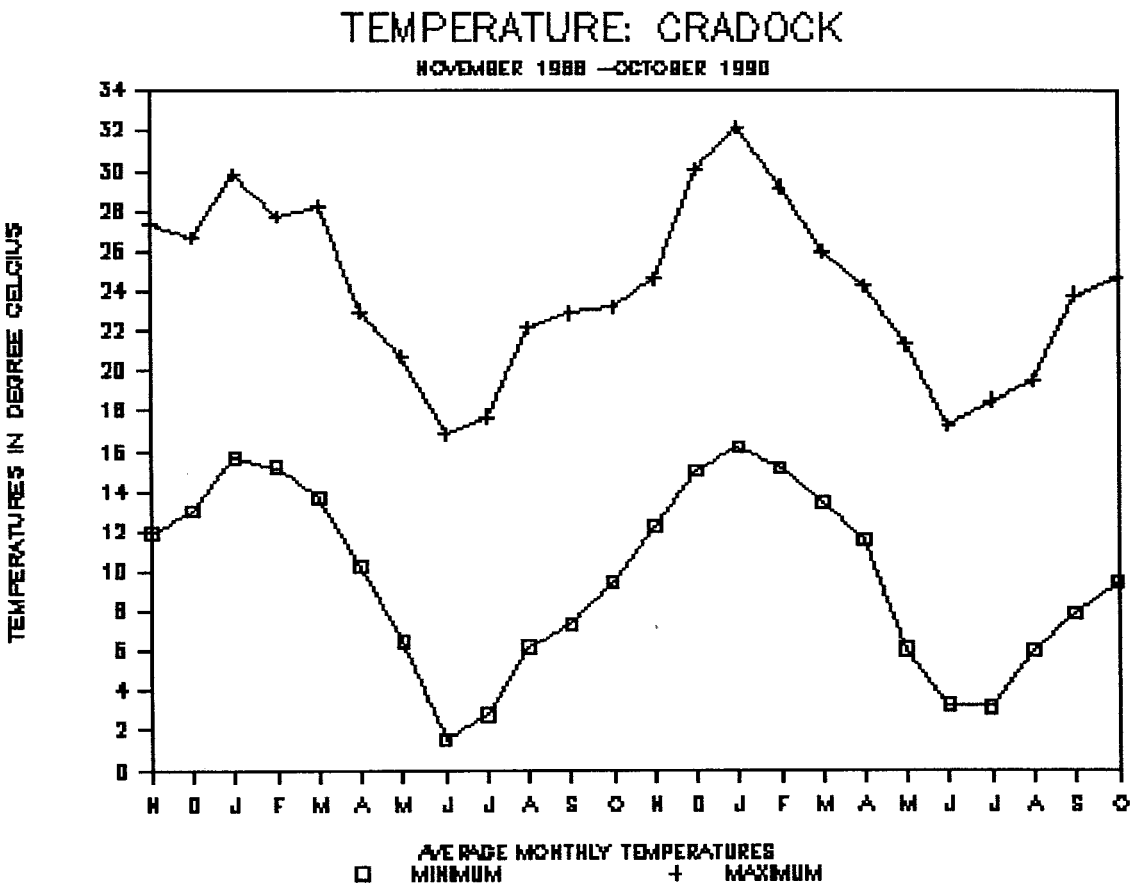

Figure 45 - Average temperature recorded in Cradock during the period 1 November 1988 to 30 October 1990.

information for June 1989, February, March, April, September and October 1990 was not available. In June 1990 records were available for the first 24 days of the months. In July 1990 records were available for the last half of the month and in August 1990 the information was available until the 27th of the month (figure 46 and 47).

The temperature and rainfall for Uitenhage will not be discussed separately due to its proximity to Port Elizabeth.

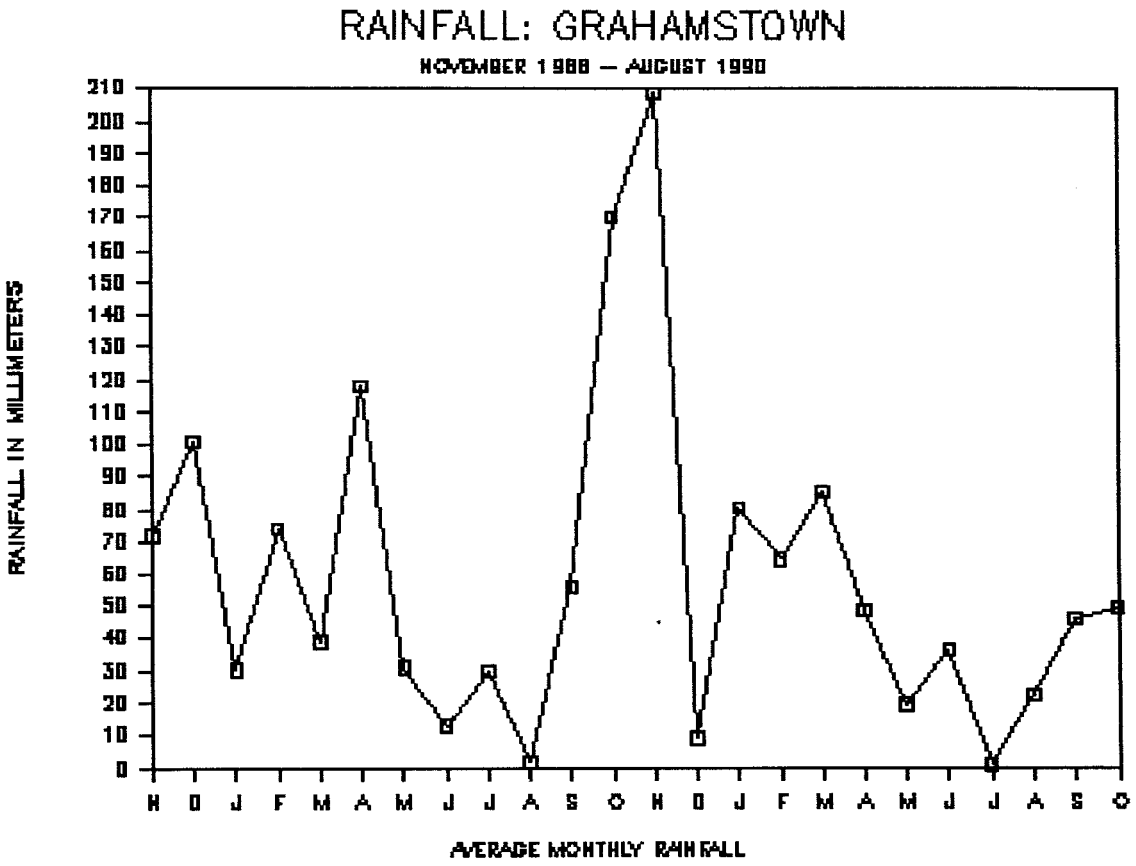

Figure 46 - Rainfall recorded in Grahamstown during the period 1 November 1988 to 30 October 1990

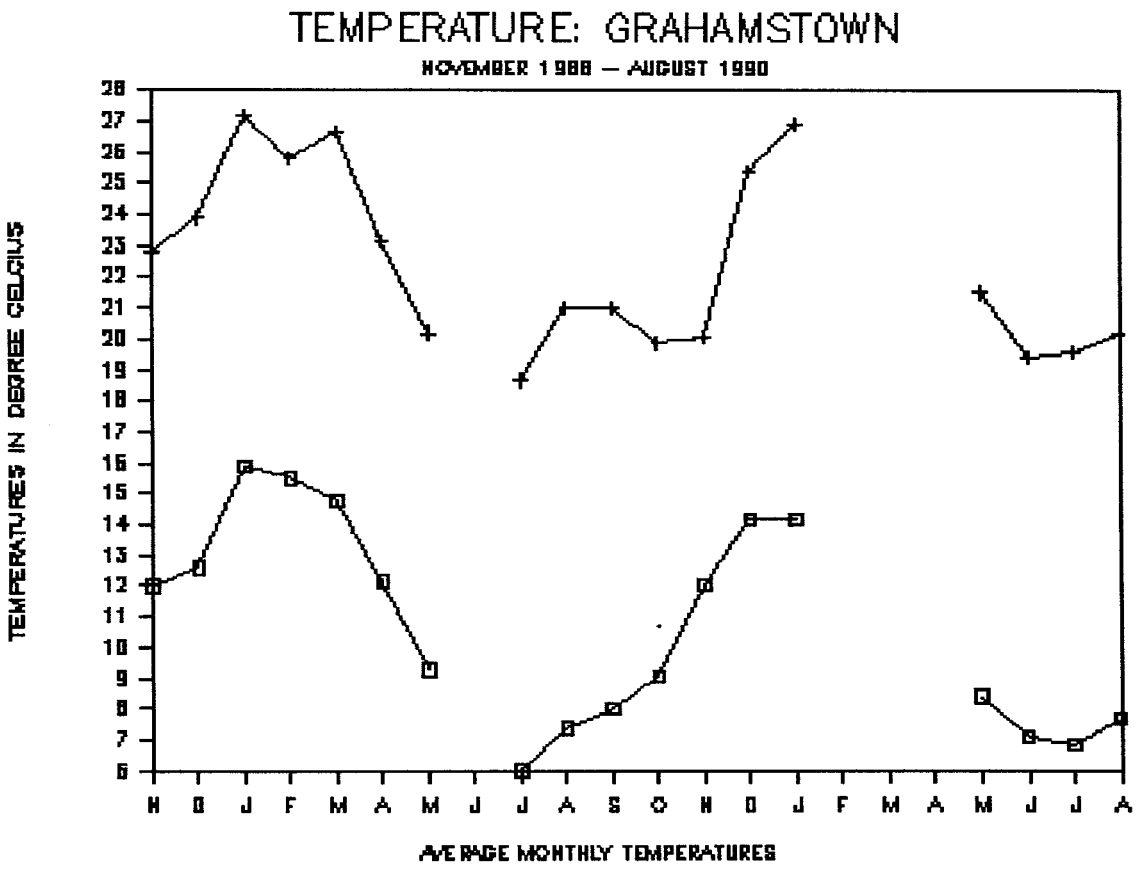

Figure 47 - Average temperature recorded in Grahamstown during the period 1 November 1988 to 30 October 1990.

