ENVIRONMENTAL FACTORS INFLUENCING THE DISTRIBUTION HOOKWORM INFECTION IN KWAZULU – NATAL, SOUTH AFRICA

by

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FRONTSPIECE

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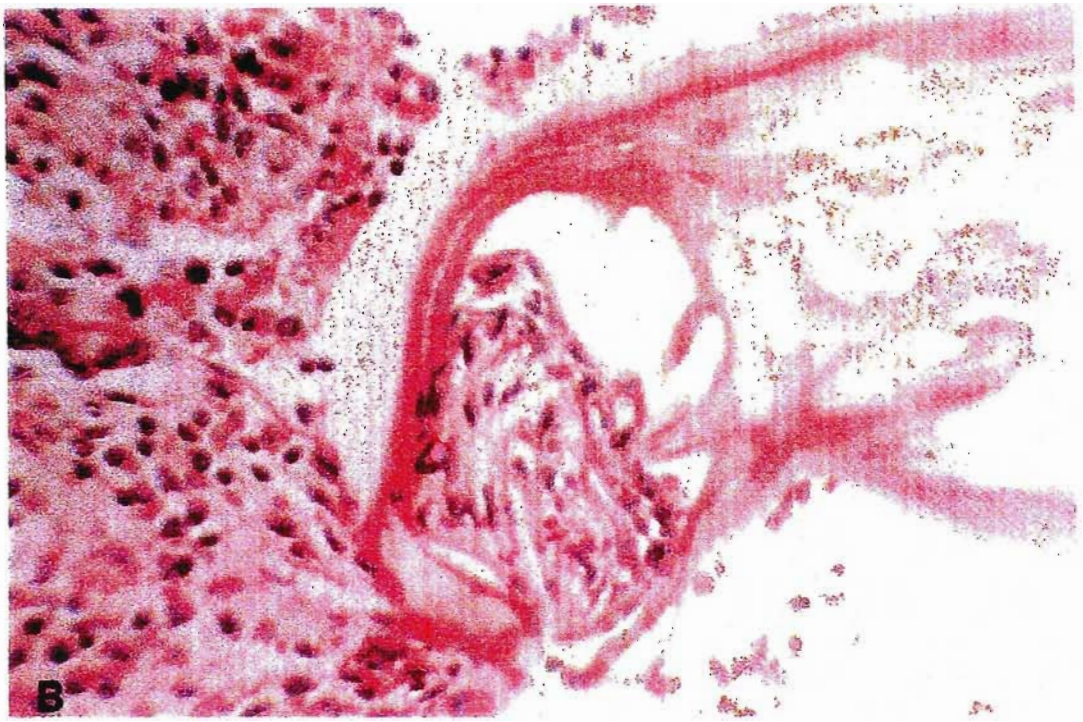

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A Mouth parts of adult hookworm showing the buccal capsule with cutting plates and
B Longitudinal section of hookworm attached to intestinal mucosa (From Yamaguchi, 1981)

ABSTRACT

The aim of this study was to investigate the occurrence of the soil transmitted parasitic nematode *Necator americanus* ("Old World" hookworm) in soils of different texture in KwaZulu-Natal. The key questions being asked were: (i) Is hookworm infection in KwaZulu-Natal confined to the coastal plain? (ii) Is there any association between hookworm prevalence and the different soil types in the province? (iii) Since several examples exist in the province of soil types on which hookworm is transmitted on the coastal plain, occurring inland, what is the status of infection in communities situated in these areas? (iv) What properties of soil are important in the transmission ecology of hookworm larvae?

All available hookworm prevalence data of KwaZulu-Natal were mapped on Land Type maps of the province (Land Type Survey Staff, 1986). Several additional surveys were carried out to supplement this database. Faecal egg counts were obtained by the Formal-Ether Concentration Method and positive infections were confirmed as *N. americanus* by larval morphology after coproculture using the Harada-Mori Technique. Univariate analysis was carried out for significant associations between hookworm prevalence, altitude, climatic variables (rainfall and temperature) and soil type. The results showed that areas ≤ 150m above sea level (i.e. the coastal plain) support high prevalences ($\bar{x}$ = 45 %), and are characterised by low-clay textured soils, warm temperatures and relatively high rainfall. Areas > 150 m (i.e. inland) have low hookworm prevalences ($\bar{x}$ = 6 %), and are characterised by high-clay textured soils, cool temperatures and moderate rainfall.

Hookworm prevalence also decreased southwards as climatic conditions (rainfall and temperature) become unfavourable, and the coastal plain also narrows in this direction. Multivariate analysis was done to determine which environmental factors combine best to provide favourable conditions for hookworm transmission. From the variables used, prevalence of infection was most significantly correlated with the mean daily minimum temperature for January followed by the mean number of rainy days for January. This points to the importance of summer conditions in the transmission of hookworm infection in KwaZulu-Natal.

Moderate hookworm prevalences ($\bar{x}$ = 17.3 %) were found in the inland sandy areas, dropping to low prevalences ($\bar{x}$ = 5.3 %) in the surrounding non-sandy areas. The intensity-related data could not be significantly correlated with the environmental variables used in this study. The Spearman Correlation Coefficient was used to test for relationships between hookworm prevalence and soil variables. In the results, only the fine and medium sand fractions showed positive correlations with hookworm prevalence. Clay showed a significant negative correlation with hookworm prevalence. No significant correlations were found between soil pH or its organic matter content and hookworm prevalence. Age and sex related infection data could not be drawn into the analysis due to the small sample size of study localities.

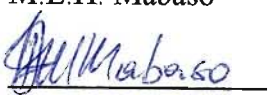
Clay soils retain more water than sandy soils and this is a consequence of the relationship between texture and moisture. Clay becomes sticky and plastic when wet because it is cohesive and as a result, clay soils tend to be less aerated or more poorly drained than sandy soils and thus do not serve as good media for hookworm transmission. As hookworm larvae are obligatory aerobes, they require unhindered movement through the soil, allowing them to move towards the host or to deeper into the soil if conditions in the surface layer become inimical for their survival. Lower temperatures inland probably suppresses transmission and this thermal effect is probably greater in areas situated on clayey soil types.

PREFACE

The work described in this thesis was carried out in the Department of Biology, University of Natal, Durban, under the supervision of Professor C.C. Appleton. It was co-supervised by Professor J.C. Hughes, Department of Agronomy, University of Natal, Pietermaritzburg.

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

M.L.H. Mabaso



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Finally, I would like to thank the Medical Research Council (MRC) for financial assistance.

This work is dedicated to my mother and my girlfriend for their untiring support during the most trying moments, may God bless.

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CHAPTER 1

Introduction

Parasitic nematodes, some of which are intestinal parasites of humans, exhibit varying degrees of complexity both in their relationships with their hosts and in their life cycles. Categorized according to their modes of transmission, these helminths mostly fall into one of the following five groups: (a) *contagious or faecally borne* - eggs or larvae are infective when deposited at or passed through the anus, therefore infection is transmitted directly from person to person; (b*) soil transmitted (geohelminths)* - eggs or larvae become infective after a period of incubation in soil; (c) *arthropod transmitted* - the infective stage of the nematode develops in arthropod intermediate hosts which then transmit the infection by biting or being ingested by people; (d) *snail transmitted* - the infective stage of the nematode develops in snail intermediate hosts or in secondary intermediate hosts after partial development in the soil; (e) *food or meat borne* - infective stages of nematodes which develop in the flesh of domestic stock (WHO, 1964).

In many areas worldwide, the most prevalent and damaging helminths are those of the soil transmitted group (WHO, 1961). From the standpoint of their overall prevalence and severity of the diseases they cause, the most important soil transmitted helminths are: *Ascaris lumbricoides* (roundworm); *Trichuris trichiura* (whipworm); *Necator americanus* ("New World" hookworm); *Ancylostoma duodenale* ("Old World" hookworm) and *Strongyloides stercoralis* (threadworm). Except for *A. duodenale*, these nematode species are also

important scourges of man in South Africa. The focus of the present study is in KwaZulu-Natal, where hookworm (*N. americanus*) is of particular importance due to its high prevalence rates (Schutte *et al.*, 1981; Maurihungirire, 1993; Appleton and Gouws, 1996).

The name "hookworm" is said to have arise from dorsal bent position of the anterior end, giving worms a hook-like appearance (Noble and Noble, 1964; Faust *et al.*, 1975). The two species of hookworm affecting humans have different geographical distributions: *A. duodenale* is found predominantly in North Africa, Southern Europe, the Middle and Far East and Australia; *N. americanus* is predominant in the Americas and sub-Saharan Africa. However, the original distribution of these species has been masked by the effect of human migrations and there is now a considerable overlap. Both species have been shown to be present in some regions of Africa, South America and Asia, although one or other usually predominates (Meyers *et al.*, 1976; Miller, 1979; Ukoli, 1984).

Despite the widespread occurrence of these parasites, they have been largely neglected by both researchers and research funding authorities (Wakelin *et al.*, 1990). It is only in the recent few years that there has been a resurgence of interest that is unmatched since the efforts of the Rockefeller Commission in the 1920s (Muller, 1990). Sadly however, like most helminthiases, the hookworms do not attract the medical headlines, largely because they are diseases of the developing world and provoke low mortality. At the same time they are typically associated with considerable misery through general debilitation, pathology and direct and indirect malnutrition (Wakelin *et al.*, 1990).

1.1 Hookworm life cycle

1.1.1 Eggs and larvae

Hookworms, like other parasitic nematodes, generally produce large numbers of eggs daily. Adults live attached to the mucosa of the small intestine. After fertilization by the male, each female *N. americanus* can produce about 10 000 eggs per day whereas female *A. duodenale* produce some 20 000 eggs per day (Meyers *et al.*, 1976). The eggs of the two species are almost indistinguishable differing only slightly in size; *N. americanus* eggs are usually somewhat longer, 64-76 µm x 36-40 µm, than those of *A. duodenale*, 56-60 µm x 35-40 µm (Gilles, 1984). However, this difference in size cannot be used to separate the species. Hookworm eggs have a characteristic shape. They are oval with broadly rounded extremities and have a clear shell membrane with a very fine vitteline layer; there is also a clear space between the embryo and the egg shell (Woodruff, 1974; Faust *et al.*, 1975; Nnochiri, 1975; Brown and Neva, 1983). Eggs are laid in the lumen of the host's intestine and begin to embryonate immediately after they are laid, and have already undergone the two, four or eight cell stage of cleavage by the time they are deposited outside the body. Under suitable conditions of warmth, moisture, shade and oxygen, the eggs hatch within 24 to 48 hours, releasing the first stage larvae (Nnochiri, 1975; Schad, 1991); Pawlowski *et al.*, 1991).

Free-living hookworm larvae resemble the juvenile state of many microbivorous soil-dwelling nematodes. Depending on the stage, these vary in size from minute (approximately 250 to 300 µm long) to the bigger infective larvae measuring 300-600 µm (Gilles, 1984; Schad,

1991). The first stage larvae are known as rhabditiform (L_1) larvae and they feed on faecal microflora (bacteria and detritus). After about three days they moult into the second rhabditiform stage (L_2) larvae which continue to feed. By the fifth day, these larvae moult to produce the third stage larvae known as filariform (L_3) larvae, which move away from the faeces into the soil. This third stage, which is infective, retains the sheath of the L_2, does not feed but relies on stored food in its intestine (Lapage, 1957; Ukoli, 1984; Schad, 1991; Pawlowski *et al.*, 1991).

The infective (L_3) larvae are found in moist soil to a depth of 15 mm to about a metre and are very sensitive to desiccation and freezing temperatures (Ukoli, 1984). They are capable of considerable vertical movement up and down the soil profile, depending on its moisture content and temperature, and can survive for several weeks under ideal conditions (Gilles 1984; Ukoli, 1984). Given suitable conditions of moisture and warmth the infective larvae accumulate on the soil surface where they infect their human hosts by penetrating unprotected skin (Schad, 1991). According to Croll (1970) and Croll and Mathews (1977), these infective larvae show behavioural responses (thermotactic and tactile stimuli) which increase their chances of making contact with a host. On contact with the human skin, the filariform larvae penetrate the epidermis and the dermis, entering the venules in the subcutaneous tissue and are carried passively through the bloodstream to the heart and capillaries of the lungs. There they leave the vascular system, emerge into the alveoli, migrate up the bronchioles, bronchi and trachea, pass over the epiglottis and down the oesophagus (Ukoli, 1984; Schad, 1991; Pawlowski *et al.*, 1991). Upon arrival in the intestine, there is another moult (to the fourth stage, L_4) during which a temporary buccal capsule develops, permitting attachment to the

intestinal mucosa. This temporary capsule is shed with the cuticle when the larvae undergo their final moult to produce adult hookworms. Sometimes the third moult, producing the fourth stage (L_4) larvae, takes place in the lungs (Lapage, 1957; Miller, 1979; Ukoli, 1984; Schad, 1991; Pawlowski *et al.*, 1991). In the intestine, the larvae differentiate sexually, moult, mature and copulate. The female begins to lay eggs about 5 to 6 weeks after penetrating the skin (Noble and Noble 1964; Meyers *et al.*, 1976; Miller, 1979; 1984; Ukoli, 1984) and thus completes the life cycle. The life span of the adult hookworm is about 2 years, although some are known to have lived for 5 years (Nnochiri, 1975; Ukoli, 1984), and the maximum recorded survival is 15 years (Palmed, 1955).

1.2 Pathogenesis of hookworm infection

Hookworm infections give rise to a wide range of clinical symptoms which are indicative of a number of pathological conditions loosely termed "hookworm disease" (Ukoli, 1984). Human infections are usually through the soft skin of the foot, hands and buttock-areas of the body that are in frequent contact with the soil. According to Miller (1979) and Ukoli (1984), morbidity from hookworm infections can either be acute or chronic. Acute infections result from the migratory activities of the larvae and of the adults reaching the intestine. Chronic infections arise from the activities of feeding adults in the intestine as well as the consequences of the physiological, biochemical and haematological disturbances they cause.

1.2.1 Penetration and migration manifestation

When the infective larvae penetrate human skin, they cause a stinging sensation, followed by irritation or so-called "ground itch" (Brown and Neva, 1983; Katz *et al.*, 1988). They also produce inflammation (dermatitis) which may be intensified by secondary bacterial infection (Noble and Noble 1964; Meyers *et al.*, 1976; Miller, 1979). The migration of hookworm larvae through the body causes few pathological changes, but small haemorrhages and leucocytic or eosinophilic infiltration may occur when larvae pass through the alveolar walls of the lungs. The migration of larvae through the respiratory tract may cause coughing due to irritation of the bronchial and tracheal mucous membrane (Pawlowski *et al.*, 1991). However, where there is a massive invasion by worms or in heavily infected individuals (500-1000 worms), the damage may be so extensive as to cause pneumonitis, and hookworm pneumonia may occur when secondary infection is superimposed (Nnochiri, 1975).

1.2.2 Intestinal manifestations

In the duodenum and jejunum, hookworms attach themselves to wall of villi which are sucked into their buccal cavities. There they feed on blood from cut vessels and on the mucosal tissue itself. During the intestinal phase, infected people can experience epigastric duodenal-type pain, indigestion, loss of appetite and diarrhoea (Borrero *et al.*, 1961; Miller, 1979; Pawlowski *et al.*, 1990). The severity of symptoms depends on the intensity i.e. (number of worms) and duration of infection as well as natural resistance and age of the host (Belding, 1958; Meyers

et al., 1976). Injury to the host results from the mechanical and chemical destruction of tissue at the point of attachment (Gilles, 1984).

The biting and sucking of worms on the wall of the small intestine produces haemorrhage and necrosis at the site of attachment. The end-result is multiple scars on the mucous membrane. Secondary bacterial infection may also occur (Nnochiri, 1975). At the time of the bite, worms secrete strong enzymes capable of breaking down the tissue at the point of attachment. The combination of this mechanical and chemical attack not only ruptures small blood vessels in the mucous membrane, but also releases nourishing components of the wall (Hotez and Pritchard, 1995). The worms secrete an anticoagulant which facilitates bleeding (Pawlowski *et al.*, 1991; Hotez and Pritchard, 1995). This anticoagulant remains active even after the worms have moved to a new location and the previous attachment site continues to bleed for several days. Thus, if the infection is not treated, significant blood loss may continue for many years leading to the depletion of body's iron stores and consequently, iron deficiency anaemia.

The worms swallow much of the leaking blood, but a lot is wasted as well. Estimated quantities of blood removed daily per worm are 0.16-0.34 mℓ for *A. duodenale* and 0.03-0.05 mℓ for *N. americanus* (Tasker, 1961; Martinez-Tores *et al*, 1967; Mahmood, 1966; Hotez and Pritchard, 1995). Intestinal blood loss has been associated with heavily infected individuals or chronic infections (Meyers *et al.*, 1976; Ukoli, 1984). This is supported by Hotez and Pritchard (1995) who state that while each worm empties a fraction of a teaspoon from the circulation every day, when 20, 100 or even 1000 worms drain this much blood simultaneously (in the last case, almost drawing a cup of blood), the consequences are severe.

However, the progression of hookworm disease varies with the physical state of the patient i.e. his nutritional and iron reserve status, age (size), and the presence of other complicating diseases especially those causing anaemia, e.g. malaria and schistosomiasis (Foy and Kondi, 1960b; Banwell *et al.*, 1967; Miller, 1979). The aetiology of anaemia is obviously multifactoral, with the prevalence and causes varying considerably in the different parts of the world (Shulman *et al.*, 1996), and particularly in tropical and sub-tropical environments (Pritchard *et al.*, 1991; Stoltzfus *et al.*, 1996). As a result the role of a single agent such as hookworm in Africa has been difficult to determine. In Kenya, increases in *Schistosoma haematobium* (urinary schistosomiasis) and hookworm egg counts as well as malaria parasite counts, were all considered to be important causes of iron deficiency anaemia (Stephenson, 1985). In Gambela, south-west Ethiopia, Bulto *et al.* (1992) found a strong association between high hookworm infection and anaemia but accepted that this might be due to malaria which was also endemic to the area. In a study on anaemia in pregnant women in the district of Kilifi on the coast of Kenya where both malaria and hookworm are endemic, Shulman *et al.* (1996) found that iron deficiency anaemia and hookworm were the main factors associated with anaemia in multigravidae, whereas in primigravidae, malaria parasitaemia was the most important risk factor identified. The reasons for this remain uncertain.