CHAPTER 4

4. DAY CARE CENTRE SURVEY

4.1. INTRODUCTION

Day care centres play an important part in the present life style of working parents, but also constitute a reservoir for a large number of infectious diseases.

The number of day care centres has steadily increased over the years. In the United States of America an estimated 11 million children attended day care centres in 1975[26].

The number of published articles dealing with the health of children in day care centres has increased since 1974 when the Index Medicus first listed child care as a separate category[131].

Acute diarrhoeal diseases are second only to acute respiratory infections as the most common group of infections in young children[132].

Studies have indicated that diarrhoea occurs more frequently among children at day care centres than among age-matched children in family care[131].

Various factors can contribute to the spread of enteropathogens in day care centres. The children are in close contact with one another, using communal toys and other objects. The incidence of diarrhoea in day care centres is higher in children under three years of age. These are possibly the non-toilet trained children[132] and are at the greatest risk of transmitting diseases spread by faecal-oral route.

In some cases, the staff members of some day care centres are overworked, relatively poorly trained and may inadvertently transmit disease.

Diarrhoeal disease does not only produce morbidity among children in day care centres but also among family contacts. Pickering et al reports that 11% of family members developed diarrhoea associated with the occurrence of gastroenteritis in children in 6 day care centres evaluated for this problem[26].

Shigella sp., *Rotavirus* and *Giardia lamblia*
have been most commonly implicated in causing diarrhoeal disease probably because of the low inoculum necessary to produce disease[131].

In this study the carrier rate of enteropathogens was to be determined as children in day care centres act as reservoirs for some of these pathogens which are apparently well tolerated by the carrier children[107].

4.2. MATERIALS AND METHODS

Two studies were conducted at day care centres of three population groups. The first study was conducted in May 1989 (autumn) and the second study during February 1991 (summer). Stool samples were collected from 52 Black children, 65 Coloured and 47 White children during the first survey and from 49 Black, 55 Coloured and 36 White children during the second survey.

The samples of stools from Black children came from two different day care centres in the two surveys. In both studies the White children were from the same day care centre, the Coloured children attended nine different day care centres during each of the studies.

Stool samples were screened for *Salmonella sp.*, *Shigella sp.*, *Campylobacter sp.* and *Yersinia enterocolitica* using standard bacteriological techniques (See pg 29 of thesis for Materials & Methods).

Stool samples were also examined for intestinal parasites using a concentration technique as well as a wet preparation for motile parasites. A modified Ziehl Neelsen stain was used to detect the presence of *Cryptosporidium* oocysts.

During the first survey parents were requested to complete a questionnaire concerning health status of the children and

their eating habits.(See appendix 27) In certain cases nursing staff assisted in completing the forms.

The consistency of the stool specimens was noted during the second survey.

4.3. RESULTS

The questionnaire to the parents concerning the health of their child was returned for 133 children out of the 164 in the survey. Only 54 of the questionnaires contained information regarding the health status of the child. From these 54 children, 35 were reported as suffering from one or more of the following symptoms, stomachache, vomiting, weight loss or abnormal eating habits e.g. eating sand. Eighteen of these 35 children with symptoms were found to carry one or more pathogens (table XXIII).

In the first survey no pathogens were detected in 29/47 (61,7%) White, 36/64 (56,3%) Coloured and 25/52 (48,0%) Black children. In 49 of all the children single infections were detected. Twenty-five children had double or triple infections (table XXIV).

In the survey undertaken during February and March 1991 no pathogens were isolated from 15/49 (30,6%) Black, 33/55 (60%) Coloured and 20/36 (55,5%) White children. In 44/140 children single infections were detected and in 28/140 double and triple infections were detected.

The faecal samples were graded as formed, semi-formed, soft and fluid. Fifty-six of the samples were formed, 55 semi-formed, 23 soft and 6 samples were fluid (table XXV).

TABLE XXIII - SYMPTOMS AND PATHOGENS DETECTED DURING MAY 1989 AT DAY CARE CENTRES.

SYMPTOMS	ORGANISMS DETECTED	WHITE (n=47)	COL (n=65)	BLACK (n=52)
Chest problems	Giardia lamblia	1	0	0
Continuous cough	Ascaris lumbricoides	0	0	1
	A. lumbricoides + T. trichiura	0	1	0
Abnormal eating habits	A. lumbricoides + T. trichiura	0	1	1
	A. lumbricoides + G. lamblia	0	0	1
Weight loss only	G. lamblia	0	1	1
	T. trichiura + Entamoeba coli.	0	0	1
Stomach-ache only	G. lamblia + Entamoeba coli.	0	0	1
	G. lamblia	0	0	1
	A. lumbricoides + G. lamblia	0	0	1
Stomach-ache with diarrhoea, weight loss.	T. trichiura + G. lamblia	0	2	1
	G. lamblia	0	0	1
	A. lumbricoides + T. trichiura	0	0	1
	G. lamblia + Cryptosporidium	0	0	1

Col = Coloured

TABLE XXIV - RESULTS OF SURVEY AT DAY CARE CENTRES DURING MAY 1989.

ISOLATES	WHITE (n=47)	COL (n=65)	BLACK (n=52)
No pathogens	29	36	25
Giardia lamblia	16	5	6
G. lamblia + T. trichiura	0	2	0
G. lamblia + Entamoeba coli	1	1	1
G. lamblia + Cryptosporidium	0	0	2
G. lamblia + A. lumbricoides	0	0	4
T. trichiura	0	5	1
Shigella sonnei	0	1	0
A. lumbricoides + T. trichiura	0	5	2
Entamoeba coli	1	1	7
Cryptosporidium + A. lumbricoides	0	0	1
A. lumbricoides	0	1	1
T. trichiura + Ent. coli	0	1	1
Campylobacter species	0	0	1
Aeromona hydrophila	0	1	0
Cryptosporidium	0	1	0
T. trichiura + H. nana	0	1	0
T. trichiura + Shigella boydii	0	1	0
Shigella flexneri	0	1	0
A. lumbricoides, T. trichiura + Ent. coli	0	2	0

TABLE XXV - STOOL CONSISTENCY AND PATHOGENS DETECTED AT DAY CARE CENTRES DURING FEBRUARY 1991.

ISOLATES	CONSISTENCY	WHITE (n=36)	COL (n=55)	BLACK (n=49)
No pathogens		20	33	15
Giardia lamblia	Soft	0	1	2
	Semi	2	6	3
	Form	7	2	2
	Fluid	0	0	1
	TOTAL	9	9	8
Ascaris lumbricoides	Soft	1	2	4
	Semi	1	0	0
	Form	1	0	0
	TOTAL	3	2	4
G. lamblia + T. trichiura	Soft	0	0	2
A. lumbricoides, T. trichiura + Ent. coli	Semi	0	0	1
A. lumbricoides + G. lamblia	Semi	2	0	2
	Form	1	0	0
	TOTAL	3	0	2
A. lumbricoides + T. trichiura	Soft	0	1	0
	Semi	0	1	7
	Form	0	0	1
	TOTAL	0	2	8

TABLE XXV - CONTINUED

T. trichiura, G. lamblia + Cryptosporidium	Soft	0	0	1
T. trichiura, G.lamblia + Ent. coli	Semi	0	0	1
T. trichiura	Form	0	1	1
Blastocystic hominis	Semi	0	1	0
	Form	0	0	1
	TOTAL	0	1	1
Ent. coli	Soft	0	0	1
	Form	1	1	1
	Fluid	0	0	1
	TOTAL	1	1	3
G. lamblia + Ent. coli	Semi	0	0	1
T. trichiura + H. nana	Soft	0	1	0
T. trichiura, H. nana + Cryptosporidium, Ent. col	Soft	0	1	0
Salmonella enteritidis	Semi	0	1	1

CHAPTER 5

5. DISCUSSION

While the overall isolation rate for enteropathogens found in this study falls generally within the rate seen in other countries, there were considerable differences in the percentage positive findings in the various population groups in the Eastern Cape.

These differences indicate that there are marked variations in the life-style, living conditions and environmental circumstances.

5.1. SALMONELLA SPECIES

The isolation rate of *Salmonella sp.* in the Black population group compares with the other studies done in South Africa. In 1977 an isolation rate of 7,9% was reported from Baragwanath Hospital in Johannesburg[19]. In 1986 Smith and van den Ende reported finding 12% of the samples examined in Natal positive for *Salmonella sp.*[119].

In other African countries e.g. Nigeria the isolation rate reported in 1985 was 4,9%[103] and in Kenya 3,7%[120].

This is similar to the findings in the Coloured population group of 3,19%. In other countries e.g. Sweden a 5% rate was detected[121], Australia 5,7%[122] and in Britain, in the city of Liverpool, 6,1% of samples were positive[120]. Lower isolation rates were reported from the Central African Republic [110] and Bangladesh[75] being 1,5% and 1% respectively.

In this study out of the four areas, Cradock, Grahamstown, Uitenhage and Port Elizabeth, the percentage isolation rate at Cradock was the lowest at 0,6%. This can be compared to the findings of the studies made in Bangladesh.

The isolation rate at the Livingstone laboratory was very much higher being 6,4%, but the rate for the rest of the Port Elizabeth area was 2,0% slightly lower than that of Grahamstown at 3,47%.

When considering the distribution of *Salmonella sp.* according to age, it was noted that no infections were detected in the samples submitted from the White population in the age group >12-18, but only 30 samples were examined. Children under this age group had an infection rate almost twice that of the White adults. Too few samples from the Asian population group were examined for comment. In the Coloured population group children, in the age range 12 and under had the highest percentage isolation. The same was true for the Black population group but the percentage of the children infected was about

twice as high at 7,8%. The percentage of positive findings of *Salmonella sp.* among the White, Coloured and Black population groups was approximately in the ratio 1:2:3 respectively with a significantly higher percentage of infection occurring in the children.