In KwaZulu-Natal, the prevalence and causes of anaemia and particularly severe anaemia are not well documented, at least with respect to hookworm and other blood-feeding parasites. In the northern part of the province where hookworm is among the most prevalent parasitic diseases, Mayet *et al.* (1985) found that most *N. americanus* infestations were light and therefore appeared not to be an important contributor to anaemia. Although, hookworm

infection does not appear to be the overriding public health problem that its prevalence alone might suggest, it is nevertheless of significance since as Gelfand and Warburton (1967) suggested in Zimbabwe, even a mild hookworm load may be serious by acting as an aggravating factor in a patient who is already suffering from another type of anaemia. The spread of the human immunodeficiency virus infection (HIV/AIDS) in KwaZulu-Natal further complicates the issue. As it makes the body susceptible to other infectious diseases and commonly results in tuberculosis (TB) causing anaemia.

In addition to anaemia, loss of protein (hypoproteinaemia) is another manifestation of hookworm disease. Heavy infections may cause lassitude, fainting and even death especially in infants (Meyers *et al.*, 1976). Even worse, according to Hotez and Pritchard (1995), children chronically infected by hookworms may suffer from a lack of proteins and iron causing severe retardation of growth and impaired cognitive behaviour and motor development, sometimes irreversibly so. Another functional consequence of this disturbed iron balance and impaired nutritional status is low productivity because of incapacity to work (Ukoli, 1984; Crompton 1989). Cardiac oedema is evident in advanced chronic infections with malnutrition, hypoproteinaemia and anaemia (Beet, 1956; Meyers *et al.*, 1976; Ukoli, 1984).

1.3 Prevention and control

Hookworms undergo four stages in their life cycle (see section 1.1), i.e. escape of eggs into the environment, development and survival of larvae in the environment, the infection of another human host, and adult life within the host's body where eggs are produced to start another cycle. According to Hawkins and Feachem (1978), if any one of these stages can be effectively blocked, the continuous transmission of the parasite will be interrupted, and human infections will fall or may even disappear. They proposed three ways of controlling hookworms by interrupting their life cycle: (1) blocking transmission of hookworm eggs to the environment; (2) controlling the hookworms within the environment (soil) and (3) blocking transmission of hookworm larvae back to man.

1.3.1 Blocking transmission of eggs to the environment

Transmission of hookworm eggs to the environment can be blocked by chemotherapy which kills the adult worms within the human body. Several anthelmintics are available for use against both species of hookworm that infect man e.g. Pyrantel pamoate, Levamisole, Albendazole, Thiabendazole and Mebendazole (Gilles, 1984; Rossignol, 1990; Pawlowski *et al.*, 1991). They are relatively non-toxic, well tolerated, effective and can be given orally. However, multiple intestinal helminth infections are common in the rural tropics and subtropics and in these circumstances, broad spectrum anthelmintics that are effective against more than one species of nematode are useful. Among anthelmintics available for human use, Mebendazole (Vermox®) and Albendazole (Zentel®) are considered as having the broadest

spectrum, being effective against the three most common intestinal geohelminths; *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (Gilles, 1984; Albonico *et al.*, 1994, 1995). These are the only two registered for use in South Africa and the KwaZulu-Natal Health Department has received permission to buy Albendazole for its helminth control programme (J. Friggens, pers. comm. 1998)

1.3.2 Control within the environment

Sanitation is an important means of controlling hookworm in the environment (Foy and Nelson, 1963; Pawlowski *et al.*, 1991). The pit latrine is the simplest excreta disposal system and the most efficient for conditions in the rural tropics and subtropics (Foy and Kondi, 1961a; Hawkins and Feachem, 1978). Even though Feachem *et al.* (1983) showed the type of latrine cannot influence hookworm prevalence. In Zimbabwe, Chandiwana et al (1989) found that if latrines are inadequate and unhygienic, they played no significant role in curbing helminth transmission. It is important therefore to mantain and improve existing latrines. Digging of shallow holes should be discouraged and latrines should be built away from the homestead (Hawkins and Feachem, 1978). The conventional pit latrine, however, tends to be smelly and allows flies to breed in excreta in the pit, and carry pathogens to food or utensils. There is evidence to suggest that these pathogens may include hookworm eggs and larvae (Cairncross, 1990). Odour and fly problems are very much reduced in the ventilated improved pit (VIP) latrine which was developed in Zimbabwe in the 1970s (Cairncross, 1990; Morgan, 1990). Alternatively, if the owners cannot afford the vent pipe required for a VIP latrine, a tight-fitting lid on the toilet seat can assist in fly control.

In the Burma Valley of Zimbabwe however, despite a marked increase in the number of sanitation units installed between 1985 and 1990, no relationship was found between latrine availability and reinfection intensity (Bradley *et al.*, 1993). This is consistent with observations that the effect of improved sanitation is slow to develop, and that anthelmintic interventions should be repeated until sanitation has an impact on transmission (Bradley *et al.*, 1993). However, ± 90 % of people should use latrines (exclusively) to have marked impact (C.C. Appleton, pers. comm.).

1.3.3 Blocking transmission to man

Hookworm larvae are transmitted by skin contact with faecally contaminated soil, and this can be avoided by wearing shoes (Chaves, 1938; Foy and Nelson, 1963; Hawkins and Feachem, 1978; Marquardt and Demaree, 1985). In Dar es Salaam, Tanzania, the prevalence of hookworm infection in school children (who wear shoes) was found to be 2 %, compared with 12 % among children not attending school (Killewo *et al.*, 1990). The socio-economic status of the two groups was similar and there was no programme of chemotherapy nor of sanitation for school children. The only difference was the requirement that all school children wear shoes and in the process become accustomed to wearing them even after school. In southern Thailand, a study on predictors of the risk of hookworm infection (Chongsuvivatwong *et al.*, 1996) found that the only significant factor was wearing of shoes.

However, according to Chaves (1938), Foy and Kondi (1960a), and Hawkins and Feachem (1978), cost and lack of cultural acceptability will often make this strategy impracticable.

Thus health education should be the basis on which the control efforts operate (Crompton, 1990). Health education should be carried out and every attempt made to discourage indiscriminate defaecation and encourage instead, personal hygiene and the reason for habitual and proper use of latrines (Nnochiri, 1975; Davis, 1976). Education of the public regarding the mode of hookworm transmission could also lead to wearing of shoes or cheap forms of protective footwear to reduce exposure to the infective larvae (Davis, 1976; Marquardt and Demaree, 1985).

1.3.4 Anaemia therapy

Hookworm-related anaemia in the poorer sections of the infected communities has frequently been attributed to a poor diet (Foy and Kondi, 1960a; Miller, 1979; Gilles, 1984). In such situations, it may be necessary to encourage the community to eat the right types of food, especially those rich in iron and protein (Miller, 1979; Gilles, 1984), for example green vegetables such as soya beans, spinach and cabbage. Alternatively, if the diet of the country is deficient, particularly in protein or iron, then efforts should be made to improve it through for example, food fortification (Scott, 1960; Foy and Nelson, 1963; Marquardt and Demaree, 1985). A nutritionally-enhanced biscuit has recently been developed in South Africa, specifically for use in rural areas where children are malnourished (Evans *et al.*, 1997). While chronic hookworm-induced anaemia can be prevented, alleviated or even cured by dietary administration of supplementary iron, without removal of the hookworms, Roche and Lyrisse (1966) likened this to a bank president who, rather than firing a dishonest teller who every day pockets some of the organisation's money, chose to replace the daily loss from a special fund.

This is more so because improved nutritional status does not lead to a decrease in worm burden (Tripathy *et al.*, 1971), and in fact is similar to feeding the worms. Hence an integrated control programme, using all available resources (environmental, health education, chemotherapy and dietary supplementation), is the most rational and satisfactory approach.

Nevertheless, while hookworm disease is most prevalent in the developing countries of the tropics and subtropics and are treatable, it is important to note that in many of these places, especially in rural areas, anthelmintics and sometimes iron supplements may not be available or too expensive (Hotez and Pritchard, 1995). Since the populations of developing countries suffer from a multitude of disadvantages such as poverty, malnutrition, low living standards, high birth rates and illiteracy, the high morbidity levels caused by parasitic diseases impact on the countries' economies (Goldsmid, 1991).

South Africa typifies such countries, and previous studies, particularly in KwaZulu-Natal, have shown that hookworm infection is an important feature of the parasitic diseases of the region. There is thus a pressing need to understand the biology of hookworms as it has important implications for epidemiological control and treatment (Schad, 1991). Sadly however, as Schad (1991) noted, scientific knowledge of hookworms lags behind that of many other helminth parasites of man. Several aspects of the biology of hookworms are poorly understood, particularly the relationship between the free-living stages and their environment, to their hosts and other factors co-occurring in the soil habitat.

1.4 Motivations and objectives of this study

The distribution of hookworm infections can probably be attributed to the habitat requirements of their free-living stages. Altitudinal transects across KwaZulu-Natal by Appleton and Gouws (1996) and C. C. Appleton and Kvalsvig (unpublished data) showed prevalence of hookworm infection to be highest on the coastal plain. Changes in prevalence along the transect by C. C. Appleton and Gouws (1996) were significantly correlated with a rainfall-derived variable (mean monthly precipitation for July, MMP Jul). In another study, Maurihungirire (1993) found that hookworm prevalence decreased along a latitudinal transect down the coastal plain of KwaZulu-Natal from north to south. This decrease in prevalence with increasing latitude along the coastal plain was significantly correlated with temperature-derived variables (M. Maurihungirire and C. C. Appleton, unpublished data). Maurihungirire (1993) also reasoned that the decrease in prevalence down the coastal plain must be due to the soil types on this plain, which narrows and completely disappears just south of the Eastern Province border. This suggested that the coastal sands of this plain are a more suitable habitat for hookworm transmission than the inland weakly developed soils which are acidic and have higher clay contents.

The absence of human hookworm infection along the sandy parts of the coast of the Eastern Province and Western Cape, where the prevalence of other soil transmitted nematodes remains high (Maurihungirire, 1993), further complicates the issue. This phenomenon has been attributed to the climatic differences, especially of temperature, between the Cape

provinces and KwaZulu-Natal. In the Cape the rainy season (winter) is cold (10-15 ° C) and the summer is dry and warm (20-23 ° C) (Clement *et al.*, 1987). In KwaZulu-Natal the summer (rainy season) is warm (22-32 ° C) and the winter (dry season) is cool (15-19 ° C) along the coastal plain (Clemence *et al.*, 1987). According to WHO (1961), soil serves these parasites in essentially the same manner as an intermediate host. The soil receives non-infective stages and it provides conditions under which the larvae can develop to the infective stage. In addition, it provides protection for the infective stage until contact is made with the host. Perhaps it is logical that since some other parasites require specific intermediate hosts, soil transmitted worms require a specific soil type and tolerate only a narrow range of physical conditions within that soil (WHO, 1961).

It is clearly important to investigate soil types and the soil environment in respect of the development of the infective stages. Thus the focus of this study is on the way environmental factors, especially climate and soil type, influence the distribution of hookworm infection in KwaZulu-Natal. It also tests the hypothesis that soil type determines the worm's prevalence in a given area. The approach here is threefold: (1) to confirm that the parasite occurs predominantly on the coastal plain by reassessing all available data on the prevalence and distribution of infection in KwaZulu-Natal in relation to the soil type map of the province (Land Type Survey Staff, 1986); (2) to determine hookworm prevalences in selected communities located on inland areas of suitable soil types to see whether transmission occurs off the coastal plain and if so, how the change in location and climate (most importantly temperature) effects it; and (3) to analyse characteristics of the different soil types in the areas sampled for possible correlation with epidemiological data. This should help to identify areas

of the province and also of the country which are potentially suitable for hookworm transmission, i.e. "risk areas".

CHAPTER 2

Factors influencing hookworm distribution, development and transmission

It is necessary in any host-parasite system for certain biological conditions to be fulfilled before endemicity can be established. The parasite must produce a sufficient number of eggs or larval stages, and these must be capable of surviving until they have passed from one host to the next. There must also be available a sufficient number of hosts to acquire and perpetuate the infection.

As noted in the previous chapter (1.4), the intermediary vehicle of hookworm transference is the soil. Consequently, it is the soil which constitutes the infective medium to the host, that is, man. A focus of transmission becomes established in the soil and is maintained there for a shorter or longer time depending on the prevailing environmental conditions. Development in the soil is thus of fundamental epidemiological significance for species such as *Ascaris lumbricoides*, *Trichuris tichiura* and the hookworms which are also known as the geohelminths or soil-transmitted nematodes. It may be useful, therefore, to examine those physical and chemical properties of the soil which might be expected to influence the activities of nematodes. A report by WHO (1964) reviewed work on the transmission of these species but little seems to have been done since then. A valuable recent review by Smith (1990) quantitatively analysed the available data for larval hookworms.

The free-living part of the hookworm's life cycle and the climatic and biological factors affecting it, are critical aspects governing transmission of the infection to man (May, 1958). This may be enhanced by the domestic habits and recreational behaviour of people such that small, localized differences either in local climates or in local cultures are very important in determining endemicity from the public health point of view.

2.1 Geographic factors

The climate of a country may vary widely over short distances according to the topography and height above sea level and, therefore, nematode distribution might be expected to vary accordingly (Wallace, 1963). In respect to hookworm, May (1958) noted that the heaviest hookworm infection rates seemed to be confined to lowland areas. For example, in Venezuela the heaviest rates occur in the lowlands where the soil is sandy and where poor communities are found. A point of particular importance is the type of soil present in given areas because soil type may influence nematode distribution within localized regions (Wallace, 1963).

In South Africa, recent studies conducted in KwaZulu-Natal are consistent with these findings. Based on studies by Maurihungirire (1993) and Appleton and Gouws (1996), hookworm infection is apparently confined to the sandy coastal plain. The question arises as to whether this is a result of the macro-climate becoming progressively cooler and drier inland, or is it a result of different soil types.

The geography of hookworm disease is governed by a large array of factors that characterize the environment at various stages of the parasite's development, and not all are understood (May, 1958). Soil is probably the most important such factor, since it is required for the development of the free-living generations of these nematodes which, in turn, produce the infective larvae. Communities that cultivate the land are therefore vulnerable to infection and also to reinfection which they perpetuate. Domestic conditions such as lack of sanitation, and unhygienic habits such as walking barefooted allow for continuous reinfection.

2.2.2 Domestic and cultural factors

Habits such as defaecation vary from place to place and must play an important role in determining the risk of infection. The desire for privacy during defaecation makes people choose areas under or concealed by bushes. These areas are also likely to be moist and shaded and therefore provide favourable conditions for the development of hookworm larvae (Pawlowski *et al.*, 1991). It is frequent visits to such faecally contaminated areas that increase the risk of reinfection. This behaviour is most evident in rural villages where there is a lack of sanitary facilities. May (1958) cites Pakistan, where in many areas defaecation sites are selected around villages, and in Spain, on the plains of Jarana near Madrid, where clothes are washed in huts and spread to dry near faecal heaps. Nwosu (1981) made similar observations in Nigeria, where a primary determinant of transmission appeared to be the degree of indiscriminate defaecation. A similar kind of practice occurs in most rural parts of KwaZulu-Natal, where clothes washed in rivers or streams are left to dry on low bushes, most of which grow in faecally contaminated soil (personal observation). In densely populated areas such as

informal settlements, latrines or faecal heaps are poorly maintained and/or isolated so that children often play in contaminated soils. These areas are characterized by poor living standards and young children are seldom restricted in where they play and are also not selective in where they defaecate. These are important elements of soil pollution and create conditions ideal for transmission of hookworm infection (May, 1958).

In a study near the coast in Kwale district south of Mombasa, Kenya, a high prevalence of hookworm infection was found in a rural population whose principle occupation was farming (Pugh *et al.*, 1981). In many countries it has been found that the conditions favouring the development of hookworm larvae coincide with those favouring the growing of rice, coffee, tea, sugar cane, cotton and bananas (May, 1958). Generally speaking it is the crops that are grown on loamy soils that provide shade and ideal conditions for transmission of hookworm infection (Pawlowski *et al.*, 1991). Agricultural activities in certain areas along the KwaZulu-Natal coastal plain involve cultivation of bananas and sugar cane, and it might well be that, as in other countries, these influence the pattern of hookworm infection. According to Pawlowski *et al.* (1991), endemic hookworm infection in rural villages may often be related to the use of human faeces as fertilizer on agricultural plots. Thus when a large labour force is in the field for long periods, far from sanitary facilities, heavy faecal pollution of the soil is inevitable. In Zimbabwe, the Burma Valley, which is an important farming area, is characterized by sandy soils, a hot humid climate and a short rainy season. Chandiwana *et al.* (1989) recorded a 61 % hookworm prevalence here among labourers working under intensive irrigation which made farming possible all year round rather than seasonally. Bradley and Chandiwana (1990) suggested that farm labourers may have encountered high concentrations

of infective larvae in the plantations where they worked, as these plantations (tobacco, coffee, bananas and cotton cultivation) offer suitable cover for defaecation and provide an ideal environment for the development and survival of hookworm larvae.

Irrigation may encourage hookworm transmission in areas that would otherwise be too dry for the larvae to survive (Pawlowski *et al.*, 1991). May (1958) found that this is more prevalent in areas where a dense human population and irrigation co-exist or where suitable irrigation and warmth occur for most of the year. Pawlowski *et al.* (1991) stated that the use of fresh or inadequately composted human faecal manure may contribute significantly to hookworm transmission, and that digging faeces into the soil breaks up both the soil and the faecal mass, encouraging embryonation, hatching and larval development. The use of faecal material as a fertilizer is a well-established Chinese practice in the cultivation of rice and mulberry, such that wherever and whenever this infective material comes into contact with human skin for any prolonged time, infection becomes possible (May, 1958). Although human socio-cultural behaviour related to hookworm transmission has not been investigated in KwaZulu-Natal, it would seem that it is of utmost importance. Behavioural factors which influence the degree of exposure to soils contaminated with viable hookworm larvae are likely to play a primary role in generating the observed pattern of infection (Pugh *et al.*, 1981).

2.2.3 Climatic factors

Hookworm larvae are free-living and free-feeding for a minimum period of five days, during which they are so greatly influenced by climate that in the absence of suitable conditions, survival in the soil is impossible (Cameron, 1958). In terms of climatic and soil factors, WHO (1964) reported that there was a narrow range of both which promoted maximal growth and development rates and allowed survival for the maximum time.

The influence of temperature on nematodes can be divided into: (1) non-lethal low temperatures at which activity is inhibited; (2) optimum temperatures; (3) non lethal high temperatures at which activity is inhibited; (4) lethal low temperatures and (5) lethal high temperatures (Wallace, 1963). Theoretically, therefore, every species should have a temperature tolerance range corresponding to each of these categories. Tropical climates typically have a narrow range of mean monthly temperature but a wide range of relative humidity. In contrast temperate climates experience a wide range in temperature and a narrow range in relative humidity. Consequently, a parasite which depends on moisture may be widely distributed in temperate areas while one depending more on temperature will be predominantly tropical. Following WHO (1964), it is this range of optimum temperature which may account for the more northerly distribution of *A. duodenale* which can tolerate temperatures approaching 0 °C, whereas *N. americanus* is killed by even brief periods of chilling at temperatures well above freezing. *Necator* can however, develop at temperatures above the tolerance range of *A. duodenale*.

The optimum temperature ranges for hookworm development are 28-32 °C and 23-24 °C for *N. americanus* and *A. duodenale,* respectively. Once the infective stage has been reached, both species are relatively resistant to temperature extremes (WHO, 1964). The most favourable soil temperature for development and migration of nematode larvae generally lies between 20 °C and 30 °C. At 10 °C, development in many species of hookworm is inhibited and at 45-50 °C, larvae are killed (WHO, 1964).

Sunlight acts on eggs and larvae by direct heating and radiation on the surface of the soil. Wallace (1963) suggested that sunlight may affect those nematode species which live in faecal material deposited at ground level, but was doubtful as to whether it had any influence on larvae in the soil. WHO (1964) stated that the effect of sunlight was greatest in sandy soils and penetrated to a depth of 1 cm or more. Hominick *et al.* (1987) agreed and also found sunlight to be both ovicidal and larvicidal so that shaded sites provided a more favourable habitat for hookworm survival than unshaded sites.

Desiccation kills hookworm larvae rapidly (WHO, 1964) and since the free-living larvae live in the thin film of water around soil particles, moisture is nearly as important as temperature (Thomson and Cameron, 1988). The review by Smith (1990) indicated that larval food reserves may come under increasing pressure during repeated cycles of soil wetting and drying due to movement of larvae in response to changing environmental moisture. Hominick *et al.* (1987) suggested that moisture was probably important both as rainfall and as soil water. For example, in Nigeria greater numbers of *N. americanus* L_3 larvae were recovered during the rainy season (much higher levels of hookworm infection were consequently found during this

period), and there was a tendency for larvae to be confined to the top layer of soil (Udonsi *et al.*, 1980). Rainfall and the number of rainy days per month have proven to be suitable predictors of parasite abundance, at least in the case of *N. americanus* (Smith, 1990). In Nigeria, Nwosu and Anya (1980), found a significant correlation between a wetness index, comprising the product of monthly precipitation and number of rainy days, and hookworm infection. Water is usually supplied to the soil as rain, but for development, rainfall must coincide with favourable temperatures, and the amount of rainfall which is necessary to maintain larvae depends on the water retention properties of the soil (WHO, 1964; Smith, 1990).

2.2.4 Soil factors

Several studies have suggested that there is an association between soil type and the distribution of some nematode species (Wallace, 1963; Tedla, 1986). According to Wallace (1963), the influence of soil type on nematodes may be highly complex because physical and chemical characteristics of soils vary widely between localities, even where the texture of the soil is more or less similar. In relation to consistency of the soil, it has been observed (WHO, 1964) that there is an inverse relationship between prevalence of hookworm infection and density of the soil. In heavy clay soils prevalence is low, and it is generally considered that sandy soils are most suitable for hookworm larvae. It is believed that this may be related to the vertical flow (draining) of water by percolation and capillary flow in sandy soils (WHO, 1964). Hookworm larvae move against the flow of water (negative rheotaxis), so that when water is percolating down through the soil they are moving upwards towards the surface, and

as the upper layers of the soil dry out and water moves upwards by capillarity from the deeper levels, the larvae migrate downwards (WHO, 1964).

Wallace (1963) suggested that it might be the water-retention properties of different soil types that influence nematode distribution, and that the influence of soil moisture is sometimes evident in the relationship between rainfall and nematode population change. In general, therefore, clay soils often have a lower permeability than sandy soils, because their interstitial pores are smaller. Consequently clay soils tend to be wetter and less well aerated than sandy soils under the same climatic conditions. However, during periods of drought, the surface layers of sandy soils may become too dry and so inhibit nematode activity (Wallace, 1963).

Nematode larvae require moisture and a certain particle size range for migration (Wallace, 1963; WHO, 1964). Hence particle structure is an important determinant of the degree of movement which larvae can undergo in soil. There is of course, wide variation in the arrangement of particles within soil types depending on the amount of cultivation and application of agricultural manures and lime which influence soil structure and "crumb" formation (Wallace, 1963). For example, in well-tilled soil where crumb structure is evident, larvae will be able to migrate more freely than in uncultivated soil which may be more highly compacted (WHO, 1964). Thus, two different textural types of soil such as clay loam and sandy loam might have more or less similar structure in terms of particle size, allowing more or less similar degrees of nematode migration, but the water holding properties of clay and sand particles are different (Wallace, 1963). A clay soil under cultivation may have suitable crumb structure and be well drained whereas in an adjacent locality, the same soil type may be

lacking in crumb structure and may even be water logged because of a high water table and an absence of field drains. It is therefore necessary when discussing soil in relation to hookworm larvae, to know the soil texture as well as the soil moisture and soil structure.

When soil pores are filled with water, aeration becomes limited, and inhibits larval migration WHO (1964). When the only water remaining in the soil is within crumbs, larval activity is also low. Indeed, in very dry soil, water may be actually drawn from the larvae themselves and they desiccate. Between these two extremes there are soil conditions which are particularly favourable for development and movement of larvae. This is the condition in which soil-water exists as small droplets at the points of contact between the soil crumbs (WHO, 1964). This condition occurs at field capacity (after the soil has been wetted but excess water has drained from the pores) (WHO, 1964). Wallace (1963) stated that nematode larvae are so small that pore size is probably insignificant in determining their distribution. In addition, nematode larvae live in the film of water covering the particles of the soil and not in the air spaces within the pores themselves (WHO, 1964). There is little doubt of the importance of this moisture in nematode movement in soil, and there is evidence that the rate of larval movement increases with pore size (Wallace, 1963). It is likely that the major effect of pore size on nematode activity is in its influence on soil moisture and aeration. It is assumed that in some heavy soils nematodes experience anaerobic conditions, whereas in dry soils the gaseous composition of the soil water resembles that of the atmosphere, and a lack of oxygen may reduce nematode numbers in the soil (Wallace, 1963).

The effect of air temperature on the soil depends on the soil water content. A wet soil shows

a smaller rise in temperature for the same amount of heat than does a dry soil, because the thermal conductivity of a wet soil is greater and heat penetrates deeper into it than in dry soil (WHO, 1964; Smith, 1990). However, there are also diurnal variations, for example, as WHO (1964) noted, during the day time in summer the surface of the soil is warmer than the subsurface, but at night the surface often becomes cooler than the subsurface. The frequency and amplitude of cyclical temperature changes in the microclimate at the soil surface influence or constrain the extent to which the larvae can withstand inimical conditions (Smith, 1990). Non-feeding third stage larvae live at the expense of finite food reserves which are used at a rate depending on larval activity, i.e. when moving in response to changing environmental conditions. However, the soil surface of a wet piece of ground covered with dense vegetation exhibits a much smaller diurnal range in temperature than the surface of an equivalent area of dry, bare earth (Smith, 1990). Superimposed on this is a seasonal fluctuation in temperature which is also reflected in the soil.

While it is possible to generalize by saying that hookworm infection is most likely to occur in tropical regions, it is not possible to say that it can occur everywhere in the tropics or that it cannot occur outside the tropics. As an example, Evans (1988) suggested that in Kavango, Namibia, and probably elsewhere too, dung beetles may play a role in dissemination of hookworm eggs by burying moist faecal pellets in areas of soft damp soil walked over by barefoot individuals. Furthermore, by disturbing faecal deposits in order to make pellets, they help aerate and mix faeces with moist soil. What is also important is that the micro-environment to which the free-living stages of soil transmitted nematodes are exposed may be different from the macro-environment (WHO, 1964). Air temperature, rainfall, soil type and

vegetation cover exert their effects through their influences on the microclimate of the larval habitat (Smith, 1990). There are also a number of examples of areas where hookworm can survive but adjacent or surrounding areas are unsuitable for development. On the coastal plain of south eastern United States, soil conditions obviously favoured the survival of *N. americanus* and yielded 46,000 larvae six to eight days after inoculation with 50,000 eggs (Beaver, 1953). This strongly supports the general view that sandy soils provide ideal conditions for the development and rapid migration of infective stage larvae.

Augustine and Smile (1926) had earlier demonstrated the importance of soil type in egg survival and pointed to the sandy soils of Alabama (USA) as a strikingly better environment for hookworm eggs than clay. In Botswana, Michaelsen (1985) concluded that the microclimate of sandy soils was probably more favourable for hatching of *N. americanus* eggs and for survival of the larvae than that of clay soils. In Liberia, the rate of infection by *N. americanus* and *A. duodenale* fluctuated considerably in different areas while that of other soil transmitted nematodes (e.g. *A. lumbricoides*, and *T. tichiura*) remained relatively constant (Hsieh *et al.*, 1972). Both hookworm species occurred mainly on the country's narrow coastal belt.

A similar observation has been made in KwaZulu-Natal (Maurihungirire, 1993; Appleton and Gouws, 1996), where hookworm infection seems to be confined to the sandy coastal plain, but is noticeably absent from other sandy coastal areas in South Africa such as the south-western Cape. The factors responsible for these varying and sometimes conflicting observations are not well understood and more investigations need to be conducted. The

explanation probably lies in the wide variation in the biotic, physical and chemical environment in soils with different textures. These factors may be expected to have a profound influence on the general activity of nematodes and may therefore affect their abundance in a particular area (Wallace, 1963).

2.4 Arrested development (Hypobiosis)

Arrested development or delayed maturation have been demonstrated in a wide range of animal parasites. They have been reported in the intestinal nematode of sheep, *Haemonchus contortus* in the northern United States, and *Ostertagia ostertagi* in Australia (Capitini *et al.*, 1990). During this period the infective larvae postpone maturation and remain developmentally arrested in the host tissues, either in the intestine or skeletal muscle (Michel, 1974; Gibbs, 1986). The larva's metabolic rate declines and movement ceases, and during this period it is highly resistant to anthelmintics. This resistance wanes when movement resumes and larval metabolism returns to normal levels (Stone and Willis, 1967).

Delayed development of hookworm larvae has also been reported in people infected with *A. duodenale*. In an experimental study by Prociv and Luke (1995) in Queensland, Australia, a scientist voluntarily infected himself with third stage larvae of *A. duodenale*. Hookworm eggs first appeared in his faeces after 45 days and continued for four weeks, after which the infection and symptoms were terminated by two doses of Pyrantel®. Monthly faecal monitoring confirmed complete eradication of the patent infection. Although he was not exposed to reinfection, abdominal symptoms (pain, nausea, flatulence) recurred 12 months

later, and hookworm eggs, confirmed as *A. duodenale* by larval culture, reappeared in his stool. This was attributed to hypobiosis. In West Bengal, India, Schad *et al.* (1973) and Lee and Atkinson (1976) observed that *A. duodenale* L_3 larvae in infecting their hosts shortly before the dry season, postpone their development until just before the monsoon season begins, thereby ensuring that when they are voided, their eggs have sufficient moisture for development to the infective stage.

According to Hawdon and Johnston (1996), hypobiosis confers several selective advantages on hookworm larvae. Firstly, arrested development provides a mechanism for seasonal variation in egg output from an infected host. Secondly, arrested stages will spontaneously resume development in the host, resulting in essentially continuous re-infection. Finally, and perhaps most importantly, hypobiosis provides a mechanism for vertical transmission of infective stages to the host's offspring. Arrested larvae of the canine hookworm, *Ancylostoma caninum*, resume development at parturition, enter the milk, and are passed to the nursing pups. This is a major route of transmission for this species (Hawdon and Johnston, 1996), and on the basis of epidemiological evidence, has been suggested to occur in *A. duodenale* as well (Schad, 1990).

Nwosu and Anya (1980) suggested that arrested development may be a feature of transmission foci that have well-defined seasonal temperature fluctuations and where the dominant hookworm species is *A. duodenale*. This partly supports the view of Lee and Atkinson (1976), that arrested development is governed by the environmental experience of the infective larvae. However, according to Nawalinski and Schad (1974), some hookworms

undergo arrested development regardless of their pre-parasite history, i.e. they have a genetically determined, abnormally long prepatent period. Alternatively, some hookworm larvae with genetically determined potential to undergo arrested development, may do so only after exposure to particular external conditions. Thus cold and dry seasons may intervene between periods of optimum conditions so that hookworms may show seasonal abundance.

Arrested development allows some species to overcome winter conditions (Lee and Atkinson, 1976). For example, in North America, *H. contortus* is basically a warm-weather parasite requiring warm, wet conditions for maximal development, but by utilizing hypobiosis it has adapted to environments where winter is too cold for the survival of its free-living stages (Capitini *et al.*, 1990). Full development of infective L_3 larvae to the adult stage is therefore restricted to a very short summer period. This has been achieved by *H. contortus* via different intensities of hypobiosis, i.e. low hypobiosis in summer but up to 100 % in winter (Capitini *et al.*, 1990). It is thus apparent that some nematodes are able to modify their normal pattern of development to suit the biology of their host and their geographical location (Lee and Atkinson, 1976).

Hawdon and Johnston (1996) suggested that hypobiosis facilitated the introduction of *A. duodenale* to North America from Asia, before European contact, via the former land bridge across the Bering Straits (Beringia) rather than via the trans-Pacific route. They suggested that hypobiosis would have enabled hookworms to survive the inhospitable environmental conditions of Beringia in an arrested state among migrating populations. Levels of infections were probably low, but seasonally variable numbers of *A. duodenale* might have survived for

generations under sub-Arctic conditions by vertical transmission. This is more so as the climate along coastal Asia and North America, almost certainly the migratory route, was undoubtedly more hospitable to both humans and parasites than the interior, especially in spring. In addition the "artificially warm microclimates" created by humans attempting to live in the harsh environment of Beringia, coupled with habitual defaecation patterns and faecal contamination of domestic areas, would have facilitated the survival and transmission of hookworm, at least at low levels (Hawdon and Johnston, 1996). In a study of gastrointestinal parasites of known members of a baboon troop in the central Namib desert, Namibia, only an infant and its mother were infected by hookworm (unidentified) (Appleton and Brain, 1995). This suggests vertical transmission as a probable mode of infection, since the unfavourable dry conditions in this region are probably not conducive for soil transmission.

The presence of hypobiotic strains of *A. duodenale* is of considerable public health significance, since arrested development has an important influence on hookworm epidemiology and the effectiveness of eradication programmes (Schad, 1990). This is because no currently available anthelmintic drug has been shown convincingly to have an effect on arrested larvae in the intestine or lodged in tissue (Prociv and Luke, 1995).

It is clear from the foregoing that abiotic factors (temperature, rainfall and soil type) and biotic factors such as hypobiosis all play an important role in determining hookworm transmission and distribution. In this study I have focused on the effect of soil type because available data suggest that hookworm infection occurs largely on the densely populated coastal plain of KwaZulu-Natal, South Africa.

CHAPTER 3

Materials and methods

This study involved the collection and mapping of hookworm prevalence data (see Chapter 4) and the selection of inland study localities based on soil type, collection of soil samples and recovery of nematodes from these areas followed by an analysis of soil samples from each study locality (see Chapter 5). Methods used for the sampling and the processing of stools are similar for both Chapters 4 and 5. Except for the Generalised Soil Pattern maps of South Africa and the province of KwaZulu-Natal, all other maps were plotted using Geographic Information System software (GIS Atlas). Generalised Soil Maps were used to provide an overview of the distribution of soil types in the province. More detailed Land Type Maps were used to investigate the relationship between soil type and hookworm distribution. However, because the maps were not available for the entire coastal plain of KwaZulu-Natal, study sites had to be selected in areas for which published maps exist (the Umkuze and the Durban Land Type maps). It was not until later that unpublished maps for the Port Shepstone, Kokstad, Harding and Richards Bay areas were obtained from the Agricultural Research Council, Cedara College of Agriculture , Pietermaritzburg.

3.1 Description of the study area

The province of KwaZulu-Natal is situated in the east of South Africa, stretching from approximately 28° 30′ S to 30° 30′ S. In the north it is bordered by Moçambique and in the

south by the Umtamvuna river and the Eastern Cape. The eastern side is bordered by the Indian Ocean and the western side by the Drakensberg mountain escarpment.

"KwaZulu-Natal is notable for the variety of scenery presented by the varied physiography, ranging from the impressive mountains to plateaux, plains, deeply incised river valleys and picturesque coastal hinterlands" (Phillips, 1973). The landscape is indeed characterized by steep environmental gradients between these features and is suitable for the investigation of relationships between environmental variation and hookworm prevalence. For the purpose of this study the province was divided into six regions according to (Phillips, 1973): (1) Mountain region (MR); (2) Plateau region (PR); (3) Upland region (UR); (4) Basin plainlands (BP); (5) Intermediate region or coastal hinterland (IR) and (6) Coastal region or coastal lowlands (CR) (Figure 1).