Salmonella sp. were isolated in this study throughout the year. The higher incidence was recorded in spring 1989 and during winter in 1990. The peak periods of isolations in the White and Coloured population groups differ from those in the Black population group. In 1989, a peak in the seasonal pattern was seen in summer and in 1990, autumn had the higher percentage positives. In the Black population group the higher incidence was recorded in spring 1989 and in winter 1990. This pattern is similar to elsewhere in South Africa. In Johannesburg[19] *Salmonella sp.* were more frequently recovered in the cooler months and in Cape Town it was less common in summer. In Egypt[111] *Salmonella sp.* were also isolated more frequently in the cooler months.

Salmonella typhiminium was the most frequently isolated in all the population groups. It has been reported that *Salmonella enteritidis* is the cause of outbreaks of gastroenteritis in many parts of the world[18]. Other *Salmonella sp.* have also been isolated during outbreaks of diarrhoea. In the Cape Flats Salmonella group C1 (6,7:d:1,5) was predominant in an outbreak of diarrhoea and made up 49% of all the Salmonella isolates[130].

5.2. SHIGELLA SPECIES

Shigella sp. have a well established role in the aetiology of diarrhoea.

In this study the overall isolation rate was 8,54% which for bacteria is second only to the enteropathogenic *E. coli*. The Asian population group had the highest percentage positives, but the number of samples tested was limited. The Black population and Coloured population group had a higher isolation rate than the White population group. This compares to surveys done in developed and developing countries. In Australia a 1,3%[122] was reported and in Sweden 0,7%[121]. In Africa, Nigeria reported 9,4%[109] and Zimbabwe 5%[13] isolation rates.

In South Africa other workers have reported rates between 4 and 8%[11,19,124]. In Durban a low incidence was reported in 1986[119].

In the Uitenhage and Grahamstown areas low percentage isolations of *Shigella sp.* were recorded. In Cradock the isolation rate was 18,9% the highest for bacterial infections. The percentage of *Shigella sp.* isolated at Livingstone was 12,2%. Again the highest of the bacteria at this laboratory.

In all the population groups the isolation rate of *Shigella sp.* was the lowest in the infants, <=2 year

old group. This differs from the other bacterial infections where the infants were more affected.

In both the White and Black groups seasonal peaks were detected in the summer months. This is similar to reports from Cape Town[11] and Egypt[3]. The Coloured population had the higher incidence in the winter months. This correlates with the findings in the Black group in Johannesburg[19].

Shigella flexneri was the most frequently isolated in all the population groups. In the <=2 year old group in the White population *Shigella sonnei* was detected more frequently. *Shigella boydii* was not detected in the White and Asian group and also had the lowest percentage in the other population groups.

5.3. YERSINIA ENTEROCOLITICA

The world wide incidence of *Yersinia enterocolitica* diarrhoea is low.

In this study the White population group had the highest incidence 4/1 761 (0,23%) compared to the Coloured and Black population groups with 1/1 268 (0,07%) and 3/4 140 (0,07%) respectively. *Yersinia enterocolitica* has been reported in only a few surveys. The highest incidence was reported from Sweden[121] where an incidence of 5,6% was detected in inpatients and 1,5% in

outpatients. In a later study in 1988[123] a 3% isolation rate was reported. In 1985 Nigeria reported 1,4%[103] which is the same as Italy in 1987[34].

The high incidence in Sweden could be related to their living conditions during winter when farmers drive their pigs into their houses for shelter. In South Africa it may be that the higher incidences of *Yersinia enterocolitica* could be related to the keeping of pigs and the eating of pork.

The age groups mostly affected were the <=2 year olds and the >18 year old group in both the White and Black population groups.

Seasonal patterns may play a role in the infection, but due to the limited number isolated in this study a reliable pattern could not be discerned.

5.4. CAMPYLOBACTER SPECIES

Campylobacter sp. are more prevalent in developing countries than in developed countries[52,53]. This can be noted by the high isolation rate in Bangladesh with 14%[75] Kenya 9,9[120], and Zimbabwe 8%[13]. In South Africa high isolation rates were also reported from Cape Town 18%[11], Soweto 35% and 31%[57] respectively and Ga-Rankuwa 16%[124].

In this study the isolation rate was low compared to other centres in South Africa. In 1986[119] another low rate of

1,7% was reported from Durban. In this study the White population group has the highest percentage of 0,68%. This is lower than the reported studies from developed countries. Australia reported 7,4% in 1983[122] and Britain, in the city of Liverpool 5% in 1986[117]. The incidence reported in Sweden differs between two studies in 1981/1982 from 2,8% reported in the one study[121] to 18% reported in the other study[123].

The majority of isolates in this study was detected in the younger children <=2 and adults >18 years. In the Black population group all the isolates were found in the young children. In the White population group the adults were more affected. The probability of self-infection in children from contaminated soil has been suggested[58].

Campylobacter sp. were not isolated in the areas outside Port Elizabeth.

Seasonal variance was not detected due to the limited number of isolates.

5.5. AEROMONAS HYDROPHILA

A high percentage of ***Aeromonas hydrophila*** isolates were reported from first world countries. In 1983 Australia reported 10,8% in patients and only 0,7% in the control group of patients[122]. In a study done in the city of Liverpool in 1986 13,7% of the faecal samples were positive for ***Aeromonas hydrophila***[117].

In this study the 0,08% of isolates detected is much lower than the findings in Kenya[120] of 2,5% and in Egypt of 0.8%[111]. ***Aeromonas hydrophila*** appears to be more prevalent in the first world countries as it was not reported in many of the studies done in Africa and South Africa. In the studies reported from the third world countries no special selective media were used for the isolation of ***Aeromonas hydrophila*** and this could explain the low isolation rates and the absence of reports on ***Aeromonas hydrophila***.

In this study the isolates of ***Aeromonas hydrophila*** were detected in the Coloured and Black population groups. The majority of isolates appeared in the over 18 year old group. The significance of these isolates is unknown. In the countries with a high prevalence of ***Aeromonas hydrophila*** a higher incidence was detected in late summer[70,111]. In this study no seasonal pattern could be determined due to the low percentage of isolates.

5.6. ENTEROPATHOGENIC ESCHERICHIA COLI

Enteropathogenic *E. coli* are known to cause diarrhoea and gastro-enteritis in infants and young children world wide.

In this study enteropathogenic *E. coli* displayed the highest isolation rate for bacterial infections. The Black population group had the highest percentage of positives (10,3%). The White population group had a 8,37% isolation rate compared to the 4,85% of the Coloured population group.

In South Africa the isolation rates varied in different surveys. A high isolation rate of 28,8% was reported in 1977[19]. In the winter of 1981 an average isolation rate of 18% was recorded from surveys done at three different hospitals in Joahnnesburg[55]. Studies at Ga-Rankuwa[124] reported a 36% isolation[124] rate and Cape Town reported 40,2% with 27% positive in healthy controls[11]. In other developing countries high isolation rates have also been reported. Bangladesh reported 20%[74] and Zimbabwe 11% in 1987[13]. This might confirm the suggestion that enteropathogenic *E. coli* are more common in developing countries. Low isolation rates were reported from Australia with 1,9%,[122] Liverpool 4,1%[117] and Sweden 4,2%[121]. However, in certain developing countries low isolation rates have been reported and also elsewhere in South Africa. Kenya reported 1,2%[120] and

in Cape Town during 1991 a 7% isolation rate with 1% carrier rate was reported (D. Coltman. Personal Communication). As shown in this study the Black population group was more affected than the White population group, yet the Coloured population group had the lowest percentage of enteropathogenic *E. coli*. These findings may indicate that infection with enteropathogenic *E. coli* is dependant on many factors and not only on living conditions and the immediate environment.

In the Uitenhage, Grahamstown and Port Elizabeth laboratories enteropathogenic *E. coli* were the most frequently detected of the bacterial infections. At the Cradock and Livingstone laboratories this was second to *Shigella sp.*

The seasonal incidence of enteropathogenic *E. coli* differs between the different population groups. In 1989 the Black and Coloured population groups showed isolation peaks in winter and the White population group a peak in summer. A summer peak was also noted in Johannesburg[19] and Cape Town (D. Coltman. Personal Communication). In other countries a summer peak was also detected[121]. In 1990, however, an overall peak was detected in autumn, with no isolates in the Coloured population group in winter and with the Black population group only slightly higher in autumn and winter. In rural Egypt no discernible seasonal pattern was detected[3].

5.7. ROTAVIRUS

Since the discovery of human ***Rotavirus*** in 1973[80] it has been identified as possibly the most important aetiologic agent in acute non-bacterial gastroenteritis among infants and young children world-wide[82,83]. During the time of the study, screening for ***Rotavirus*** was not being carried out routinely but only on special request by the doctors concerned. Therefore permission was obtained to screen faecal samples received at the Livingstone laboratory for ***Rotavirus***.

The ***Rotavirus*** showed the highest overall percentage of positives. This compares with findings elsewhere in South Africa and other countries. In 1986 22,8% was reported for Durban[119]. Steele et. al. (1986) reported an isolation rate of 32,8%[101] from Ga-Rankuwa hospital in 1982 and 23,1% during 1983 to 1985[102]. In 1990 Cape Town reported an 18% isolation rate with the control group showing <1% (D. Coltman. Personal Communication). In both developed and developing countries a high percentage of positives were reported. Australia reported 12,7%[122], the city of Liverpool 33,5%[117] and Bangladesh 15,5%[126] in 1990.

In this study the White population group had a higher isolation rate, but only limited samples were tested. The routine testing for ***Rotavirus species*** should be introduced as it is possibly an important agent in the cause of diarrhoea among all the population groups. Reports from elsewhere have described a seasonal

distribution of *Rotavirus* incidence with a peak during winter months[99,100]. In South Africa studies conducted in and around Johannesburg have indicated that a peak in the colder months was detected in the White population group and that the Black population have a low threshold constant throughout the year[105].