3.2 Data collection

In an attempt to explain and possibly predict the distribution of hookworm in KwaZulu-Natal, mapping of all available hookworm prevalence data was done on soil maps prepared by the Department of Agriculture and Water Supply (Land Type Survey Staff, 1986). Unpublished soil information for the Port Shepstone, Kokstad, Harding and Richards Bay areas was accessed with the help of Mr. V. Roberts (A. R. C, Cedara college of Agriculture, Pmb). The prevalence data used were taken from a study conducted by Schutte *et al.* (1981) in Maputaland and from a series of altitudinal transects done by Appleton and Gouws (1996) and by C. Appleton and J. Kvalsvig (unpublished data) in KwaZulu-Natal, and from a altitudinal

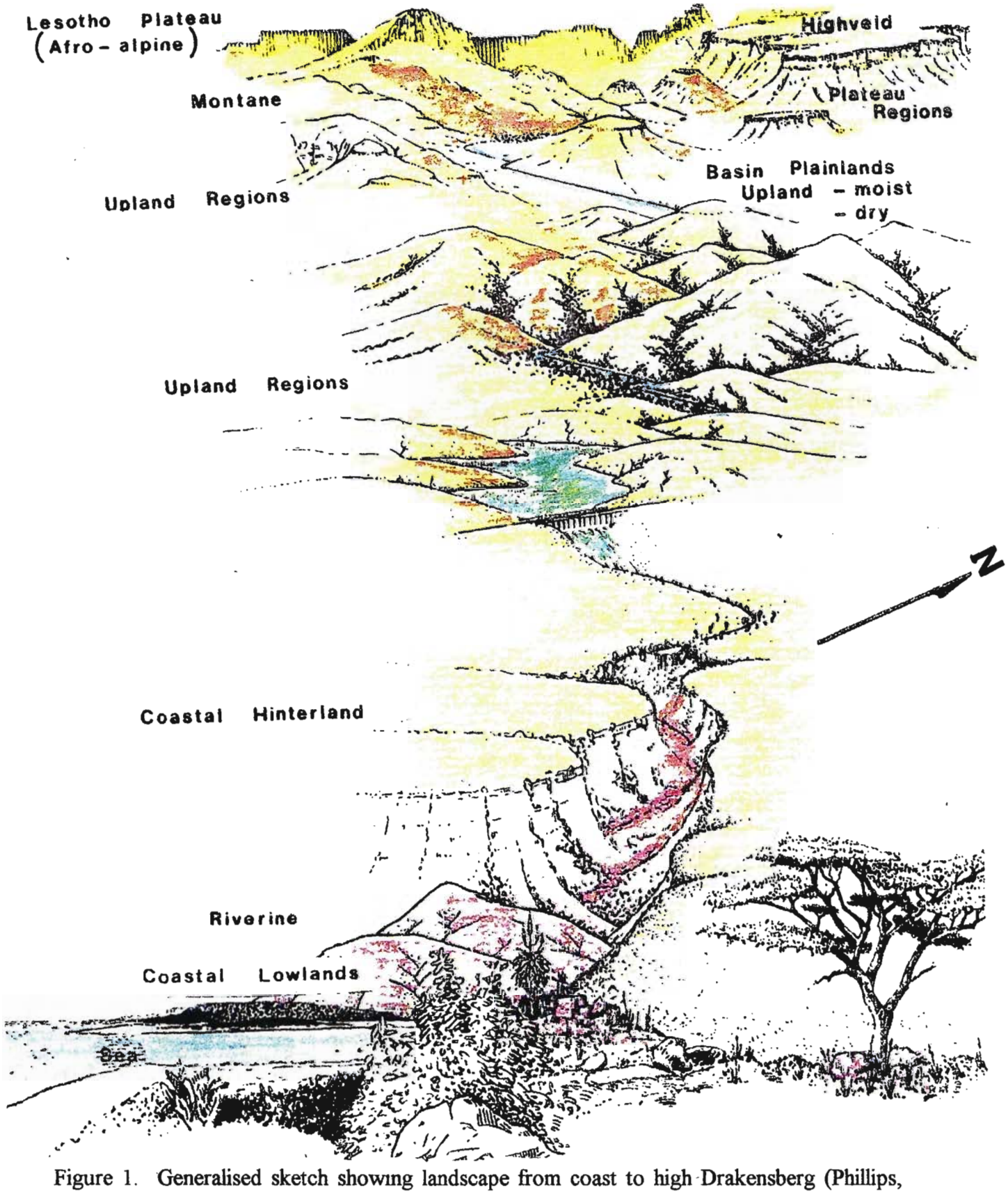


Figure 1. Generalised sketch showing landscape from coast to high Drakensberg (Phillips, 1973).

transect in KwaZulu-Natal by Maurihungirire (1993). Several other hookworm surveys were carried out to supplement this database. In total, data were available from 74 localities in the province (Figure 2).

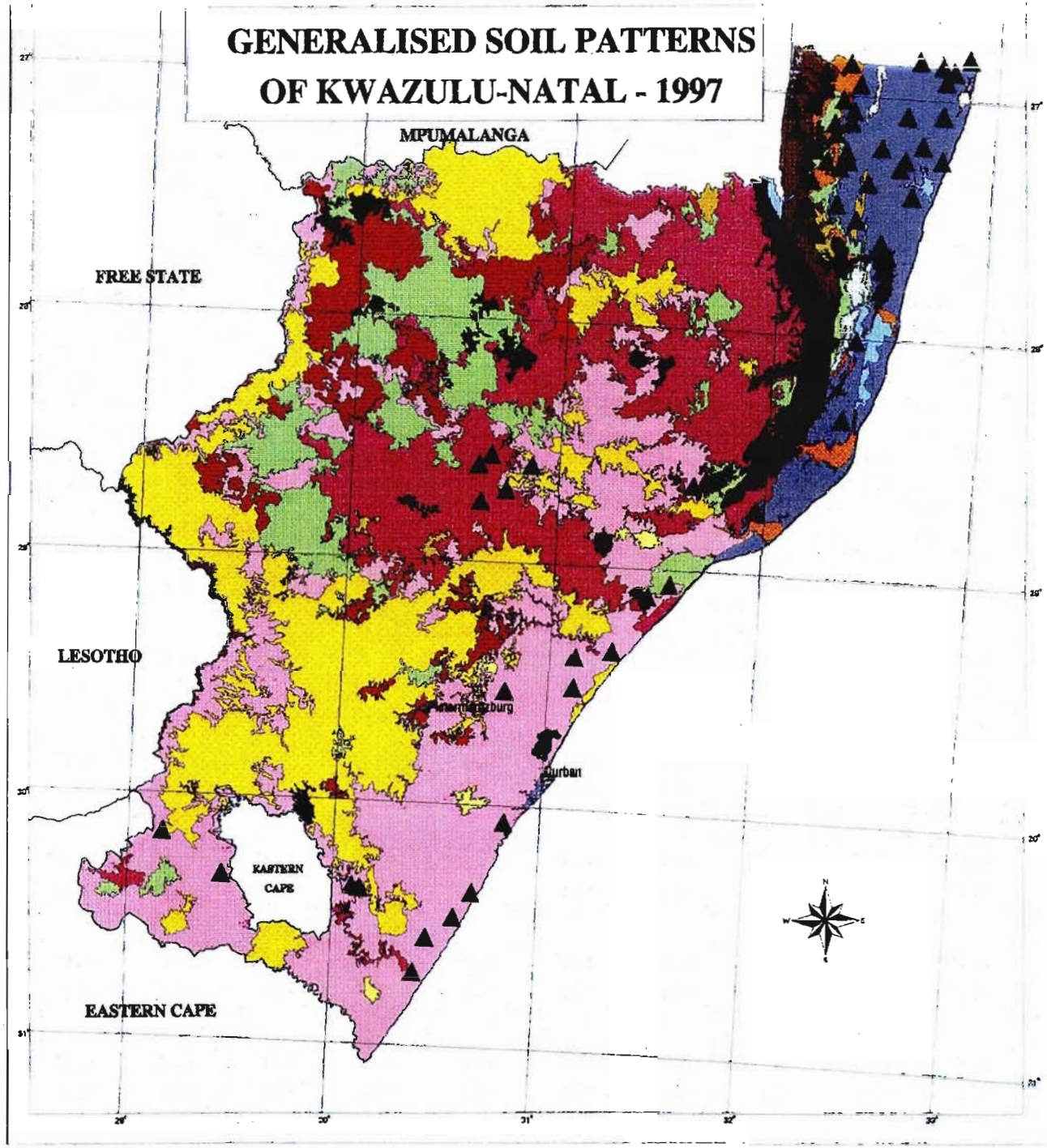
From the centre of each locality datapoint, the major (dominant) soil type(s) within a 5 km radius were recorded as per the Land Type Maps. These were then differentiated according to texture and classified according to clay content, i.e. low-L (< 15 %), medium-M (15-45 %), and high-H (> 45 %) clay content based on the Memoirs of the Land Type Survey Staff (1986). Also taken into account were rainfall- and temperature-related variables for the respective localities (from the climatological data plotted by Schulze, 1982) and their altitude and physiographic positions following (Phillips, 1973).

3.3 Sampling

Sampling was based on the method of Maurihungirire (1993) and Appleton and Gouws (1996), where attention was focused on primary school children. These children are usually the group that has the highest infection rates as well as the highest worm burdens so that they contribute greatly to the contamination of the environment (WHO, 1996). For this reason, primary schools provide the most effective and efficient way to reach large portions of the infected population. Participating scholars were only from grades one to five, as this group was found to be the most co-operative. Twelve boys and twelve girls were selected randomly from each grade and their ages noted. Each individual was then allocated a number and issued with a correspondingly numbered jar for a single stool specimen. The jars were handed out at

Figure 2. Generalised soil pattern map of KwaZulu-Natal, with overlay showing the position of the data points localities (triangles) in relation to the coastal plain soil type (ED) and other soil types. Due to the generalised nature of this map, some soil types shown in the legend and which are refered to in the text, are not shown.

GENERALISED SOIL PATTERNS OF KWAZULU-NATAL - 1997



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LEGEND

RED-YELLOW WELL DRAINED SOILS LACKING A STRONG TEXTURE CONTRAST

- AA — Red and yellow soils with a hydric horizon
- AB — Red and yellow, moderate or weak structured soils with low to medium base status
- AC — Red, moderate or weak structured soils with high base status
- AD — Red, excessively drained sandy soils with high base status. Dunes present

SOILS WITHIN A PLINTHIC CATENA

- BA — Red, yellow and greyish soils with low to medium base status
- BB — Red, yellow and greyish soils with high base status

SOILS WITH A STRONG TEXTURE CONTRAST

- CA — Soils with a marked clay accumulation, strong structure and a non-reddish colour. In addition one or more vertic, melanic and plinthic soils could be present
- CB — Soils with a marked clay accumulation, strong structure and a reddish colour

SOILS WITH A HIGH CLAY CONTENT

- DB — Black and red, strongly structured clayey soils with high base status

SOILS WITH LIMITED PEDOLOGICAL DEVELOPMENT

- EA — Soils with minimal development, usually shallow on hard or weathering rock, with or without intermittent diverse soils. Lithic rock or absent in the landscape
- EB — Soils with minimal development, usually shallow on hard or weathering rock, with or without intermittent diverse soils. Lithic rock generally present in part or most of the landscape
- EC — Red and yellow, sandy well drained soils with high base status
- ED — Greyish, sandy, excessively drained soils
- EE — Soils with negligible to weak profile development usually occurring on recent flood plains

PODZOLIC SOILS

- FA — Soils with a sandy texture, leached and with subsurface accumulation of organic matter, iron and aluminium oxides, either deep or on hard or weathering rock

ROCKY AREAS

- Rock with limited soils
- NAS — Not mapped
- Water bodies

Based on the Land Type Series. 1972 - 1997
Refer to this map as:
Land Type Survey Staff, 1997.
Generalised Soil Patterns of South Africa
1:250 000
ISCW, Pretoria.

SCALE 1 : 1 000 000

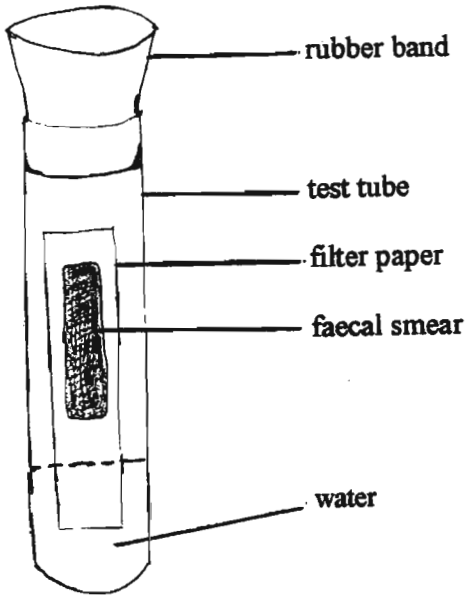
80 0 20 40 60 80 100 km

school at ± 09h00 to give subjects the opportunity to supply stools more easily after their breakfast eaten before school (Maurihungirire, 1993). The samples were taken back as soon as possible to the laboratory for parasitological examination (see below).

3.4 Laboratory methods

Immediately on arrival at the laboratory, a thin (1 to 2 mm) film of faeces from each sample was prepared for culture according to the modified Harada-Mori test tube cultivation technique (Figure 3) as described by Goldsmid (1967) and outlined in Appendix A. The remaining faecal material was preserved with 10 % formalin to kill larvae and to prevent hatching of any ova present. Weighed sub-samples (0.5-1.0 g) of the preserved samples were then processed according to the modified formal-ether stool examination concentration method of Allen and Ridley (1970) (see Appendix B). These "wet preparations" were examined under an Olympus compound microscope (40x objective and 10x eyepiece). Hookworm prevalence rates were assessed and eggs were quantified for each child, and the presence/absence of the other common intestinal helminths, *Ascaris* and *Trichuris,* were also recorded.

For the purposes of photo-microscopy and making detailed drawings using an Olympus photomicroscope, rhabditiform and filariform larvae were recovered from the coprocultures after being kept at 25 °C for 10 days (see Plate 1and Figure 4).



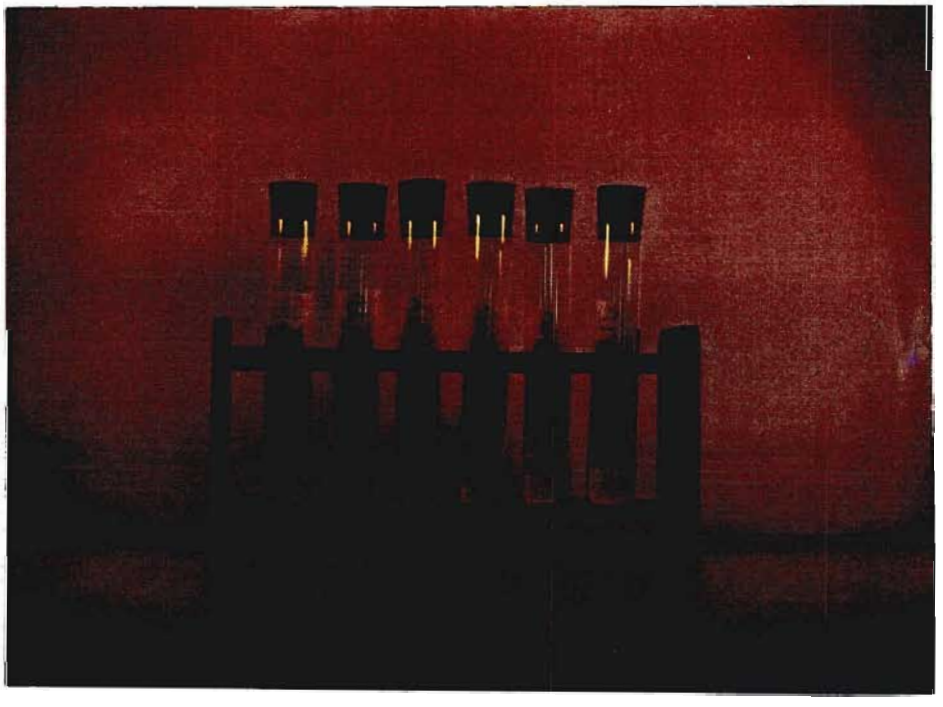
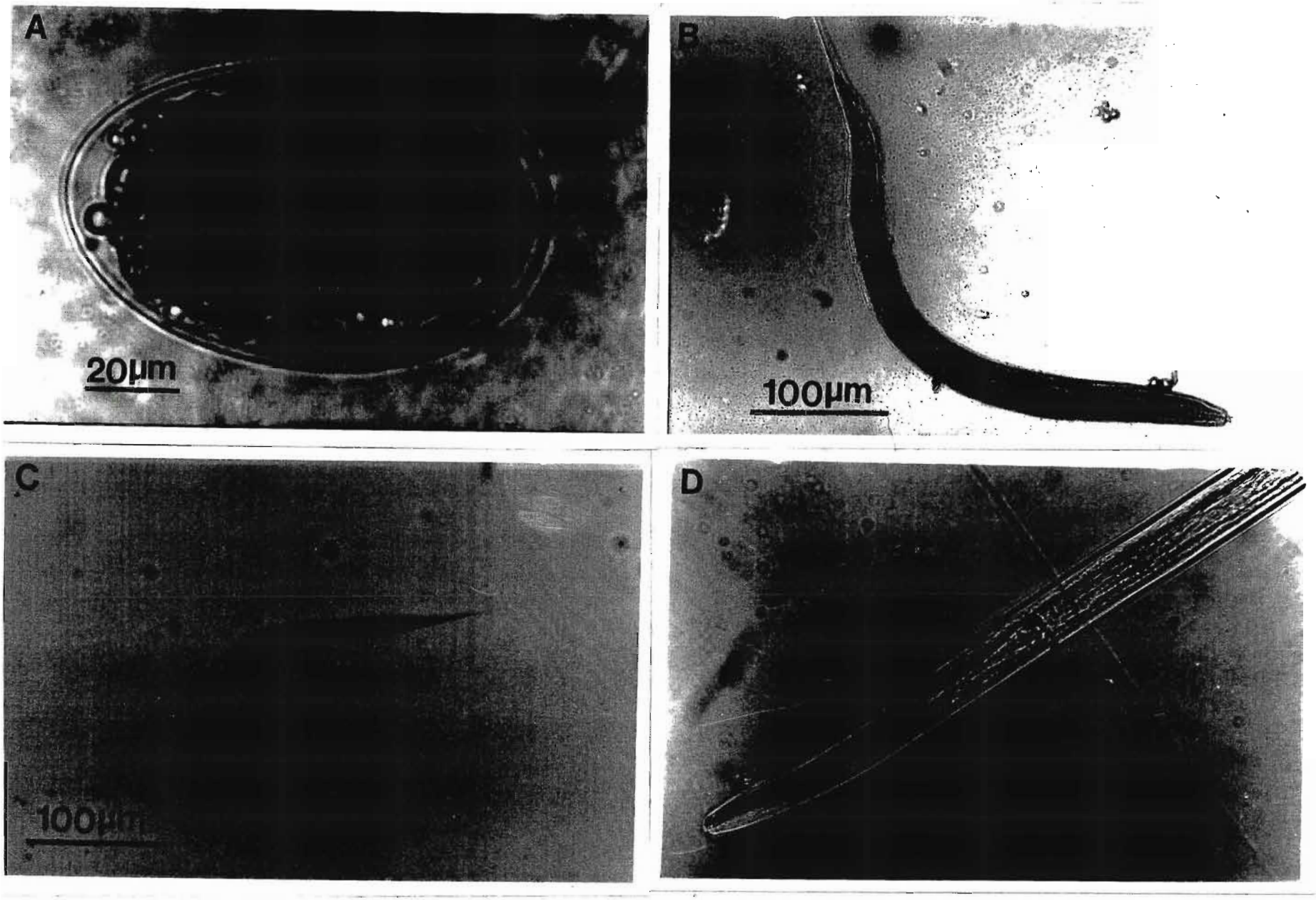


Figure 3. Harada-Mori test tube cultivation method used for diagnosis of hookworm and other intestinal parasites.



Pate 1. Photomicrographs showing *N. americanus* (A) egg, (B) rhabditiform larva, (C) filariform larva and (C) head region of filariform larva (arrow showing oesophagointestinal junction).

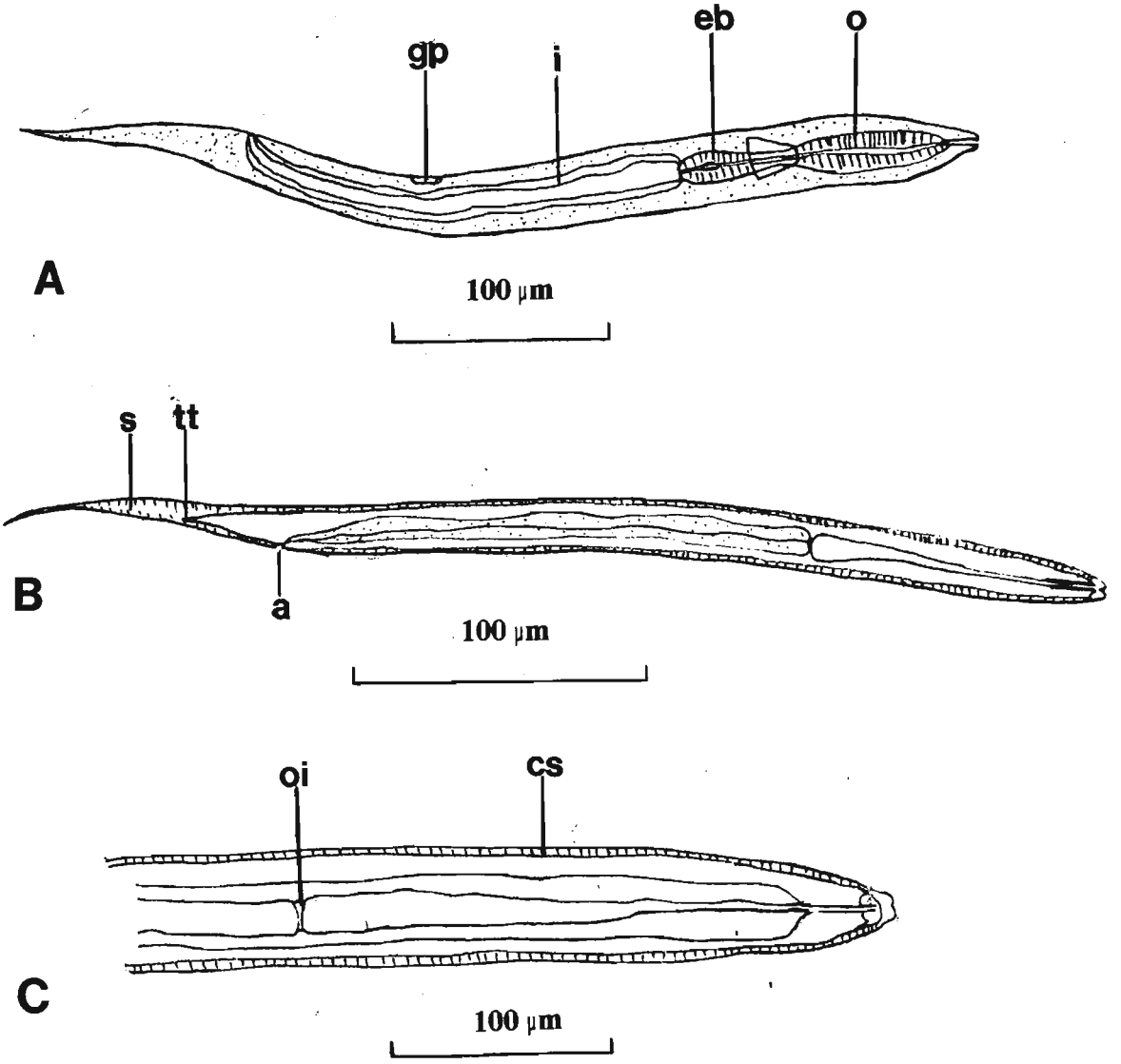


Figure 4. Diagrams of *N. americanus,* (**A**) rhabditiform (L_2) second stage larva (from K. Padayachee and D. Chinasamy, unpublished data), (**B**) filariform larva and (**C**) head region of filariform larva; **o**-oesophagus, **eb**-esophangeal bulb, **i**-intestine, **gp**-genital primodium, **a**-anus, **tt**-tip of tail, **s**-sheath, **cs**-cuticular striations, **oi**-esophagointestinal junction.

3.5 Selection of inland study localities

To assess the presence of hookworm infection in areas of sand situated inland of the coastal plain in KwaZulu-Natal, the Durban Land Type Map (Land Type Survey Staff, 1986) was used to select seven rural communities within the Maphumulu district (see Chapter 5, Figure 10). One school was chosen from each community with the help of Mr. Ndlovu, a senior environmental health officer in the area. Three schools (Nyamazane, Mushane, and Tshelabantu) were selected from communities situated on soil type Ab (low clay soils) and four from outside this area of interest, on high clay soils. Two of the latter were in the north (Empungeni and Thimuni) on soil type Fa and the other two in the south (Mary Gray and Nsuze-Gcwensa) on soil type Fb. The hypothesis was that if hookworm is present in the area of interest (Ab soils) its prevalence should at least be higher than in the surrounding areas (Fa and Fb soil types). Data from two schools (Sakhesethu and Sizani) situated on Ab soils on the coastal plain (Maurihungirire, 1993) were included in the analysis for comparative purposes.

3.6 Recovery of soil nematodes

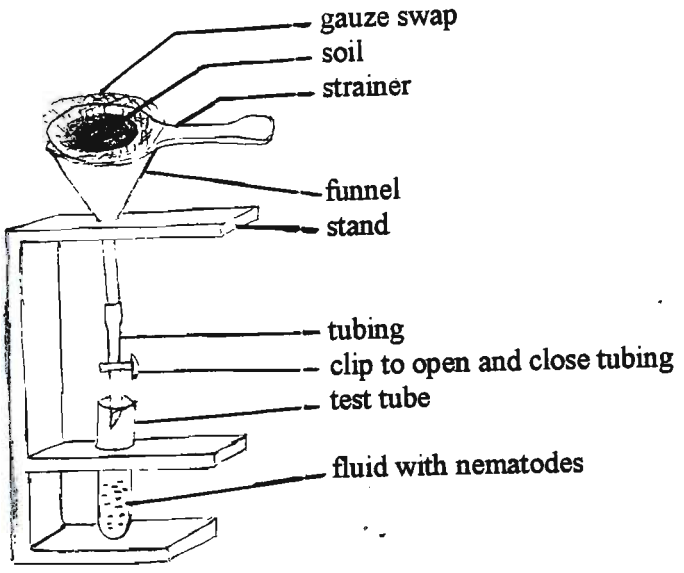
Counts of total nematode loadings in soil samples from the nine study localities named in section 3.5 were used to provide an indirect measure of the suitability of these soils to support the free-living and infective stages of *Necator americanus*. Ten 200g soil samples were collected from each locality, using a spade to scoop soil from the surface horizon (0 - 5 cm depth). Each sample was transferred into a separate jar (Figure 5).

Figure 5. Soil sampling at one of the study localities (Nyamazane). Some of the equipment used in the process is shown.

Samples were collected from points at least 100 m apart in school yards, play grounds, and about 300 m from the school premises in yards of surrounding local households. These were then taken to the laboratory, Durban, where nematodes were recovered using a series of modified Baermann apparatus (see Appendix C), in four specially constructed wooden stands each capable of supporting five funnels (Figure 6). In this way, 20 samples could be processed at a time, but because they had to be processed as quickly as possible, only two study localities could be visited per day.

3.7 Soil analysis

To investigate characteristics of the different soil types for possible correlation with epidemiological data, 500g portions of soil samples from all nine study localities were analysed for the following: organic carbon by the Walkey-Black procedure (Walkey and Black, 1934); particle size by the pipette method for [clay (< 0.002 mm), silt (0.002-0.05 mm), fine sand (0.05-0.25 mm), medium sand (0.25-0.50 mm) and coarse sand (0.50-2.00 mm)] (Gee and Bauder, 1986). pH was measured with a glass electrode using water and KCl and Exchangeable Acidity (Thomas, 1982).



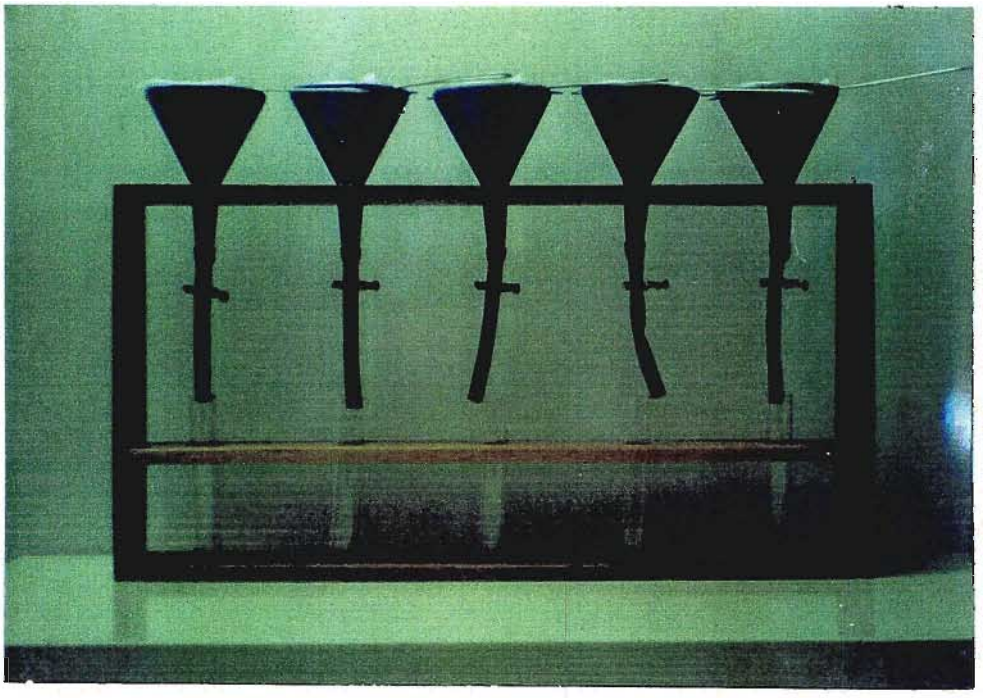


Figure 6. Modified Baermann apparatus used to extract nematodes from soil. Note the funnels and the test tubes supported by a wooden stand.

3.8 Statistical analysis

Basic statistical summaries were generated for all selected environmental variables. A univariate analysis was then carried out to assess the relationships between hookworm prevalence and individual climatic variables. In an attempt to identify components of the environment which influence the distribution pattern of hookworm, prevalence was tested against soil type, clay content (texture), physiographic position and climatic variables (temperature and rainfall-derived variables) using ANOVA, t-test and χ^2. Multivariate analysis was conducted to assess the environmental features which combine best to create the conditions necessary for hookworm survival and transmission.

To correlate hookworm data with nematode loadings in the soil as well as with soil variables (Chapter 5), epidemiological data were pooled for all the schools that were situated on similar soil types. Statistical differences in prevalence and mean intensity were analysed using ANOVA and χ^2. These were carried out for both age and sex data. Kruskal-Wallis (non-parametric equivalent of ANOVA) was used to test for differences in soil variables between groups of study localities that occur on different soil types. Finally, an attempt was made to correlate epidemiological data with soil variables and nematode loadings using the Spearman Correlation Coefficient. In this study prevalence (proportion of the population infected at a given time) is defined as the number of infected individuals divided by the total number of individuals examined and expressed as a percentage, and mean intensity as the total number of eggs per gram of faeces (e.p.g.) divided by the number of infected individuals (Anderson, 1982).

CHAPTER 4

Soil type, climate and the distribution of hookworm infection in KwaZulu-Natal

4.1 Introduction

From Chapter 2 it can be seen that different environmental variables may play roles in determining hookworm distribution in different parts of the world, but the precise factors responsible have not been identified. One of the factors commonly implicated is soil - type (WHO, 1961; WHO, 1964; Pawlowski *et al.*, 1991) and it seems probable (see Chapter 2) that the distribution of *Necator americanus* in tropical Africa is determined by the availability of suitable soil types and climate and, of course, an infected population.. One of these factors might be of prime significance in itself, or a combination acting together might regulate the extent of hookworm distribution. This chapter is dedicated to investigating this possibility. The key questions being asked are: (i) what is the hookworm distribution in KwaZulu-Natal? And, (ii), which environmental features are significantly associated with this distribution pattern?

4.2 Materials and Methods

The methods used in the collection and mapping of prevalence data and the field and laboratory methods employed were outlined in Chapter 3.

4.3 Results

4.3.1 Hookworm prevalence

The highest hookworm prevalences are in the northern and broadest part of the coastal plain (see Page 55), and these gradually decline southwards as the coastal plain narrows in this direction. There is also a sharp decline in hookworm prevalence in an inland (westward) direction. It seems probable therefore, that hookworm is limited in its distribution by a preference for the environmental conditions offered by the coastal plain.

4.3.2 Statistical analysis

Descriptive statistics for the environmental variables tested are summarized in Table 4.1. For the categorical information it was necessary to group soil types because some individual categories had very small sample sizes. These soil types were grouped into broad categories based on the Memoirs of the Land Type Survey Staff (1986), viz: I apedal freely drained soils (Ab, Ac, Ae, Ah); II dark coloured or red soils with Glenrosa or Mispah forms (Ea, Fa, Fb) [soil type Ea was included in this group on the basis of texture, i.e. high clay content]; III grey regic sands (Ha, Hb); and IV miscellaneous land classes (Ia, Ib). Similarly, the upland region (UR) was grouped with the mountain region (MR) (see Chapter 3). Two soil types (Db and Dc) were omitted from the analysis due to the sample size being too small even when taken together. Hence the soil type percentages ($\leq$ 150 m above sea level) in Table 4.5 do not sum up to 100 and the sample size was changed to n = 72 data points.

Table 4.1. Summary of all variables used in the analysis (data from Schulze (1982), except for ET**). [*HU = accumulated mean temperatures above a threshhold value of 10 °C and below an upper limit of 30 °C; **ET = (8 MAT + 14 AR) / (AR + 8), where MAT is the mean annual temperature and AR the annual temperature range (Stuckenberg, 1969)].

VARIABLES (for the whole study area)	MEAN (SD)	RANGE
Altitude (m above sea level)	296 (240.7)	(50-1700)
Rainfall-derived (mm)		
MAP (mean annual precipitation)	796 (159.9)	(100-1200)
MMP Jan (mean monthly precipitation for January)	118 (19.1)	(12-160)
MMP Jul (mean monthly precipitation for July)	25 (8.9)	(10-70)
MNR Jan (mean number of rainy days > 1mm for January)	8 (2.2)	(4-12)
MNR Jul (mean number of rainy days > 1mm for July)	2 (0.8)	(1-4)
Temperature-derived (°C)		
MAT (mean annual temperature)	20 (1.1)	(15-22)
MMT Jan (mean monthly temperature for January)	24 (1.9)	(19-26)
MMT Jul (mean monthly temperature for July)	16 (1.9)	(9-18)
MD max Jan (mean daily maximum temperature for January)	29 (1.8)	(25-31)
MD max Jul (mean daily maximum temperature for July)	23 (1.9)	(16-25)
MD min Jan (mean daily minimum temperature for January)	19 (2.0)	(12-22)
MD min Jul (mean daily minimum temperature for July)	9 (2.2)	(2-13)
HU Nov-Jan (heat units, November to January)*	1214 (153.2)	(700-1300)
ET (Effective Temperature)**	16 (0.7)	(14-18)
Evaporation-derived (mm)		
MPE Jan (monthly potential evapotranspiration for January)	161 (19.4)	(100-190)
MPE Jul (monthly potential evapotranspiration for July)	102 (7.9)	(90-110)

4.3.2.1 Univariate analysis

The relationships between hookworm prevalence and the individual environmental features listed in Table 4.1 were assessed by using the Pearson Correlation Coefficient and the statistically significant results are given in Table 4.2. The criterion for acceptance of a variable in the univariate analysis was a p value < 0.0001 and only those with $r > 0.5$ were entered into the multivariate model. Associations with $p < 0.05$ (t-test) were used for pairwise comparisons.