Ka-Ngwane[81] also reports the higher incidence in winter. A study done in Cape Town reported the higher incidence in autumn and lower in the winter months[82]. In this study in 1989 the highest incidence in the Black population was reported in winter and in 1990 the isolation rate in summer and autumn were higher than the winter of 1989. This might indicate an increase of infection in 1990. In the Coloured population *Rotavirus* was detected only in the warmer months.

5.8. **INTESTINAL PARASITES**

The severity of infestation with intestinal parasites as well as the clinical picture may vary from one part of the world to another. This may be related to age, nutritional status, the immune response of the host as well as the pathogenicity of the parasites. *Trichuriasis* is a common infestation found in the tropics and the carrier rate is high. Infestation with over 5 000 eggs per gram of faeces is considered pathogenic. Most of the people with egg counts above 20 000/g of faeces develop severe diarrhoea or dysentery[140].

5.9. PROTOZOA

Giardia lamblia and *Entamoeba histolytica* are known to have high carrier rates in certain countries especially those in the tropics. Isolation rates between 26% and 44% were reported for *Giardia lamblia* from different countries in Africa. [13,109,110,111].

In this study the total percentage of protozoa detected in all the population groups was 1,5%. This compares with the lower percentage of 3%[124] found in Ga-Rankuwa. The highest percentage occurred in the White population group. *Giardia lamblia* were the most commonly detected.

The >2 to 12 year old age group had the higher percentage positives. The spread of *Giardia lamblia* in day care centres is well documented and this might be the cause of the higher number of *Giardia lamblia* detected in the White population. (See Chapter 4, pg 107 on day care centres)

The Cradock and Grahamstown areas had higher isolation rates than the other areas. This is probably due to the same conditions that are mentioned with helminths infestations.

Protozoa were detected throughout the two years. In the White population a seasonal incidence occurred. The

majority of protozoa were detected in the warmer months. A decline in incidence was also detected in 1990. In the other population groups protozoa were detected throughout the year with the Coloured population showing a higher percentage in the cooler months.

Entamoeba histolytica were very seldom detected. High isolation rates are reported from the tropics where asymptomatic carriers are the main source of contamination[114].

5.10. **HELMINTHS**

In this study 5,17% of samples examined were found positive for helminths in all population groups. The higher incidence of helminths in the Coloured population could indicate a high carrier rate and that environmental factors may contribute to infestation. The highest incidence of helminths occurred in children between >2 and 12 years of age who are known to be the most commonly affected. The same age group had the highest percentage of infestation in the Black population group. The infestation in the White population group is low and this could indicate a difference in living conditions. This is emphasized by the fact that Cradock had the highest infestation and in Grahamstown the infestation with helminths is second only to ***Cryptosporidium*** infection. Perhaps in the rural areas poorer living conditions, lack of potable water supplies, bad sanitation

and little available health education contribute to the spread of parasites.

Helminths were detected throughout the two year study period but infestation in the White population group declined in 1990 whereas in the Coloured and Black population groups the percentages of helminths detected were higher than in 1989.

Overall *Ascaris lumbricoides* infestation was the most frequently detected in all the population groups. In the >2 to 12 year old age group, in the Coloured population, *Trichuris trichiura* was more frequently detected. Infestation rates with *Trichuris trichiura* reported from the Central African Republic are lower than found in this study[110]. In Namibia, however, 35% of 31 Bushmen tested were found to have *Trichuris trichiura* infections[125].

In another study conducted in the Kavango area no *Ascaris lumbricoides* or *Trichuris trichiura* were detected. It was noted that the arid climatic conditions probably present very narrow limits for the survival of these two parasites[125].

Percentage infestations with *Taenia species* and *Hymenolepis nana* were low in all the population groups.

5.11. CRYPTOSPORIDIUM OOCYSTS

Since the increased awareness of *Cryptosporidium sp.* in the early 1980's[114] several studies have shown that *Cryptosporidium sp.* are a frequent cause of diarrhoea[116,117] especially in children in the developing countries[119].

This is confirmed by the results in this study. In the Black and Coloured population groups the incidence is much higher than in the White population group. A study conducted at Ga-Rankuwa in 1990 reported that in 24% of the faecal samples *Cryptosporidium* oocysts were detected[124]. In the Grahamstown and Uitenhage area *Cryptosporidium* oocysts were the most frequently detected enteropathogen in all the faecal samples screened. The samples in the Cradock area were not screened for *Cryptosporidium* at the time of the study. *Cryptosporidium* oocysts were also the most frequently detected parasite in samples examined at the Livingstone laboratory.

The 9,4% prevalence of *Cryptosporidium* in the Black population group is lower than reported previously in this area[127]. Higher rates of 18,4% have been recorded at Baragwanath Hospital and 15% at King Edward Hospital[119,128]. In the developed countries reported incidence has been low[114].

Cryptosporidium appeared more frequently in the summer months at Ga-Rankuwa and in Cape Town it was more prevalent in autumn. In the Black population group it was detected more in spring in 1989 and in the winter of 1990. The Coloured population group had the higher incidence in the winter of 1989 and in the autumn of 1990. This was similar to the findings in Cape Town.

5.12. TEMPERATURE AND RAINFALL

The temperature and rainfall were recorded for the study period to determine whether a climatological corrolation could be observed in relation to seasonal incidence.

Temperature and rainfall patterns might influence the spread of certain enteropathogens in the Eastern Cape more in one population group than others.

In Port Elizabeth the greater amounts of rainfall were recorded during spring in 1989 and during the winter in 1990. This matched the seasons when the higher isolation of *Salmonella sp.* were recorded among the Black population group. The same did not apply for the Coloured and White population groups where the higher incidence occurred in summer and autumn. The availability and use of water in streams and pools, contaminated with *Salmonella sp.* during these times, might be considered as a source of infection.

The same suggestion might apply to the isolation of *Shigella sp.* to a certain extent. Although the isolation of *Shigella sp.* in the Black population group was more common in the summer months, the month with the highest isolation rate was June 1990 which also recorded the highest rainfall in Port Elizabeth. In the Coloured population group the highest incidence of *Shigella sp.* was also associated with the highest rainfall.

In the Grahamstown and Cradock area very little rain was recorded in the winter. In the spring of 1989 a higher rainfall was recorded and this might effect the higher isolation of *Salmonella* and *Shigella sp.* in that year.

The incidence of *Rotavirus* was the higher in the months after higher rainfalls were recorded.

The incidence of enteropathogenic *E. coli* did not appear to be effected by the rainfall.

The occurrence of an elevated incidence of protozoa appeared in the summer months and showed a closer correlation to the higher temperatures.

The effect of the rainfall on the spread of *Cryptosporidium* is not known. During the spring of 1989 with the higher rainfall *Cryptosporidium*

oocysts were detected more often, yet the month, April 1990, with the highest isolation rate in both the Coloured and Black population groups, had a very low rainfall. It is possible that at the beginning of periods of high rainfall oocysts which have lain on the bottom of pools of water become suspended in the swirling water and are collected by the water carriers. The oocysts in contaminated pools would in any case be collected in increasing concentration in periods of drought.

The infestation with helminths in the Cradock and Grahamstown areas could be related to the higher temperatures in summer, but is more likely to be related to the poor living conditions and the lack of adequate clean water supplies. The ova of the helminths lie dormant in the mud and transmission of the ova occur more frequently.

5.13. **DAY CARE CENTRE**

In this study the presence of ***Giardia lamblia*** in the White day care centre is higher than the centres of the other two population groups. This is similar to findings in South Carolina were similar studies were conducted on White and Black children[108]. The studies were conducted at a day care centre for Black children and three day care centres for White children. The twenty-two Black children and their four attendants were found to be free of ***Giardia lamblia***. The three day care centres for White children showed a 17%, 26% and 32% prevalence of

Giardia lamblia respectively. The study also included screening of children before entering a school. The Black children were free of *Giardia lamblia* while 10,3% of the White children had Giardia-positive stools. Those who attended day care had a 6,7 higher incidence of Giardia than those who did not attend day care. Suggestions have been made to possibly explain the higher incidence of *Giardia lamblia* infestation in the White children. Sealy et. al. (1983) considered the possibility of racial immunity, ascariasis, toilet training and the threshold number of index cases[107].

The first two suggestions might relate to this present investigation. In the first survey in 1989 the White children had the higher incidence of *Giardia lamblia* 17/47 (36%) compared to the 8/65 (12%) of the Coloured and the 13/52 (25%) of the Black children. In both studies the infestation with helminths in both the Coloured and Black population groups were high. The Coloured population group showed a 26/65 (40%) infestation in 1989 and 16/55 (29%) in 1991. The Black population group showed a lower rate namely 12/52 (23%) in 1989 and the higher in 1991 namely 30/49 (61%). Should the presence of helminths inhibit *Giardia* this might explain the findings.

The high percentage of *Giardia* infestation in the White population group during 1989 could indicate spread within the day care centre. A slightly lower rate was

detected in February 1991. This might be due to new children who were enrolled at the day care centre and the spread of *Giardia* had possibly not affected them. The higher incidence of *Giardia lamblia* among the Black children compared to the Coloured children in the first study might indicate a higher carrier rate among the Blacks compared to the Coloured children or possible spread of *Giardia lamblia* within the day care centre. In the second study the detection rate in the Black children was also higher than in the Coloured children. As a higher incidence of *Giardia lamblia* is detected in summer months this might explain the increase of *Giardia lamblia* in the Black children. The Coloured children were from different day care centres and this might not give a correct picture of the problem at a day care centre.

The high infestation of helminths in the Coloured and Black children is possibly related to poorer living conditions. The second survey in 1991 might confirm this suggestion. The Black children screened during the second survey had a higher percentage of helminths infestation (61%) These children mainly came from an area where the lack of water and toilet facilities existed more so than the children in the first survey.

The detection rates for *Cryptosporidium* oocysts was low during both studies. This might indicate a low carrier rate among all the children. In other centres diarrhoeal outbreak due to *Cryptosporidium* have been

described[133]. The possibility of such an outbreak cannot be overruled.