Table 4.2 Significant correlations ($p < 0.0001$) between hookworm prevalence and environmental variables.

VARIABLE	r
Altitude (m above sea level)	- 0.56579
MNR Jan -mean number of rainy days > 1mm for January	- 0.54481
MMT Jan-mean monthly temperature for January (°C)	0.64939
MD max Jul-mean daily maximum temperature for July (°C)	0.59929
MD min Jan-mean daily minimum temperature for January (°C)	0.76535

Although five significant correlations were found, the scatter of points for some of them ranged widely about the line of fit, indicating a weak relationship. For further analysis therefore the dataset was split into two altitudinal areas: < 300 m and ≥ 300 m. This is in line with the planned helminth control programme which targets areas below 300 m as the zone of most severe transmission (C.C. Appleton, personal communication).

4.3.2.2 Univariate associations

The aim of this part of the analysis was to identify significant associations between hookworm prevalence and individual environmental variables. Analysis of variance showed that there was a significant difference ($p = 0.0001$) in hookworm prevalence between the different soil types, with soil type groups I and III supporting the highest mean prevalences (Figure 7). A pairwise comparison (t-test) between the soil type groups showed that there was no significant difference in mean prevalence between groups I and III, nor between groups II and IV. Any combination of these two groups did, however, show a significant difference. A pairwise comparison between the the three clay content categories given in Chapter 3 for the four soil type groups, viz: low (L) in groups I and III, medium (M) in group II and high (H) in group IV, showed no significant differences ($p > 0.05$) in hookworm prevalence between the soil type groups with high and medium clay content. A comparison between these soil groups and those with low clay content did however show significant differences. In respect of the physiographic regions, a t-test pairwise comparison showed no significant differences in mean hookworm prevalence between regions CR and BP where soil groups I and III occur, and regions IR, MR/UR and PR where soil groups II and IV occur.

Analysis of variance also showed that there was a significant difference ($p = 0.0001$) between hookworm prevalences in the two altitudinal zones (< 300 m and ≥ 300 m). High prevalences ($\bar{x}$ = 44.4 %) were found at low altitudes (< 300 m) whereas higher altitudes (≥ 300 m) supported much lower low prevalences ($\bar{x}$ = 4.2 %). The χ^2-test showed that a significant proportion of soil groups I and III were found at low altitudes, while groups II and IV were found mainly at higher altitudes (Figure 8).

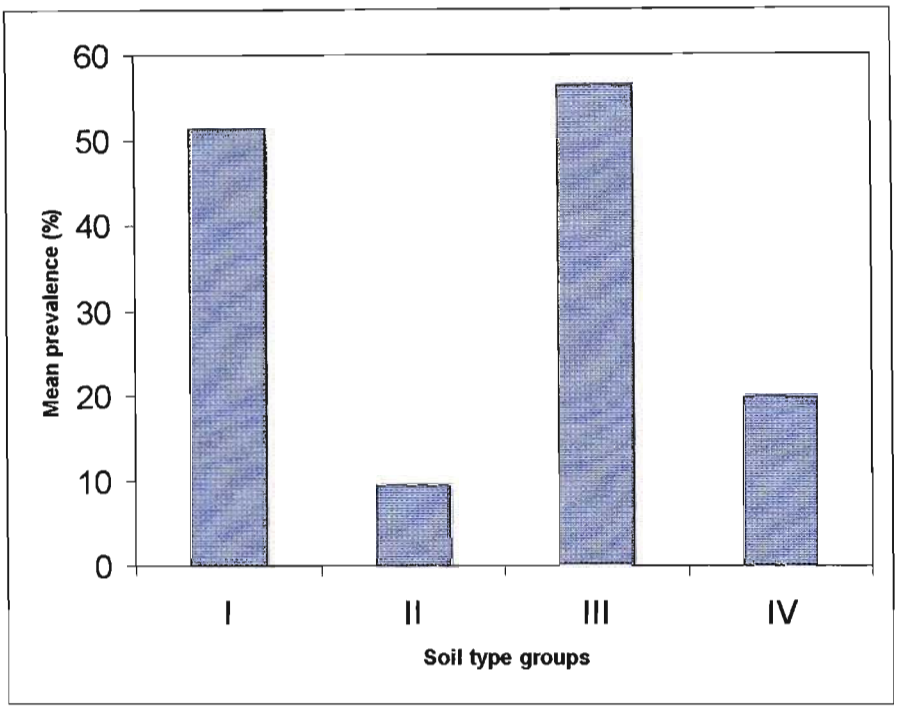


Figure 7. Prevalence of hookworm infection in the different soil type groups; I (n = 7), II (n = 26), III (n = 27) and IV (n = 12). Soil types grouped into broad categories based on the Memoires of the Land Type Survey Staff (1986), viz: I apedal freely drained soils (Ab, Ac, Ae, Ah); II dark coloured or red soils with Glenrosa or Mispah forms (Ea, Fa, Fb); III grey regic sands (Ha, Hb); and IV miscellaneous land classes (Ia, Ib).

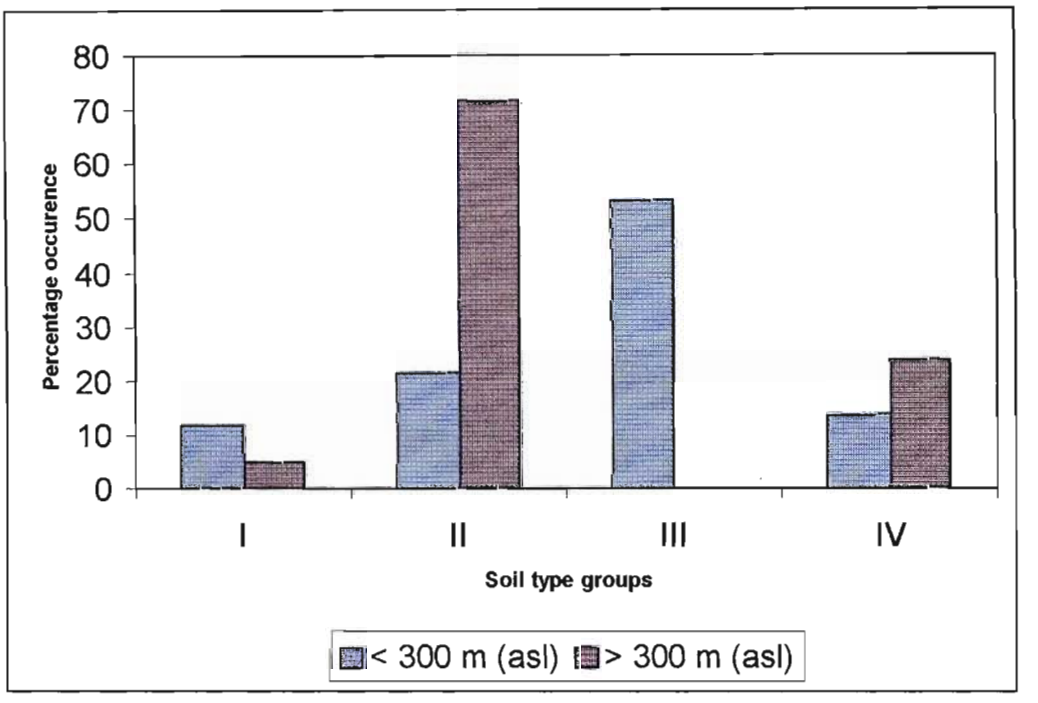


Figure 8. Percentage occurrence of the four soil type groups in the two altitudinal zones (< 300 m and ≥ 300 m above sea level). Soil types grouped into broad categories based on the Memoires of the Land Type Survey Staff (1986), viz: I apedal freely drained soils (Ab, Ac, Ae, Ah); II dark coloured or red soils with Glenrosa or Mispah forms (Ea, Fa, Fb); III grey regic sands (Ha, Hb); and IV miscellaneous land classes (Ia, Ib).

The prevailing climatic conditions (rainfall and temperature-derived variables) are similar in areas where soil groups I and III are found (54-150 m), and also where soil groups II and IV are found (321-594 m) (Table 4.3).

Table 4.3 Soil type groups in relation to altitude (54 - 594 m above sea level) and selected climatic variables listed in Table 4.2 ($p = 0.0001$).

VARIABLE	Group I (n = 7)	Group II (n = 26)	Group III (n = 27)	Group IV (n = 12)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Altitude (m asl)	150.0 (200.0)	593.8 (559.1)	53.70 (13.34)	320.8 (249.9)
MNR Jan (mm)	7.714 (1.799)	9.500 (1.334)	6.667 (1.981)	9.333 (1.923)
MMT Jan (°C)	24.86 (0.690)	22.81 (2.059)	25.63 (1.079)	23.92 (1.165)
MD max Jul (days)	23.43 (1.397)	21.73 (2.237)	24.19 (1.210)	22.92 (1.084)
MD min Jan (days)	19.71 (1.113)	17.46 (2.139)	20.48 (1.014)	18.50 (1.087)

Based on the above observations, the division of the province into two altitudinal zones of < 300 m and ≥ 300 m was replaced by a new zonation, ≤ 150 m and > 150 m above sea level, for more precise associations (see Figure 9). A t-test (Table 4.4) showed a significant difference in prevailing climatic conditions between these two newly defined areas of high and low prevalence, with areas below 150 m experiencing warmer temperatures and relatively higher winter rainfall than areas above 150 m. A χ^2-test clearly showed (Table 4.5) that there was a significant difference ($p = 0.001$) in the proportions of soil types found in the two areas, corresponding with clay content and physiographic region. The Lebombo foothills grouped under MR/UR are not strictly mountains but rather elevated hills (100-300 m) on the western margin of the coastal plain and account for 19.6 % of the area below 150 m.

Figure 9. Map of KwaZulu-Natal showing altitudinal contours (based on Appleton and Kvalsvig, 1994) and the distribution of hookworm infection (prevalence). Localities of high prevalence (yellow and green dots), intermediate prevalence (red dots) and low prevalence (blue dots) are also shown.

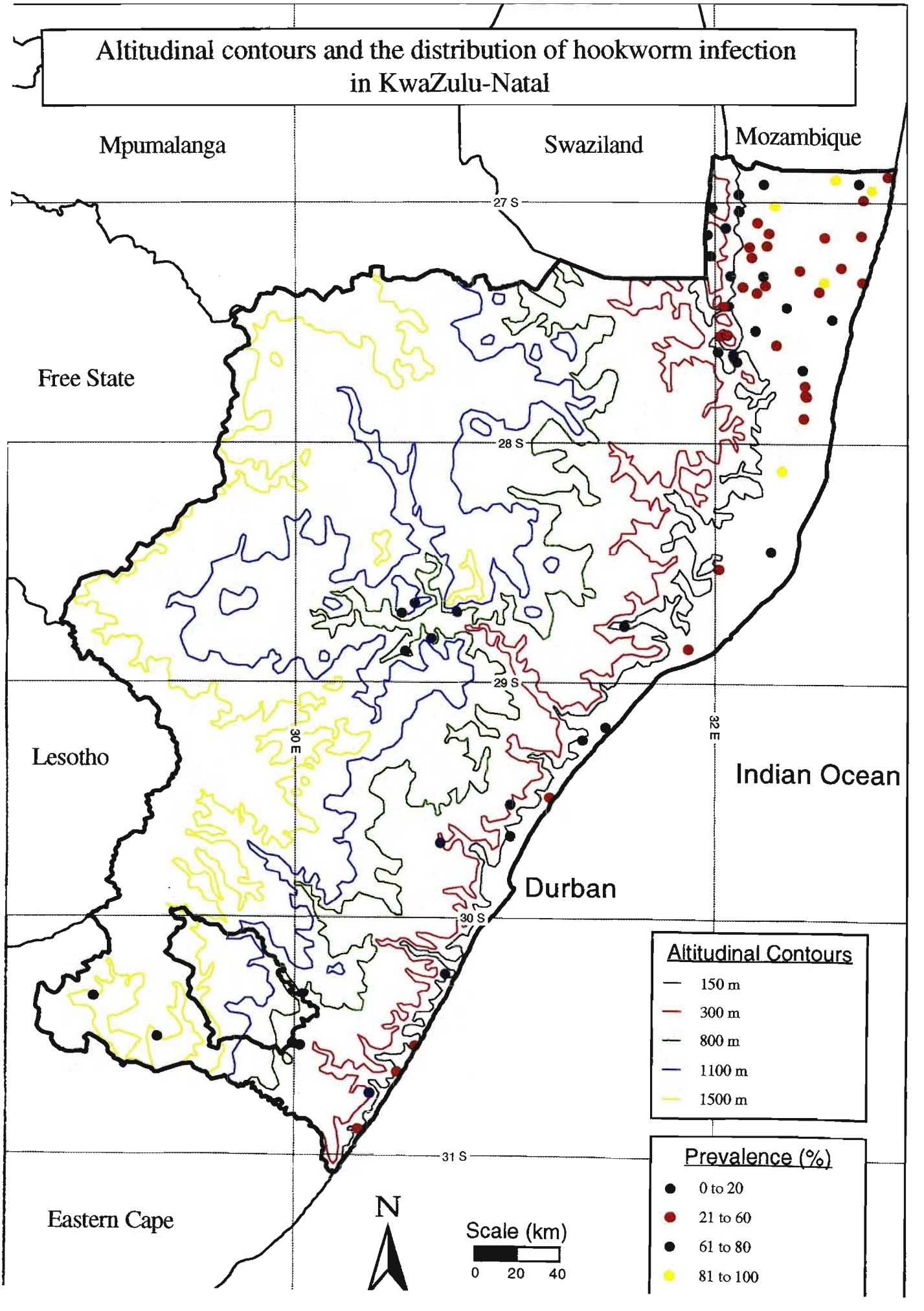

Altitudinal contours and the distribution of hookworm infection in KwaZulu-Natal
Mpumalanga
Swaziland
Mozambique
27 S
Free State
28 S
29 S
30 E
32 E
Lesotho
Indian Ocean
Durban
30 S
31 S
Eastern Cape
N
Scale (km)
0 20 40
Altitudinal Contours
150 m
300 m
800 m
1100 m
1500 m
Prevalence (%)
0 to 20
21 to 60
61 to 80
81 to 100

Table 4.4 Comparison of mean values of hookworm prevalence and climatic variables between the two major altitudinal zones. Data from Schulze *et al.*, 1982, except for ET = (8 MAT + 14 AR) / (AR + 8), where MAT is the mean annual temperature and AR the annual temperature range (Stuckenberg, 1969)].

VARIABLE	≤ 150 m asl	> 150 m asl	p-value
	Mean	Mean	
Prevalence %	45	6	0.0001
Rainfall-derived (mm)			
MMP Jul (mean monthly precipitation for July)	27	20	0.0001
MNR Jan (mean number of rainy days > 1mm for January)	8	10	0.0001
MNR Jul (mean number of rainy days > 1mm for July)	2	2	0.0306
Temperature-derived (°C)			
MAT (mean annual temperature)	21	19	0.0001
MMT Jan (mean monthly temperature for January)	25	22	0.0001
MMT Jul (mean monthly temperature for July)	17	15	0.0001
MD max Jan (mean daily maximum temperature for January)	30	28	0.0001
MD max Jul (mean daily maximum temperature for July)	24	21	0.0001
MD min Jan (mean daily minimum temperature for January)	20	17	0.0001
MD min Jul (mean daily minimum temperature for July)	9	7	0.0006
HU Nov–Jan (heat units, November to January)	1276	1078	0.0001
ET (Effective Temperature)	16	16	0.0324
Evaporation-derived (mm)			
MPE Jan (monthly potential evapotranspiration for January)	165	153	0.0140
MPE Jul (monthly potential evapotranspiration for July)	105	97	0.0001

Table 4.5 Percentage occurrence of soil types, viz: apedal freely drained soils (Ab, Ac, Ae, Ah); dark coloured or red soils with Glenrosa or Mispah forms (Ea, Fa, Fb); grey regic sands (Ha, Hb); and miscellaneous land classes (Ia, Ib), clay content (Land Type Survey Staff, 1986) and physiographic regions (Phillips, 1973) in the two major altitudinal zones.

Soil type	≤ 150 m asl	> 150 m asl
Ab	5.9	0
Ac	0	4.4
Ae	3.9	0
Ah	2	0
Ea	16	0
Fa	3.9	30.4
Fb	2	34.8
Ha	41.2	0
Hb	11.8	0
Ia	3.9	0
Ib	5.9	30.4
Clay content		
H-high (> 45 %)	29.4	30.4
M-medium (15-45 %)	9.8	69.6
L-low (< 15 %)	60.8	0
Physiographic regions		
BP (basin plainland)	19.6	0
CR (coastal region)	58.8	0
IR (intermediate region)	2	39.1
MR (mountain region)	20	34.8
PR (plateau region)	0	17.4
UR (upland region)	0	8.7

4.3.2.3 Multivariate analysis.

Covariance analysis (Table 4.6) was used to assess the effect of a combination of environmental variables listed in Table 4.2 (selected as described in section 4.3.2.1). Soil type was included as a probable key factor in hookworm transmission based on the univariate associations. However, in the multivariate analysis neither soil type nor altitude were significantly correlated with prevalence. This may be due to the fact that the analysis included areas of both high and low hookworm prevalence introducing a masking effect. The univariate analysis clearly showed (Figure 7) that specific soil types at particular altitudes supported high hookworm prevalences. So it is the effect of climatic variables acting in combination with soil type, that was detected here. Prevalence was in fact most strongly correlated with minimum daily temperature for January (MD min Jan) followed by the number of rainy days for January (MNR Jan).

Table 4.6 Associations between hookworm prevalence and the selected environmental variables (with r > 0.5) listed in Table 4.3 and soil type.

VARIABLE	**F-statistic**	**p-value**
Soil type	1.37	0.2606
Altitude (m above sea level)	3.91	0.0523
MNR Jan-mean number of rainy days >1 mm for January	7.33	0.0087
MMT Jan-mean monthly temperature for January (°C)	0.10	0.7557
MD max Jul-mean daily maximum temperature for July (°C)	4.25	0.0434
MD min Jan-mean daily minimum temperature for January (°C)	25.23	0.0001

4.4 Discussion

The present analysis of all available hookworm prevalence data in KwaZulu-Natal has shown that there is a decline in prevalence across the province, inland from east to west, i.e. in an inland direction. Prevalence was highest on the coastal plain but it also decreased from north to south. The trend of decreasing prevalence inland is probably due to the physiographic structure of the region which creates steep climatic gradients, especially along the east-west axis (coast to interior). The occurrence of high hookworm infection in low lying areas, has also been reported in other parts of the world. For example, in the Hatay province of Turkey, a number of villages near the Mediterranean coast showed 88 % hookworm infection while villages above 610m were free of hookworms (Mimioglu and Akyol, 1956). Matsusaki *et al.* (1964) found that throughout Japan, *Necator americanus* was most prevalent on the plains; while Goldsmid *et al.* (1974) found 82 % *Necator americanus* infection in the Zimbabwean lowveld. None of these studies, however, attempted to correlate hookworm prevalence with the prevailing environmental conditions.

In this study the effect of climatic differences between the two designated altitudinal zones (above and below 150 m asl) is well illustrated by the differences in mean prevalence of hookworm infection. The results showed that altitudes ≤ 150 m asl have high hookworm prevalences and are characterized by warmer temperatures and higher rainfall; areas > 150 m asl are characterized by low hookworm prevalences, cooler temperatures and moderate rainfall. These results concur with findings of other studies even though most did not identify the species of hookworm involved and in such cases "hookworm" is used in a general sense

For example in Queensland, Australia, Swayer (1921) found that hookworm infection was more prevalent throughout the region of high rainfall, between the coastal ranges and the sea, while Lambert (1921) found that the coastal belt of North Queensland had a very high rate of hookworm infection, except for the Ayr district which lies in an area belt of low rainfall. Similarly, in the eastern region of the Black Sea, the degree of hookworm infection decreases as one moves away from the coast and the low valleys (Ozsan, 1953), and in the drier areas infection disappears altogether. Wang (1959) found that hookworm infection occurred throughout all of China, except in a few provinces in the north-west which are too cold and dry.

The climatic components of rainfall and temperature, to which the inland decline of hookworm prevalence is attributed, can also be used to explain the southward decline of hookworm prevalence along the coast of KwaZulu-Natal. This is particularly because precipitation in the province decreases with increasing latitude and in summer, day-to-day cooling, the extent of decreases in temperature, becomes more frequent (Martyn, 1992). In fact De Moor *et al.* (1977) stated that while the central and northern parts of the coastal plain are subtropical and very warm, temperature fluctuations become more pronounced southwards, with a longer, drier and cooler climate. According to Meadows (1985) and Martyn (1992), the coastal area of KwaZulu-Natal has a subtropical climate and as such experiences warm, wet summers and cool, dry winter conditions, whereas the Eastern Cape has a temperate climate with long dry summers and cool wet winter conditions and thus is probably less suitable for hookworm development. Sturrock (1966) showed that in arid areas of Tanzania, where there is a long dry season and a short rainy season, the level of hookworm infection is very low.

However, the distribution pattern of hookworm observed in KwaZulu-Natal cannot be explained by climate alone. Soil type is also important. According to Fraga de Azevedo (1963), altitude, climate and soil are all important in the epidemiology of hookworm infection in Moçambique. In the present study, high hookworm prevalence was significantly associated with soil types which are common below 150 m and low prevalence with soil types occurring mostly above this altitude. The area ≤ 150 m defines the coastal plain which is 74 km wide in the north and is characterized by regic sands and the highest hookworm prevalences. In the south, it narrows to 31 km and is dominated by red dune cordon and yellow sands with high prevalences. Further south it becomes even narrower with moderate hookworm prevalences which disappear dramatically as does the coastal plain just south of the border between KwaZulu-Natal and the Eastern Cape. The coastal plain of KwaZulu-Natal was once submerged and formed part of the continental shelf, and is not yet fully exposed as its southern continuation extends underneath the sea (King, 1963). Within the study region, areas > 150 m include the Lebombo mountains in the north with their dark coloured or red soils, the coastal hinterland with weakly developed sandy soils and the intermediate region with a mosaic of weakly developed soils and black clays, all supporting very low hookworm prevalences.

The above associations can be explained by the texture of the dominant soils. The low clay-content, sandy soil types found below 150 m asl showed a significant correlation with high hookworm prevalence when compared to high clay-content soil types above this altitude. This seems to confirm speculations by a number of authors (see Chapter 2), that high soil clay content may be responsible for the very low prevalence, or even absence, of infection in

certain areas. In Tanzania, Kihamia (1977) attributed low infection rates to clay soils in the mountainous area of Kilimanjaro. In Botswana, infection rates of 86-90 % were recorded in four villages in sandy areas whereas in two villages in areas with clay soils, the infection rates were 9 % and 13 % (Michaelsen, 1985). According to Wallace (1963), the advantage for hookworm larval development in sandy soil over clay is that the former is better aerated, such that when gaseous exchange is hindered in clay soils by waterlogging, anaerobic conditions develop. This inhibits transmission because the larvae are strict aerobes. This mechanism can probably be used to explain observations made by other studies as well. For example, in Tennessee, USA, hookworm infection was associated with the presence of sandy soils (Otto *et al.*, 1931). Pampiglione and Hadjers (1965) showed in eastern Algeria that soil conditions on the coastal strip were well suited to the survival of hookworm larvae and in Georgia (USA), Martin (1972a & b) showed that hookworm is endemic to the sandy soils of the coastal plain, and that prevalences were much lower outside this region.

As already mentioned and discussed, hookworm distribution is unlikely to be determined by a single environmental factor, but by a variety of interdependent factors. When combinations of factors were taken together for multivariate analysis, temperature was correlated most prominently, followed by rainfall. This is particularly interesting because in Nigeria, Nwosu and Anya (1980) found that *Necator* transmission is a rainy season phenomenon and that temperature had little effect since it was consistently above the 10 °C ambient developmental minimum for the parasite's development. The pattern is probably similar in other regions which experience a warm tropical climate, e.g. hookworm surveys conducted in India by Maplestone (1930); Tanzania, (Sturrock, 1966); West Bengal (Nawalinski *et al.*, 1978;

Hominick *et al.*, 1987); Nigeria (Udonsi *et al.*, 1980; Nwosu, 1981); Gambia (Knight and Merret, 1981) and Cameroon (Ratard *et al.*, 1992). These authors all found that transmission was either limited to or was highest during periods, or in areas, of high rainfall. In KwaZulu-Natal rain falls at times throughout the year even though there is usually a summer maximum especially along the eastern coastal lowlands owing to the moderating influence of the warm Moçambique current in the Indian ocean (Christopher, 1982; Meadows, 1985). There is therefore no real dry season though there is usually a summer peak in precipitation. Temperature, however, has a markedly seasonal distribution with cold winters and warm to hot summers (Meadows, 1985). Thus in this region temperature seems to play an important role in determining the extent of hookworm distribution, with cold temperatures (10-15 ° C) serving as an effective means of regulating the inland and southerly distribution.

In the present results, the mean minimum daily temperature (MD min Jan), followed by mean number of rainy days (MNR Jan), both representative of mid-summer, showed strong correlations with high hookworm prevalences, with the maximum daily temperature for mid-winter showing a weak correlation. This highlights the importance of temperature and also points to the possibility of seasonal transmission. According to Wang (1959), transmission in China is often seasonal such that in cold dry provinces transmission may be limited to a few summer months, while in warm southern regions it may continue almost throughout the year. Similarly, in winter Zimbabwe experiences dry weather and low temperatures which create unfavourable conditions for hookworm survival in the soil, and this would be expected to result in a marked reduction in transmission. In contrast, transmission is likely to increase during the wet summer months because moisture and warm weather will enhance the survival

of larvae (Chandiwana, 1990). However, in KwaZulu-Natal the existence of seasonal transmission still needs to be demonstrated. Meadows (1985) stated that the type of temperature control on biological processes depends on the mechanism by which temperature affects the species concerned. In this study minimum daily summer temperature appears to be the most significant aspect of this factor. The duration of larval development is a function of temperature (Smith, 1990) and this could also be an indication that the development of hookworm larvae relies on the short-term weather pattern, i.e. day to day changes during a particular period.

Taking this a step further, Smith (1990) noted that survival of hookworm larvae depends on all components of the environment that affect larval activity. Nematodes are entirely dependent on water for their activity (Wallace, 1963). So temperature is important for "speed of hatching" and the rate of larval development for ± 5 days and moisture/damp soil is important for survival during that period. Rainfall is probably only important inasmuch as it is present as interstitial water, providing a suitable medium for larval movement and feeding. This is particularly important because when the soil dries out, this water is restricted to thin films around individual soil particles (Killmann, 1995). According to Schad (1991), the infective stage remains quiescent in moisture films until it responds to the necessary stimuli which facilitate contact with its host. In Basra, Iraq, high average temperatures and damp soil provide ideal conditions for the development of hookworm larvae and infection rates are high (Nor El-Din, 1958). According to Wallace (1963), neither extreme moisture conditions nor complete drying out favour nematode survival as this depends on the water-holding properties of the soil. For example, the absence of hookworm larvae from soil samples collected in

Egyptian villages was attributed to their failure to develop, and was explained by the excessive dryness of the soils and the compacted nature of the surface layer (Scott, 1937).

It is also important to note (Geiger, 1950; Levine, 1963; Yoshino, 1975) that standard weather stations above the ground are a considerable distance (± 1.2 m) from the actual larval habitat in the soil and their readings may therefore have little relevance to the biology of these organisms (geohelminths). This is even more so since the soil acts as a moderating influence (buffer) reducing the range of atmospheric conditions compared to those at the soil surface. The effectiveness of this buffering depends on the characteristics of the soil (Hawker and Linton, 1971). Neverthereless, based on the present work it can be concluded that the distribution of hookworm in KwaZulu-Natal is influenced primarily by soil type and superimposed on this are climatic factors, particularly temperature (minimum daily temperature for January), followed by rainfall (the number of rainy days for January MNR Jan).

CHAPTER 5

Hookworm infection in inland areas of sand, and possible influences of certain physical and chemical attributes of different soil types on hookworm distribution in KwaZulu-Natal

5.1 Introduction

The epidemiological importance of any particular developmental stage in a parasite's life cycle is directly related to the time the parasite spends in that stage (Smith, 1990). With regard to the parasite of interest in this study, *Necator americanus*, the ability of the larval stages to survive under natural environmental conditions largely determines transmission in a particular area. According to Pawlowski *et al.* (1991), the first two free-living stages; the rhabditiform larvae (L_1 and L_2), are short lived, remain in the faeces or faecally polluted soil and are not very mobile. Most die in the first few days post-hatching, while developing to the infective stage; many being ingested by predaceous nematodes, mites and other invertebrates in the soil. By contrast the non-feeding, third stage infective filariform larvae (L_3) are capable of considerable vertical movement in the soil (1m or more), although they do not move far laterally (Pawlowski *et al.*, 1991).

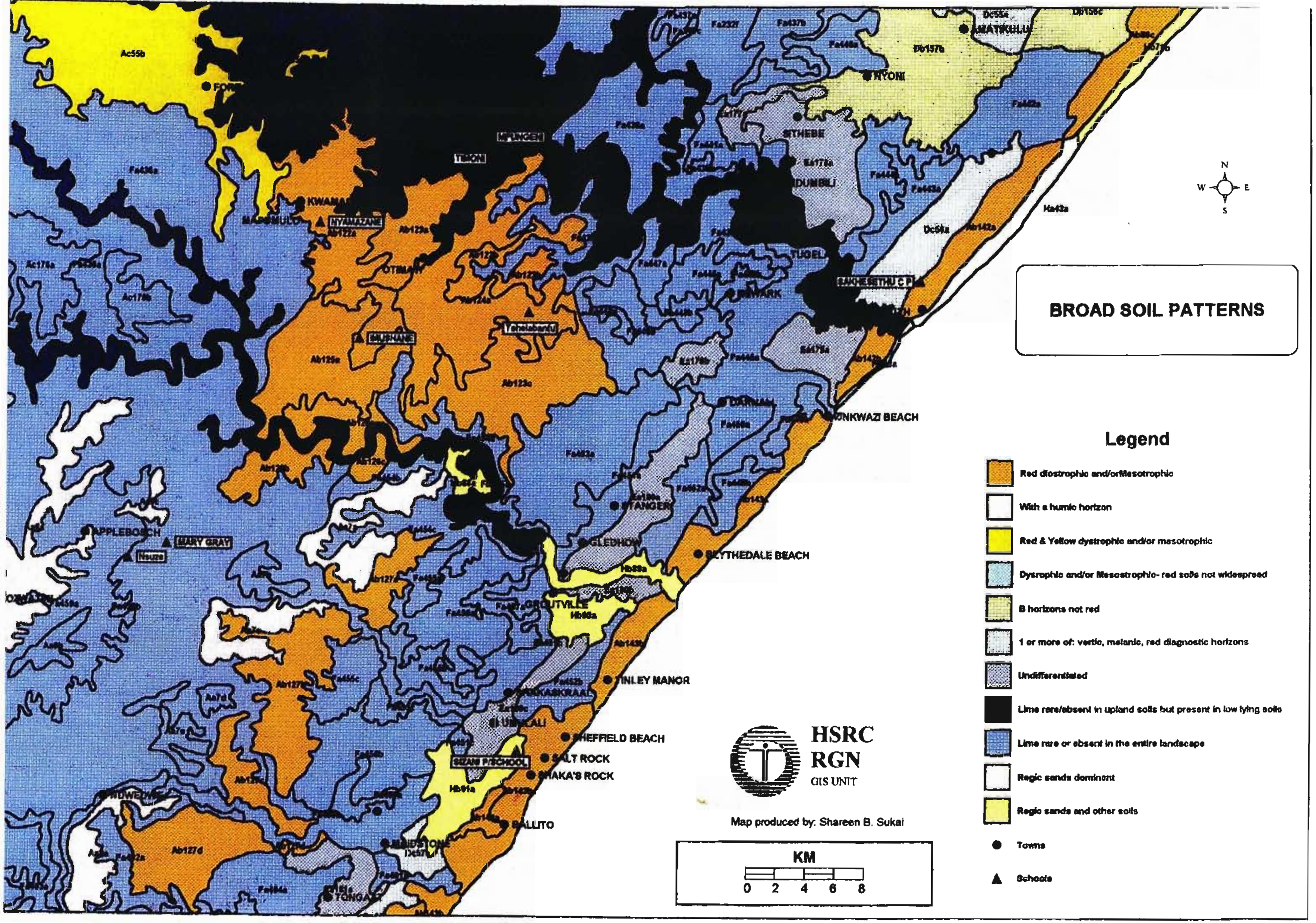
Thus in an area that is suitable for hookworm development, the probability of transmission depends on the survival of infective larvae in the soil during the period for which it awaits its host. The long-lived L_3 larvae therefore have more epidemiological importance than the

short-lived L_1 and L_2, since it is soil contaminated with L_3 larvae that is a potential source of infection. In the soil the larvae are subjected to a microenvironment which is influenced by prevailing external climatic conditions. However, the influence of climate on the soil depends largely on the nature of the soil itself (Foth, 1984).

In KwaZulu-Natal, hookworm infection is most prevalent in communities situated on the low-lying coastal plain (≤ 150 m above sea level) as shown in Chapter 4. This distribution pattern is significantly associated with certain environmental features that characterize the plain. These include climatic factors (particularly temperature and rainfall-derived variables) which coincide with the occurrence of a low clay-content of soil types the grey regic sands (Ha, Hb) and red dune cordon sand (Ab). However, several examples exist in KwaZulu-Natal where sandy soils of type Ab occur inland (Figure 10). Isolated pockets of transmission may therefore exist in communities located on these sandy areas.

The first part of this chapter is aimed at investigating the presence of hookworm infection in these areas. The second part was initially intended to look at the survival of infective hookworm larvae under different culture conditions in the laboratory, notably survival in soils of different texture and under different temperature and moisture regimes. However, preliminary trials showed that this kind of work required more time than was allocated for this study. This relates particularly to standardisation of methodology and elimination of possible confounding variables. The process would include finding a pure *Necator americanus* infection as it usually occurs together with *Strongyloides stercoralis* in KwaZulu-Natal which also has soil-dwelling larvae.

Figure 10. Broad soil patterns map 2930 Durban (Land Type Survey Staff, 1986) showing the position of the nine study localities (triangles) in relation to the different soil types. Seven of the localities are situated in the inland Mapumulo District and two on the coastal plain.


BROAD SOIL PATTERNS
N
W
E
S
Legend
Red diostrophic and/orMesotrophic
With a humic horizon
Red & Yellow dystrophic and/or mesotrophic
Dysrophic and/or Mesostrophic- red soils not widespread
B horizons not red
1 or more of: vertic, melanic, red diagnostic horizons
Undifferentiated
Lime rare/absent in upland soils but present in low lying soils
Lime rare or absent in the entire landscape
Regic sands dominant
Regic sands and other soils
Towns
Schools
HSRC
RGN
GIS UNIT
Map produced by: Shareen B. Sukai
KM
0 2 4 6 8
NYONI
TUGELA
SAKHESETHU C P
ZINKWAZI BEACH
BLYTHEDALE BEACH
GLEDHOW
GROUTVILLE
TINLEY MANOR
SHEFFIELD BEACH
SALT ROCK
SHAKA'S ROCK
BALLITO
MAIDSTONE
TONGAAT
NDWEDWE
APPLEBOSCH
MARY GRAY
NYAMAZANE
TIMON
DARNALL
DUMBILI
SITHEBE
AMATIKULU
Ac55b
Ab123c
Ab127d
Hb88a
Hb91a
Ha43a
Dc56a
Bb157b

With limited time available, another method was sought to assess the suitability of the different soil types for larval hookworm survival. This was done by using the abundance of free-living nematodes in soil samples, as an indirect measure of the suitability of the soil environment for hookworm larvae, following the method of (Appleton and Henzi, 1993).