The same applies to the bacteria isolated from the children. Although very limited numbers of bacteria have been isolated the risk of transmission, especially of *Shigella sp.*, exists as outbreaks of diarrhoea in day care centres due to *Shigella sp.* have been described[26].

The questionnaire regarding the health status of the children might indicate that some children were affected by the enteropathogens, but that a large number of the children were carriers, especially of *Giardia lamblia*, which is known to spread easily.

The results of this study assisted the staff of the day care centre in understanding the risks of the spread of infections at day care centres. Staff at day care centres should be encouraged to prevent the spread of these enteropathogens from person to person where possible because an outbreak of any diarrhoeal disease not only affects the children and the staff but also the family contacts.

This study indicates that the children at day care centres are always at risk from a possible outbreak of diarrhoeal disease.

As the number of day care centres increases, health care workers will need to become more familiar with the infections.

Effective control and prevention of infections require prompt reporting of disease by health care workers and the staff of day care centres. Preventative steps should be taken to limit the spread of disease where possible. Screening of staff at day care centres to identify carriers of enteropathogens could be implemented. The treatment of these carriers might assist with preventing the spread of diseases.

The isolation rate of enteropathogens in this study varied considerably between the different population groups. This indicates the difference in socio-economic circumstances and living conditions.

CHAPTER 6

6. CONCLUSION

Diarrhoeal disease remains a serious problem world wide. In the majority of cases the problems can be traced back to faulty conditions of hygiene. In developing countries the problem are amplified by the poor living conditions present in some areas, especially around cities.

There is a possibility of the transfer of infections from the population of one area to the population of another through the employment of casual and untrained labour in restaurants and house kitchens. *Salmonella* which occurs more frequently in poorly developed and crowded areas is a specific example. The appearance of infection peaks in the White and Coloured populations after the occurrances of peaks in the Black population group may be of significance. Similar peaks are noted for shigellae infections, but they appear more closely related, perhaps due to a smaller infective dose being responsible for producing disease. The emphasis is on food as an important vehicle for infection spread.

Although the isolation rate of *Shigella sp.* was lower in children it remains a very important enteropathogen in the lower age groups where it plays a part in the aetiology. The spread of infection to children could be directly from adult

or through contaminated food.

In this study the finding of *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Campylobacter sp.* were very low. This could be related to various factors.

During a survey when specific investigators handle the screening of the samples often higher numbers of isolates are noted than when the work is handled in a routine laboratory. Inexperienced staff may fail to recognise certain colonies suggestive of the pathogenic bacteria. The techniques and methods also vary in different laboratories. Different selective media can be used and all the media might not provide optimum isolation rates. Inadequate inhibitors in the media might also lead to low isolation rates.

Samples are not usually screened for *Aeromonas hydrophila* in third world countries and special media is not normally used. In this study no selective media was used for its isolation. Therefore the incidences reported from these countries previously and here are very low. Studies in first world countries have proved that *Aeromonas hydrophila* can be a significant pathogen in diarrhoeal disease[117,122].

As *Yersinia enterocolitica* is predominantly a zoonosis one would expect a higher incidence in the areas where direct contact with household pigs is common. A study in Sweden has shown that close contact with pigs leads to higher isolation rates[121].

The raised findings in the White population group may possibly be linked to a preferred diet that carries an increased risk of *Yersinia* infection[36].

Reports in third world countries have demonstrated high isolation and carrier rates of *Campylobacter sp.* in humans[52,53].The isolation rate in the Eastern Cape of *Campylobacter sp.* is much lower than in the other studies. It is suggested that this warrants a special study to demonstrate that the techniques and the media used for isolation of *Campylobacter sp.* are effective.

Enteropathogenic *E. coli* are detected more frequently in developing countries. In this study no differences appear with the isolation of enteropathogenic *E. coli* in the different population groups. In many first world countries, however, the search for enteropathogenic *E. coli* might not be so intense and therefore reduced isolation rates might be reported.

The finding of higher incidences of protozoa in the White population group was not completely unexpected as the higher incidence of *Giardia lamblia* appeared in the White children at the day care centres[4](table XXIII).

The infective dose for *Giardia lamblia* is small and it is easily transmitted in day care centres. The World wide incidence of *Giardia lamblia* in day care centres for White children is high[27,110]. The Black and Coloured

population groups might be exposed to these infections at an earlier age and possibly have immunity against the infection[109]. It could follow that fewer children of the age to attend day care centres develop diarrhoea caused by protozoa.

The high percentage helminths found in the Coloured and Black population groups could be due to poorer living conditions found in a percentage of their areas. Ova of the helminths can lie in contaminated soil and younger childred especially could pick up the infection while playing.

The finding of the higher percentage of helminths in the rural areas is also related to their poorer living conditions. These are similar to the less healthy living conditions that exist in the informal housing centres in the urban areas. The lack of proper sanitation and water supplies can give rise to infestation with the ova of the helminths.

The large percentage of samples containing *Cryptosporidium* oocysts in the Black population group compares to findings in other third world countries. The high incidence of *Cryptosporidium* in the rural areas again indicates poorer living conditions. Spread can readily occur through contaminated water and from person to person[133]. Monitoring for an increase in the presence of *Cryptosporidium* on a regular basis might be used as an indicator for an increase in the number in Human Immunodeficiency Virus positive

cases. Immunocompromised patients are often found to have diarrhoea caused by *Cryptosporidium*[114].

The high incidence of *Rotavirus* might indicate the need for routine testing for the *Rotavirus*. As many children excrete the virus for longer or shorter periods the significance of a finding is not known. A study could be done to determine the excretion percentage and significance in the Eastern Cape before the routine testing is introduced. The high incidence of *Rotavirus* in the White population group could be explained by the more frequent use of antibiotics in this group which could lead to the lower number of bacterial isolations. Alternatively, the same transfer of infection from untrained food handlers might be occurring as suspected with *Salmonella* and *Shigella*.

A difference in the seasonal patterns of enteropathogens was detected among the different population groups. In the White population group the majority of infections were detected in the summer months. This fact is similar to reported findings that summer diarrhoea is more prevalent in South Africa[134]. In the Coloured population group infection with enteropathogens appear throughout the year. Enteropathogenic *E. coli* and *Shigella sp.* were detected in the winter months. The seasonal findings for infection with *Shigella sp.* corresponded to that reported from Johannesburg. The seasonal appearance of enteropathogenic *E. coli* in the Black population group is the same as the Coloured population group. The incidence of *Rotavirus* in the Black population group

also appears in the winter months which is similar to findings elsewhere. The influence of the rainfall in the Eastern Cape cannot be excluded. The infection with *Salmonella* and *Shigella sp.* increased after the higher rainfall in Port Elizabeth.

The high incidence of *Salmonella sp.* isolated in the winter months is different to the finding elsewhere in South Africa[134]. This may mean that the more moderate temperature in Port Elizabeth together with the rainfall played a role in the spread of the bacteria. These findings differ from the findings of the colder drier winters of Johannesburg.

In recording the aetiological agents of diarrhoea disease in the Eastern Cape it is noted that in a large percentage of samples no aetiological agent was detected. This might be related to a deficiency in technique if one compares the low isolation rate of the *Campylobacter sp.* to findings elsewhere. This would suggest that improvements in isolation generally and technique especially for *Campylobacter sp.*, are needed.

Is it possible that some of the aetiological agents are not known and that screening methods for other less well recognised pathogenic bacteria and viruses are not yet available. Different articles have suggested that bacteria and viruses other than the ones sought in routine testing are involved in diarrhoeal disease. Agents such as *Staphylococcus aureus* could account for some of the cases that go

undiagnosed[135]. More recently enterotoxigenic strains of *Clostridium perfringens* have been implicated in some cases of antibiotic-associated diarrhoea[136].

Due to a lack of facilities it is impossible to screen routinely for *E. coli* other than the enteropathogenic *E. coli*. Different outbreaks due to verotoxin-producing *E. coli* have been described in the United States since 1983[137]. The isolation of enterotoxigenic *Bacteroides fragilis* from humans with diarrhoea have also been described[138]. Another type of diarrhoea following the consumption of raw milk has been reported. It is known as "the chronic diarrhoea syndrome" associated with raw milk. No aetiological agent has yet been isolated[139].

This survey of enteropathogens causing diarrhoea in the Eastern Cape, as has been stated, can be of value to Health Authorities and doctors. The results for example from the examination of samples for *Salmonella sp.* can be used by the authorities advantageously. Besides being obvious indicators of living areas where condition place the inhabitants at higher risk, it may be possible to find sources of contaminated water that are being used after rain. Education, warning notices and drainage of unsuitable sources could prevent outbreaks. Visit to restaurants and hotels timed to anticipate periods of high risk could reinforce hygiene procedures. The results for *Shigella sp.* appear not so seasonally dependant, indicating to authorities a more direct manner of spread. *Giardia lamblia* outbreaks could be reduced by the

education of day care staff particularly in the White population group. Likewise doctors treating children who are attending day care centres could be guided by that knowledge in their treatment. The high percentage positive findings for *Rotavirus* indicate that doctors should request screening for the virus when sending samples from children <=2 years of age for examination. Positive finding in maternity units would be especially important giving indication for disease control measures to be tightened.

A project to re-screen samples which previously have shown no causative agent, is needed.

General advise on preventing diarrhoea would still be valid. Breast-feeding, improve weaning practices, use of clean water, handwashing, management of latrines, proper disposal of babies' stools and measles immunization would limit the spread of most pathogens.

APPENDIX 1

SEASONAL PATTERN OF SALMONELLA SPECIES
ALL POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	281	4	277	1.42
DEC	322	30	292	9.32
1989				
JAN	454	18	436	3.96
FEB	470	21	449	4.47
MAR	621	21	600	3.38
APR	346	4	342	1.16
MAY	293	3	290	1.02
JUN	269	1	268	0.37
JUL	234	13	221	5.56
AUG	332	29	303	8.73
SEPT	318	36	282	11.32
OCT	280	13	267	4.64
NOV	507	21	486	4.14
DEC	316	33	283	10.44
1990				
JAN	321	10	311	3.12
FEB	345	6	339	1.74
MAR	328	21	307	6.40
APR	215	8	207	3.72
MAY	296	7	289	2.36
JUN	164	6	158	3.66
JUL	149	13	136	8.72
AUG	166	10	156	6.02
SEP	95	0	95	0.00
OCT	105	3	102	2.86

APPENDIX 2

SEASONAL PATTERN OF SALMONELLA SPECIES
WHITE POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	78	2	76	2.56
DEC	72	2	70	2.78
1989				
JAN	57	3	54	5.26
FEB	80	0	80	0.00
MAR	120	1	119	0.83
APR	94	2	92	2.13
MAY	72	0	72	0.00
JUN	97	0	97	0.00
JUL	87	0	87	0.00
AUG	49	1	48	2.04
SEPT	118	3	115	2.54
OCT	112	0	112	0.00
NOV	139	0	139	0.00
DEC	97	2	95	2.06
1990				
JAN	68	1	67	1.47
FEB	62	1	61	1.61
MAR	66	1	65	1.52
APR	37	1	36	2.70
MAY	70	2	68	2.86
JUN	21	1	20	4.76
JUL	54	0	54	0.00
AUG	77	0	77	0.00
SEP	21	0	21	0.00
OCT	18	0	18	0.00

APPENDIX 3

SEASONAL PATTERN OF SALMONELLA SPECIES
COLOURED POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TEST	POS	NEG	%POS
1988				
NOV	69	1	68	1.45
DEC	45	3	42	6.67
1989				
JAN	91	3	88	3.30
FEB	75	10	65	13.33
MAR	108	4	104	3.70
APR	60	1	59	1.67
MAY	47	0	47	0.00
JUN	43	1	42	2.33
JUL	36	1	35	2.78
AUG	63	1	62	1.59
SEPT	47	2	45	4.26
OCT	37	4	33	10.81
NOV	95	0	95	0.00
DEC	57	3	54	5.26
1990				
JAN	58	1	57	1.72
FEB	75	0	75	0.00
MAR	58	3	55	5.17
APR	41	1	40	2.44
MAY	51	0	51	0.00
JUN	36	2	34	5.56
JUL	18	0	18	0.00
AUG	23	0	23	0.00
SEP	29	0	29	0.00
OCT	20	0	20	0.00

APPENDIX 4

SEASONAL PATTERN OF SALMONELLA SPECIES
BLACK POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TEST	POS	NEG	%POS
1988				
NOV	134	1	133	0.75
DEC	204	25	179	12.25
1989				
JAN	303	12	291	3.96
FEB	313	11	302	3.51
MAR	392	16	376	4.08
APR	192	1	191	0.52
MAY	174	3	171	1.72
JUN	129	0	129	0.00
JUL	109	12	97	11.01
AUG	220	27	193	12.27
SEPT	151	31	120	20.53
OCT	130	9	121	6.92
NOV	272	21	251	7.72
DEC	162	28	134	17.28
1990				
JAN	194	8	186	4.12
FEB	206	5	201	2.43
MAR	204	17	187	8.33
APR	135	6	129	4.44
MAY	174	5	169	2.87
JUN	106	3	103	2.83
JUL	77	13	64	16.88
AUG	66	10	56	15.15
SEP	45	0	45	0.00
OCT	67	3	64	4.48

APPENDIX 5

SEASONAL PATTERN OF SHIGELLA SPECIES
ALL POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	281	31	250	11.03
DEC	322	30	292	9.32
1989				
JAN	454	34	420	7.49
FEB	470	54	416	11.49
MAR	621	44	577	7.09
APR	346	31	315	8.96
MAY	293	29	264	9.90
JUN	269	8	261	2.97
JUL	234	13	221	5.56
AUG	332	27	305	8.13
SEPT	318	20	298	6.29
OCT	280	15	265	5.36
NOV	507	43	464	8.48
DEC	316	31	285	9.81
1990				
JAN	322	31	291	9.63
FEB	321	35	286	10.90
MAR	328	30	298	9.15
APR	215	26	189	12.09
MAY	296	22	274	7.43
JUN	164	25	139	15.24
JUL	149	12	137	8.05
AUG	166	9	157	5.42
SEP	95	7	88	7.37
OCT	105	8	97	7.62

APPENDIX 6

SEASONAL PATTERN OF SHIGELLA SPECIES
WHITE POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	78	3	75	3.85
DEC	72	1	71	1.39
1989				
JAN	57	3	54	5.26
FEB	80	3	77	3.75
MAR	120	1	119	0.83
APR	94	5	89	5.32
MAY	72	2	70	2.78
JUN	97	2	95	2.06
JUL	87	0	87	0.00
AUG	49	5	44	10.20
SEPT	118	2	116	1.69
OCT	112	0	112	0.00
NOV	139	4	135	2.88
DEC	97	5	92	5.15
1990				
JAN	68	0	68	0.00
FEB	62	3	59	4.84
MAR	66	1	65	1.52
APR	37	2	35	5.41
MAY	70	0	70	0.00
JUN	21	0	21	0.00
JUL	54	0	54	0.00
AUG	77	4	73	5.19
SEP	21	0	21	0.00
OCT	18	0	18	0.00

APPENDIX 7

SEASONAL PATTERN OF SHIGELLA SPECIES
COLOURED POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	69	5	64	7.25
DEC	45	1	44	2.22
1989				
JAN	91	5	86	5.49
FEB	75	8	67	10.67
MAR	108	5	103	4.63
APR	60	11	49	18.33
MAY	47	6	41	12.77
JUN	43	4	39	9.30
JUL	36	2	34	5.56
AUG	63	11	52	17.46
SEPT	47	5	42	10.64
OCT	37	4	33	10.81
NOV	95	11	84	11.58
DEC	57	7	50	12.28
1990				
JAN	58	2	56	3.45
FEB	75	4	71	5.33
MAR	58	3	55	5.17
APR	41	6	35	14.63
MAY	51	2	49	3.92
JUN	36	8	28	22.22
JUL	18	5	13	27.78
AUG	23	2	21	8.70
SEP	29	3	26	10.34
OCT	20	2	18	10.00

APPENDIX 8

SEASONAL PATTERN OF SHIGELLA SPECIES
BLACK POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	134	23	111	17.16
DEC	204	27	177	13.24
1989				
JAN	303	26	277	8.58
FEB	313	43	270	13.74
MAR	392	38	354	9.69
APR	192	15	177	7.81
MAY	174	21	153	12.07
JUN	129	2	127	1.55
JUL	109	11	98	10.09
AUG	220	11	209	5.00
SEPT	151	13	138	8.61
OCT	130	11	119	8.46
NOV	272	28	244	10.29
DEC	162	19	143	11.73
1990				
JAN	194	29	165	14.95
FEB	206	28	178	13.59
MAR	204	26	178	12.75
APR	135	17	118	12.59
MAY	174	19	155	10.92
JUN	106	17	89	16.04
JUL	77	7	70	9.09
AUG	66	3	63	4.55
SEP	45	4	41	8.89
OCT	67	6	61	8.96

APPENDIX 9

SEASONAL PATTERN OF ENTEROPATHOGENIC ESCHERICHIA COLI
ALL POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	113	8	105	7.08
DEC	188	22	166	11.70
1989				
JAN	263	16	247	6.08
FEB	261	11	250	4.21
MAR	385	17	368	4.42
APR	199	17	182	8.54
MAY	154	8	146	5.19
JUN	161	13	148	8.07
JUL	133	13	120	9.77
AUG	217	32	185	14.75
SEPT	156	10	146	6.41
OCT	111	11	100	9.91
NOV	245	23	222	9.39
DEC	174	18	156	10.34
1990				
JAN	188	20	168	10.64
FEB	200	22	178	11.00
MAR	199	18	181	9.05
APR	109	17	92	15.60
MAY	164	28	136	17.07
JUN	76	11	65	14.47
JUL	66	9	57	13.64
AUG	69	4	65	5.80
SEP	41	1	40	2.44
OCT	58	7	51	12.07

APPENDIX 10

SEASONAL PATTERN OF ENTEROPATHOGENIC ESCHERICHIA COLI
WHITE POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	21	1	20	4.76
DEC	21	5	16	23.81
1989				
JAN	17	2	15	11.76
FEB	29	5	24	17.24
MAR	46	5	41	10.87
APR	39	7	32	17.95
MAY	29	5	24	17.24
JUN	51	8	43	15.69
JUL	36	2	34	5.56
AUG	16	0	16	0.00
SEPT	34	0	34	0.00
OCT	30	0	30	0.00
NOV	47	1	46	2.13
DEC	34	1	33	2.94
1990				
JAN	26	2	24	7.69
FEB	26	0	26	0.00
MAR	39	4	35	10.26
APR	15	0	15	0.00
MAY	34	2	32	5.88
JUN	7	0	7	0.00
JUL	21	1	20	4.76
AUG	25	2	23	8.00
SEP	6	0	6	0.00
OCT	8	0	8	0.00

APPENDIX 11

SEASONAL PATTERN OF ENTEROPATHOGENIC ESCHERICHIA COLI COLOURED POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	23	1	22	4.35
DEC	25	2	23	8.00
1989				
JAN	49	1	48	2.04
FEB	46	0	46	0.00
MAR	64	1	63	1.56
APR	21	0	21	0.00
MAY	20	1	19	5.00
JUN	17	1	16	5.88
JUL	19	5	14	26.32
AUG	31	3	28	9.68
SEPT	21	1	20	4.76
OCT	9	2	7	22.22
NOV	44	3	41	6.82
DEC	29	0	29	0.00
1990				
JAN	32	3	29	9.38
FEB	50	0	50	0.00
MAR	29	0	29	0.00
APR	13	2	11	15.38
MAY	25	3	22	12.00
JUN	12	0	12	0.00
JUL	5	0	5	0.00
AUG	9	0	9	0.00
SEP	15	0	15	0.00
OCT	11	1	10	9.09

APPENDIX 12

SEASONAL PATTERN OF ENTEROPATHOGENIC ESCHERICHIA COLI
BLACK POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	69	6	63	8.70
DEC	142	15	127	10.56
1989				
JAN	196	13	183	6.63
FEB	185	6	179	3.24
MAR	275	11	264	4.00
APR	139	10	129	7.19
MAY	105	2	103	1.90
JUN	93	4	89	4.30
JUL	77	6	71	7.79
AUG	170	29	141	17.06
SEPT	99	9	90	9.09
OCT	72	9	63	12.50
NOV	154	19	135	12.34
DEC	111	17	94	15.32
1990				
JAN	129	15	114	11.63
FEB	124	22	102	17.74
MAR	131	14	117	10.69
APR	81	15	66	18.52
MAY	105	23	82	21.90
JUN	57	11	46	19.30
JUL	40	8	32	20.00
AUG	35	2	33	5.71
SEP	20	1	19	5.00
OCT	39	6	33	15.38

APPENDIX 13

SEASONAL PATTERN OF ROTAVIRUS
BLACK POPULATION GROUP

FEBRUARY 1989 - APRIL 1990

MONTH	TESTED	POS	NEG	%POS
1989				
FEB	9	0	9	0.00
MAR	116	11	105	9.48
APR	69	9	60	13.04
MAY	50	9	41	18.00
JUN	31	9	22	29.03
JUL	34	5	29	14.71
AUG	87	9	78	10.34
SEPT	44	2	42	4.55
OCT	20	1	19	5.00
NOV	45	9	36	20.00
DEC	27	7	20	25.93
1990				
JAN	60	8	52	13.33
FEB	60	10	50	16.67
MAR	23	5	18	21.74
APR	5	1	4	20.00

APPENDIX 14

SEASONAL PATTERN OF ROTAVIRUS
COLOURED POPULATION GROUP

FEBRUARY 1989 - APRIL 1990

MONTH	TESTED	POS	NEG	%POS
1989				
FEB	7	2	5	28.57
MAR	19	1	18	5.26
APR	6	0	6	0.00
MAY	2	0	2	0.00
JUN	8	0	8	0.00
JUL	6	0	6	0.00
AUG	12	0	12	0.00
SEPT	2	0	2	0.00
OCT	1	0	1	0.00
NOV	5	0	5	0.00
DEC	3	1	2	33.33
1990				
JAN	6	0	6	0.00
FEB	19	4	15	21.05
MAR	0	0	0	0.00
APR	3	0	3	0.00

APPENDIX 15

SEASONAL PATTERN OF PROTOZOA
ALL POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	255	2	253	0.78
DEC	307	5	302	1.63
1989				
JAN	449	5	444	1.11
FEB	456	4	452	0.88
MAR	601	8	593	1.33
APR	339	3	336	0.88
MAY	296	12	284	4.05
JUN	268	6	262	2.24
JUL	234	2	232	0.85
AUG	333	3	330	0.90
SEPT	306	6	300	1.96
OCT	273	4	269	1.47
NOV	494	7	487	1.42
DEC	306	7	299	2.29
1990				
JAN	319	5	314	1.57
FEB	345	6	339	1.74
MAR	323	6	317	1.86
APR	215	3	212	1.40
MAY	295	6	289	2.03
JUN	164	5	159	3.05
JUL	149	1	148	0.67
AUG	159	1	158	0.63
SEP	96	1	95	1.04
OCT	103	1	102	0.97

APPENDIX 16

SEASONAL PATTERN OF PROTOZOA
WHITE POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	77	0	77	0.00
DEC	72	0	72	0.00
1989				
JAN	57	4	53	7.02
FEB	74	4	70	5.41
MAR	115	5	110	4.35
APR	95	0	95	0.00
MAY	71	8	63	11.27
JUN	95	2	93	2.11
JUL	87	0	87	0.00
AUG	49	0	49	0.00
SEPT	111	0	111	0.00
OCT	110	2	108	1.82
NOV	130	2	128	1.54
DEC	90	1	89	1.11
1990				
JAN	69	1	68	1.45
FEB	62	4	58	6.45
MAR	63	0	63	0.00
APR	37	0	37	0.00
MAY	69	1	68	1.45
JUN	21	0	21	0.00
JUL	54	0	54	0.00
AUG	73	1	72	1.37
SEP	21	0	21	0.00
OCT	17	0	17	0.00

APPENDIX 17

SEASONAL PATTERN OF PROTOZOA
COLOURED POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	52	1	51	1.92
DEC	46	0	46	0.00
1989				
JAN	92	1	91	1.09
FEB	73	0	73	0.00
MAR	105	1	104	0.95
APR	57	0	57	0.00
MAY	47	2	45	4.26
JUN	44	1	43	2.27
JUL	36	1	35	2.78
AUG	64	2	62	3.13
SEPT	45	2	43	4.44
OCT	36	0	36	0.00
NOV	97	1	96	1.03
DEC	55	0	55	0.00
1990				
JAN	57	0	57	0.00
FEB	75	0	75	0.00
MAR	58	2	56	3.45
APR	41	0	41	0.00
MAY	51	2	49	3.92
JUN	36	0	36	0.00
JUL	18	0	18	0.00
AUG	23	0	23	0.00
SEP	29	0	29	0.00
OCT	20	0	20	0.00

APPENDIX 18

SEASONAL PATTERN OF PROTOZOA
BLACK POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	126	1	125	0.79
DEC	188	5	183	2.66
1989				
JAN	297	0	297	0.00
FEB	308	0	308	0.00
MAR	380	2	378	0.53
APR	187	3	184	1.60
MAY	178	2	176	1.12
JUN	129	3	126	2.33
JUL	109	1	108	0.92
AUG	220	1	219	0.45
SEPT	148	4	144	2.70
OCT	126	2	124	1.59
NOV	267	4	263	1.50
DEC	161	6	155	3.73
1990				
JAN	192	4	188	2.08
FEB	206	2	204	0.97
MAR	202	4	198	1.98
APR	135	3	132	2.22
MAY	174	3	171	1.72
JUN	106	5	101	4.72
JUL	77	1	76	1.30
AUG	63	0	63	0.00
SEP	46	1	45	2.17
OCT	66	1	65	1.52

APPENDIX 19

SEASONAL PATTERN OF HELMINTHS
ALL POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	255	8	247	3.14
DEC	307	17	290	5.54
1989				
JAN	449	26	423	5.79
FEB	456	8	448	1.75
MAR	601	13	588	2.16
APR	339	12	327	3.54
MAY	296	15	281	5.07
JUN	268	6	262	2.24
JUL	234	7	227	2.99
AUG	333	25	308	7.51
SEPT	306	14	292	4.58
OCT	273	15	258	5.49
NOV	494	21	473	4.25
DEC	306	15	291	4.90
1990				
JAN	319	8	311	2.51
FEB	345	10	335	2.90
MAR	323	7	316	2.17
APR	215	8	207	3.72
MAY	295	17	278	5.76
JUN	164	17	147	10.37
JUL	149	9	140	6.04
AUG	159	10	149	6.29
SEP	96	6	90	6.25
OCT	103	4	99	3.88

APPENDIX 20

SEASONAL PATTERN OF HELMINTHS
WHITE POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	77	0	77	0.00
DEC	72	1	71	1.39
1989				
JAN	57	1	56	1.75
FEB	74	1	73	1.35
MAR	115	0	115	0.00
APR	95	0	95	0.00
MAY	71	2	69	2.82
JUN	95	0	95	0.00
JUL	87	1	86	1.15
AUG	49	2	47	4.08
SEPT	111	1	110	0.90
OCT	110	4	106	3.64
NOV	130	0	130	0.00
DEC	90	0	90	0.00
1990				
JAN	69	0	69	0.00
FEB	62	0	62	0.00
MAR	63	0	63	0.00
APR	37	0	37	0.00
MAY	69	0	69	0.00
JUN	21	0	21	0.00
JUL	54	0	54	0.00
AUG	73	1	72	1.37
SEP	21	0	21	0.00
OCT	17	0	17	0.00

APPENDIX 21

SEASONAL PATTERN OF HELMINTHS
COLOURED POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	52	4	48	7.69
DEC	46	3	43	6.52
1989				
JAN	92	8	84	8.70
FEB	73	0	73	0.00
MAR	105	7	98	6.67
APR	57	7	50	12.28
MAY	47	6	41	12.77
JUN	44	3	41	6.82
JUL	36	5	31	13.89
AUG	64	10	54	15.63
SEPT	45	3	42	6.67
OCT	36	7	29	19.44
NOV	97	9	88	9.28
DEC	55	8	47	14.55
1990				
JAN	57	3	54	5.26
FEB	75	4	71	5.33
MAR	58	3	55	5.17
APR	41	2	39	4.88
MAY	51	7	44	13.73
JUN	36	5	31	13.89
JUL	18	0	18	0.00
AUG	23	6	17	26.09
SEP	29	2	27	6.90
OCT	20	1	19	5.00

APPENDIX 22

SEASONAL PATTERN OF HELMINTHS
BLACK POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	126	4	122	3.17
DEC	188	13	175	6.91
1989				
JAN	297	17	280	5.72
FEB	308	7	301	2.27
MAR	380	6	374	1.58
APR	187	5	182	2.67
MAY	178	7	171	3.93
JUN	129	3	126	2.33
JUL	109	1	108	0.92
AUG	220	13	207	5.91
SEPT	148	10	138	6.76
OCT	126	4	122	3.17
NOV	267	12	255	4.49
DEC	161	7	154	4.35
1990				
JAN	192	5	187	2.60
FEB	206	6	200	2.91
MAR	202	4	198	1.98
APR	135	6	129	4.44
MAY	174	10	164	5.75
JUN	106	12	94	11.32
JUL	77	9	68	11.69
AUG	63	3	60	4.76
SEP	46	4	42	8.70
OCT	66	3	63	4.55

APPENDIX 23

SEASONAL PATTERN OF CRYPTOSPORIDIUM OOCYSTS
ALL POPULATION GROUPS

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	66	0	66	0.0
DEC	134	2	132	1.5
1989				
JAN	198	4	194	2.0
FEB	187	7	180	3.7
MAR	294	26	268	8.8
APR	171	15	156	8.8
MAY	141	4	137	2.8
JUN	115	10	105	8.7
JUL	96	6	90	6.3
AUG	200	23	177	11.5
SEPT	125	16	109	12.8
OCT	83	9	74	10.8
NOV	197	16	181	8.1
DEC	126	16	110	12.7
1990				
JAN	129	12	117	9.3
FEB	155	11	144	7.1
MAR	114	7	107	6.1
APR	71	13	58	18.3
MAY	132	9	123	6.8
JUN	74	8	66	10.8
JUL	47	5	42	10.6
AUG	47	3	44	6.4
SEP	39	2	37	5.1
OCT	50	5	45	10.0

APPENDIX 24

SEASONAL PATTERN FOR CRYPTOSPORIDIUM OOCYSTS
WHITE POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	5	0	5	0.00
DEC	4	0	4	0.00
1989				
JAN	3	0	3	0.00
FEB	5	0	5	0.00
MAR	2	0	2	0.00
APR	22	0	22	0.00
MAY	20	0	20	0.00
JUN	13	1	12	7.69
JUL	12	0	12	0.00
AUG	5	1	4	20.00
SEPT	12	0	12	0.00
OCT	8	0	8	0.00
NOV	15	0	15	0.00
DEC	10	1	9	10.00
1990				
JAN	7	0	7	0.00
FEB	14	0	14	0.00
MAR	20	0	20	0.00
APR	6	0	6	0.00
MAY	22	0	22	0.00
JUN	7	0	7	0.00
JUL	7	0	7	0.00
AUG	5	0	5	0.00
SEP	5	0	5	0.00
OCT	2	1	1	50.00

APPENDIX 25

SEASONAL PATTERN FOR CRYPTOSPORIDIUM OOCYSTS
COLOURED POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	9	0	9	0.00
DEC	16	1	15	6.25
1989				
JAN	37	0	37	0.00
FEB	36	2	34	5.56
MAR	54	4	50	7.41
APR	20	1	19	5.00
MAY	18	0	18	0.00
JUN	17	2	15	11.76
JUL	17	1	16	5.88
AUG	28	2	26	7.14
SEPT	18	2	16	11.11
OCT	8	2	6	25.00
NOV	41	1	40	2.44
DEC	21	1	20	4.76
1990				
JAN	22	1	21	4.55
FEB	40	3	37	7.50
MAR	15	2	13	13.33
APR	10	3	7	30.00
MAY	22	1	21	4.55
JUN	11	2	9	18.18
JUL	4	0	4	0.00
AUG	8	0	8	0.00
SEP	15	0	15	0.00
OCT	10	1	9	10.00

APPENDIX 26

SEASONAL PATTERN FOR CRYPTOSPORIDIUM OOCYSTS
BLACK POPULATION GROUP

NOVEMBER 1988 - OCTOBER 1990

MONTH	TESTED	POS	NEG	%POS
1988				
NOV	52	0	52	0.00
DEC	114	1	113	0.88
1989				
JAN	157	4	153	2.55
FEB	146	5	141	3.42
MAR	238	22	216	9.24
APR	129	14	115	10.85
MAY	103	4	99	3.88
JUN	85	7	78	8.24
JUL	67	5	62	7.46
AUG	167	20	147	11.98
SEPT	94	14	80	14.89
OCT	67	7	60	10.45
NOV	141	15	126	10.64
DEC	95	14	81	14.74
1990				
JAN	99	11	88	11.11
FEB	101	8	93	7.92
MAR	79	5	74	6.33
APR	55	10	45	18.18
MAY	88	8	80	9.09
JUN	56	6	50	10.71
JUL	36	5	31	13.89
AUG	34	3	31	8.82
SEP	19	2	17	10.53
OCT	38	3	35	7.89

APPENDIX 27

QUESTIONAIRE DISTRIBUTED AT DAY CARE CENTRES

CHILD'S NAME AGE SEX

IS YOUR CHILD HEALTHY AT THIS TIME? YES NO
HAS YOUR CHILD RECEIVED ANY TREATMENT RECENTLY? YES NO
IF YES, PLEASE GIVE THE REASON AND THE DATE OF THE LAST TREATMENT.

ANY OTHER FAMILY MEMBERS WITH DIARRHOEA YES NO
IF YES, PLEASE STATE MEMBER AND APPROPRIATE DATE.

IS YOUR CHILD OFTEN SUBJECT TO:-
VOMITTING STOMACH ACHE WEIGHT LOSS ITCHING
DIARRHOEA ABNORMAL EATING HABITS EG. EATS SAND, PAPER ETC.

DOES YOUR CHILD HAVE THE MAIN MEAL AT:- HOME SCHOOL

APPENDIX 28

WORLD SURVEYS (EXCLUDING S.A.) OF CAUSES OF DIARRHOEA
CAUSAL ORGANISM LISTED WITH PERCENTAGE INFECTED

COUNTRY/PUBLISHED	REF	SURVEY PERIOD	NUMBER	AGE	SALM	SHIG	YERS	AERO	CAMP	EEC	ROTA
BANGLADESH 1982]	(73)	12/1979-12/1980	3550	-	1	12	NT	NT	14	20	19
AUSTRALIA 1983	(116)	10/1980-9/1981	975	-	5,7(0,9)	1,3(0)	NT	10,8(0,7)	7,4(,6)	1,9(,1)	12,7(11,1)
NIGERIA 1985	(105)	1/1983-12/1983	142	5	4,9	9,4	1,4	NT	NT	7,7	NT
INDIA(PUNJAB) 1985	(86)	3/1982-2/1983	242	<5	8.4	2.1	NT	NT	NT	17.8	18.2
LIVERPOOL 1986	(112)	6/1983-5/1985	4028	<12	6.1	5.7	NT	13.7	5.0	4.1	33.5
KENYA 1986	(114)	3/1984	81	<3	3.7	NT	NT	2.5	9.9	1.2	NT
SAUDI ARABIA 1986	(45)	7/1983-1/1984	607	-	4	10	NT	NT	5	NT	NT
SWEDEN 1986	(115)	1/1981-12/1981	416	-	I-2.8(0) O-1.5	I-0.7(0) O-0.4	I-5.6(0) O-1.5	NT	I-2.8(0.5) O-5.9	I-4.2(2.0) O-4.0	I-53(2.0) O-41
EGYPT 1986	(107)	1/1981-6/1983	3.243	-	0.8(0.6)	2(0.6)	NT	0.8(0)	1.5(0.9)	4.4(5.7)	3(0.8)
EGYPT 1986	(3)	5/1982-4/1983	275	-	5	1	NT	NT	2	8	33
CEN AFRICA REP 1987	(106)	11/1983	133	<5	1.5(1.7)	2.2(0.8)	NT	NT	7.5(5.1)	10.2(7.5)	6(0)
REP PHILIPPINES 1987	(12)	1983-1984	2.903	-	9.2	11.6	NT	1.3	3.0	NT	30.6
ZIMBABWE 1987	(13)	11/1983-10/1984	143	<6	0(1)	5(2)	NT	NT	8(2)	11(10)	NT
ITALY 1987	(35)	1/1981-12/1985	2.500	<14	NT	NT	1.4	NT	NT	NT	NT
SWEDEN 1988	(117)	1981-1982	731	-	5	2	3	NT	18	NT	4
THAILAND 1990	(122)	8/1988-7/1989	482	<5	13	6	NT	NT	12	3	12
BANGLADESH 1991	(120)	1987-1989	5.811	-	NT	NT	NT	NT	NT	NT	15.5

I = Inpatient
O = Outpatient
NT = not tested
- = All ages
()=Controls

APPENDIX 29

SOUTH AFRICAN SURVEY OF CAUSES OF DIARRHOEA
CAUSAL ORGANISM LISTED WITH PERCENTAGE INFECTED

CITY/PUBLISHED	REF	SURVEY PERIOD	NUMBER	AGE	SALM	SHIG	YERS	AERO	CAMP	EEC	ROTA
20											
S.A. 1977	(20)	10/1974-1-/1975	191	<2	7.9(5.6)	8.4(1.7)	NT	NT	NT	28.8(16.)	14.4
S.A. 1980	(1)	12/1976-1/1977	70	<2	17	9	NT	NT	NT	43	4
DURBAN 1986	(113)	1/1985-3/1985	259	<12	6.8(3.8)	1.7(0)	NT	NT	1.7(0)	15.4(5.1)	22.8(17.9)
EAST CAPE 1987	(36)	5/1982-12/1984	1634	-	3.7	5.1	NT	NT	1.3	NT	NT
CAPE TOWN 1988	(10)	4/1981-3/1982	545	<2	1.08(7.0)	5.7(0.3)	0	NT	18(6.4)	40.2(27)	
P. ELIZABETH '89	(9)	12/1987-21988	161	<2	5.0	6.8	0	0	0	7.6	NT
GA-RANKUWA 1990	(118)	11/1987-1/1988	70	<2	1	4	NT	NT	16	10	13
KANGWANE 1990	(72)	2/1985-1/1986	312	-	6	4	NT	NT	6	36	13
CAPE TOWN 1991	(74)	1990	215	<1	6(2)	3(0)			6(4)	7(1)	18(<1)
THIS SURVEY 1991		11/1988-10/1990	7227	_	4.58	8.54	0.11	0.08	0.32	9.06	13.20
CRADOCK 1991		11/1988-10/1990	159	-	0.63	18.87	0	0	0	1.33	NT
GRAHAMSTOWN 1991		11/1988-10/1990	606	_	3.47	3.80	0.17	0	0	4.07	NT
UITENHAGE 1991		11/1988-10/1990	540	_	2.96	3.15	0	0	0	3.60	NT
P.E. (1) 1991		11/1988-10/1990	1996	_	2.00	1.15	0.20	0.05	0.65	7.14	12.90
P.E. (2) 1991		11/1988-10/1990	3926	-	6.44	12.23	0.08	0.13	0.26	11.49	13.39

NT = not tested
- = All ages
()=controls

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