The third part of this chapter reports on analyses of soil samples from the nine study localities, which were conducted in an attempt to identify characteristics of different soil types which correlate with epidemiological data.

5.2 Materials and methods

The selection of inland study localities as well as field and laboratory methods were outlined in Chapter 3.

5.3 Results

A total of 757 scholars was examined from the seven schools located up to 53 km inland and 336 from the schools on the coastal plain giving a grand total of 1093. To make statistical analysis possible, the scholars were grouped into the following age groups: 5-8, 9-12 and 13-19 years. Study localities situated on each soil type were also grouped and hookworm egg output data pooled. Four major groups were thus created: the coastal plain (CP) (n = 2), inland sandy area (SA) (n = 3) and inland non-sandy areas north (IN) and south (IS) of SA, (n = 2 in both cases).

5.3.1 Prevalence and intensity of hookworm infection

The χ^2-test (Figure 11) showed that there was a significant difference ($\chi^2 = 27.3$; $p = 0.001$) between prevalence of hookworm infection rates among the inland study localities situated in the sandy area (SA) on the red dune cordon sand (Ab) with a moderate mean prevalence of 17.3 % and surrounding localities situated north (IN) and south (IS) of SA on clayey soil types the dark coloured or red Glenrosa or Mispah forms (Fa and Fb) respectively. These latter soil types supported low mean prevalences (5.3 %). The χ^2-test also showed a significant difference ($\chi^2 = 321.1$; $p = 0.001$) between hookworm prevalences at all the inland study localities and the two study localities on the coastal plain (CP) on sandy soil type Ab, which supported a mean prevalence of 62.5 %.

Analysis of variance of the data presented in Figure 11 showed however that the intensity of infection did not vary significantly ($p = 0.1399$) among the groups of inland study localities situated either in the sandy area (SA) or on the clayey soil types (IN and IS), even though the former localities had higher intensities. Mean intensities for the coastal plain study localities were not available from Maurihungirire (1993) and therefore comparison with inland localities was not possible. Similarly age-sex intensity and prevalence-related data for these sites could not be accessed.

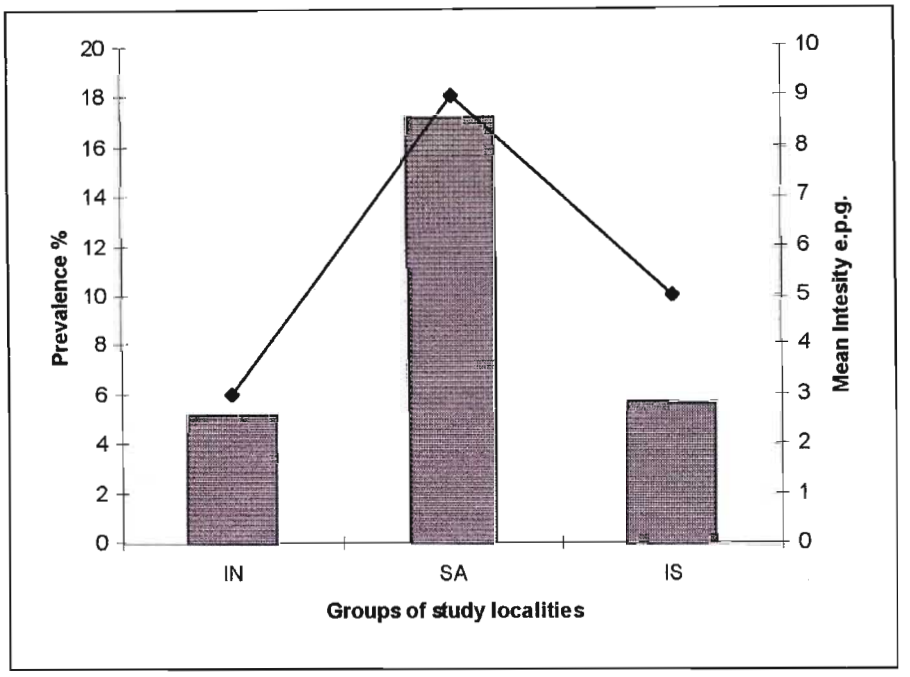


Figure 11. Prevalence and mean intensity of hookworm infection in groups of study localities situated on the inland sandy area (SA) (n =3) and surrounding non-sandy areas north (IN) and south (IS) of the SA (n = 2 for both). Bars = prevalence; diamonds = intensity.

5.3.2 Age and sex-related data

Although mean hookworm prevalence at the seven inland sites reached its peak in the 13-19 year old age group (12.0 %) (Figure 12), the χ^2-test showed that there was no significant difference in prevalence of hookworm infection between the three age groups (5-8, 9-12, 13-19 years). However, when groups of study localities were compared, the 9-12 year age group in the sandy area (SA) showed a significantly higher prevalence than those from the surrounding non-sandy areas (IN and IS) ($p = 0.001$). In contrast to prevalence, highest mean intensities occurred in the 5-8 year age group. Analysis of variance of the data in Figure 12 showed that the intensity of infection did not vary significantly ($p = 0.9276$) between the three age groups. However, the intensity of infection in the sandy area (SA) was significantly higher ($p = 0.0015$) than in the non-sandy areas north (IN) and south (IS) of SA, particularly in the 13-19 year age group.

Overall prevalences of 9.6 % and 9.3 % were recorded for male and female scholars, respectively, in the inland study area. There was no significant difference between them. When comparison was made between the different groups of study localities, the inland sandy area (SA) showed significantly higher prevalences for both genders compared to the surrounding non-sandy areas (IN and IS) (χ^2-test, $p = 0.006$ and $p = 0.001$ for males and females, respectively) (Figure 13). Similarly the intensity of infection did not vary significantly between genders within each group of sites, but localities situated on the sandy area had significantly higher mean intensities (ANOVA, $p = 0.001$) than localities on the clayey soil types (Figure 14).

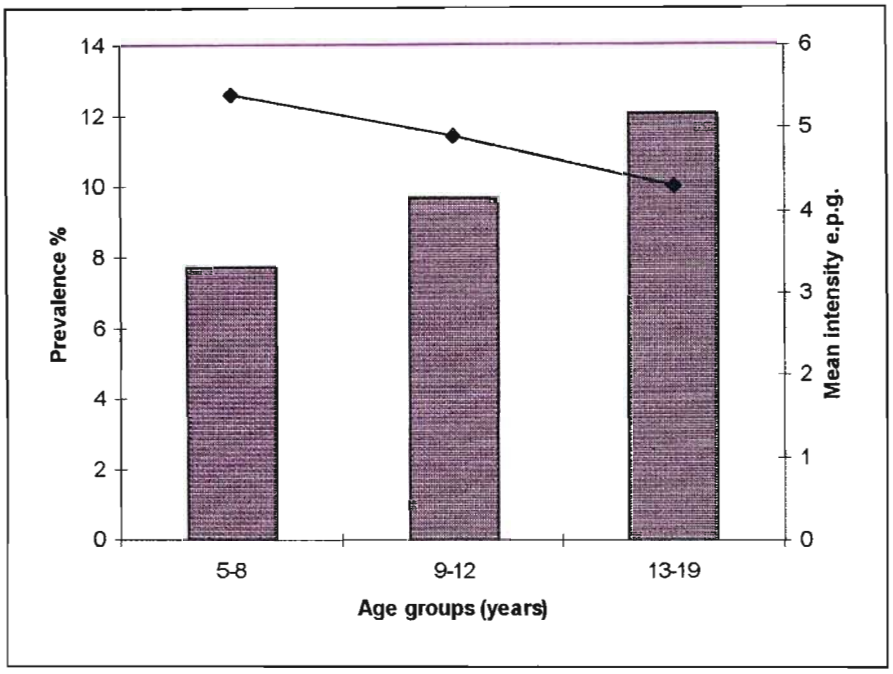


Figure 12. Mean age-prevalence and infection intensity data for the three age groups at the seven inland sites. Bars = prevalence; diamonds = intensity.

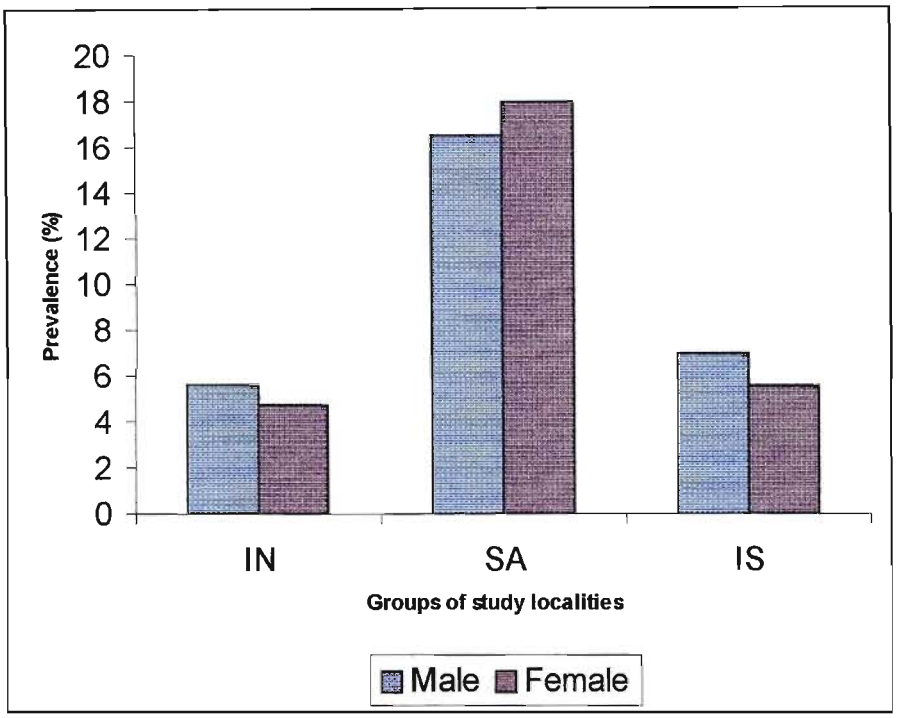


Figure 13. Hookworm prevalences in male and female scholars per study site group i.e. inland sandy area (SA) and surrounding non-sandy areas north (IN) and south (IS) of the SA.

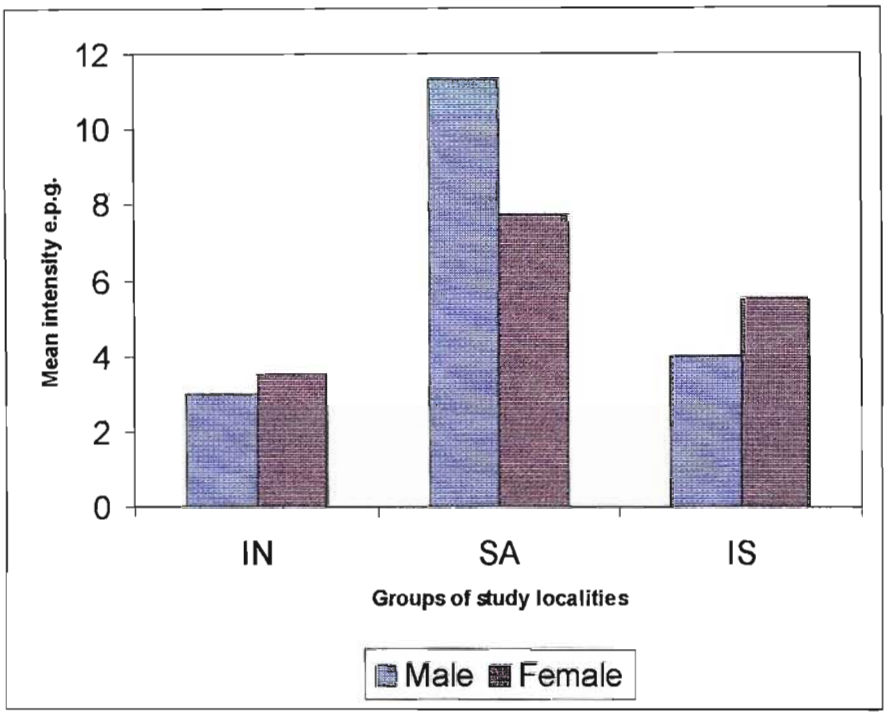


Figure 14. Mean intensities in male and female scholars in each locality group i.e. inland sandy area (SA) and surrounding non-sandy areas north (IN) and south (IS) of the SA.

5.3.3 The effect of climatic variables

To control for the effect of altitude on climate related factors (rainfall and temperature), the inland study localities (Figure 10) were selected to lie within a narrow altitude range (500 - 700 m). But the addition of two study localities within the coastal plain in the analysis made it necessary to include temperature- and rainfall-derived variables, because temperature and rainfall regimes on the coast differed from those inland. However, only those variables that showed a significant association with hookworm infection in Chapter 4 (Table 4.6) were included in the analysis. Spearman Correlation Coefficients were used to assess relationships between both prevalence and intensity of hookworm infection on the one hand and the selected climatic variables on the other (Table 5.1).

Table 5.1 Correlations between mean intensity and prevalence of hookworm infection and selected climatic variables (data from Schulze, 1982), $p = 0.0001$ and $r > 0.5$ were considered significant.

VARIABLE	**r-value**	**p-value**
Prevalence		
MNR JAN-mean number of rainy days for January (mm)	-0.829	0.0057
MDMIN JAN-mean daily minimum temperature for January (°C)	0.943	0.0001
Intensity		
MNR JAN-mean number of rainy days for January (mm)	-0.441	0.3220
MDMIN JAN-mean daily minimum temperature for January (°C)	0.577	0.1747

The analysis (Table 5.1) showed that there was a highly significant positive correlation between hookworm prevalence and the mean minimum daily temperature for January (MDMIN JAN). A highly significant negative correlation was found between hookworm prevalence and the mean minimum number of rainy days in January (MNR JAN). No statistically significant correlations were found between the intensity of hookworm infection and the climatic variables. Other transmission related-variables such as socio-economic status, culture and behaviour might be better associated with intensity-related data but these were not tested. Because of the results, however, intensity was not included in the subsequent analysis between hookworm data and soil variables.

5.3.4 The effect of soil variables

The Kruskal-Wallis test was used to see if there was any significant difference in soil variables, nematode counts and hookworm prevalences between the four groups of study localities. However, the sample size of study localities within each group was small and did not allow statistically significant comparisons to be inferred. This was the case even though there were obvious differences between the coastal plain (CP) and inland localities and also between the inland sandy area (SA) and the surrounding non-sandy areas north (IN) and south (IS) of the sandy area (Table 5.2). This was particularly true in respect of proportions of clay, silt and sand fractions as well as hookworm prevalence and nematode counts. Nevertheless, the implications of these observations are discussed in the subsequent analysis (Section 5.3.5).

Table 5.2 Comparison of mean values for soil variables (n = 10 replicates in each case), nematode counts and hookworm prevalences (p = 0.0001 was considered significant, E. Gouws pers. comm.). See Chapter 3.7 for particle size (mm) classification (Gee and Bauder, 1986). Actual soil data are given in Appendix E.

Group of study localities	Clay < .002 (%)	Silt 0.002-0.05 (%)	Coarse Sand 0.50-2.00 (%)	Medium Sand 0..25-0.50 (%)	Fine Sand 0.05-0.25 (%)	PH	Organic Matter content	Nematode counts	Hookworm prevalence (%)
CP (n=2)	5.6	5.5	2.7	42.5	43.5	5.8	1.6	348	62.5
SA (n=3)	17.4	9.3	15	18.0	40	5.6	2.5	126	17.3
IN (n=2)	32.7	6.4	20	16.1	23.9	6.4	4.3	114	5
IS (n=2)	34.4	8.4	16.1	16.6	24.3	5.4	3.4	98	5.5
P –value	0.0678	0.4020	0.1686	0.0678	0.1063	0.8066	0.5770	0.0567	0.0607

5.3.5 Correlation analysis

The Spearman Correlation Coefficient was used to test the relationships between hookworm prevalence and soil variables as well as between nematode loadings of soil samples from the respective study localities and soil variables ($p = 0.0001$ and $r > 0.5$ were considered significant) (Tables 5.3 and 5.4).

Table 5.3 Correlations between hookworm prevalence and selected soil variables with p = 0.0001 and r > 0.5 being considered significant.

SOIL VARIABLES	**r – value**	**p – value**
Clay	-0.94929	0.0001
Silt	-0.16952	0.6628
Coarse sand	-0.66111	0.0526
Medium sand	0.91539	0.0005
Fine sand	0.91539	0.0005
Organic matter content	-0.57635	0.1043
pH	0.25427	0.5091

Table 5.4 Correlations between nematode loadings and selected soil variables with p = 0.0001 and r > 0.5 being significant significant.

SOIL VARIABLES	**r – value**	**p – value**
Clay	-0.95000	0.0001
Silt	0.00000	1.0000
Coarse sand	-0.60000	0.0876
Medium sand	0.78333	0.0125
Fine sand	0.81667	0.0072
Organic matter content	-0.61667	0.0769
pH	0.20000	0.6059

Only the medium and fine sand fractions showed significant positive correlations with hookworm prevalence and nematode loadings. These two sand fractions are found in the highest proportions in study localities situated on the coastal plain (CP), followed by those localities in the inland sandy area (SA), which had high and moderate hookworm prevalences respectively (see Table 5.2). The inland localities situated on clayey soil types (IN and IS) with low prevalences and low nematode recoveries had low proportions of medium and fine sand. This is the case even though no statistically significant differences were found between study localities due to small sample sizes as mentioned in section 5.3.4. These observations are further strengthened by the fact that the soils of the coastal plain and the inland sandy area have low clay contents.

Clay content in fact showed a significant negative correlation with hookworm prevalence and nematode loadings. The coarse sand fractions also showed negative correlations with both prevalence and nematode loading (Tables 5.3 and 5.4). Organic matter too showed a correlation with both hookworm prevalence and nematode loading; this concurs with the inter-site differences observed in Table 5.2. No statistically significant correlations were found between pH and hookworm prevalence or between pH and nematode loading. In fact there were no obvious differences in pH between the different groups of study localities Table 5.2.

5.4 Discussion

The observed age and sex-related differences in mean hookworm prevalence and infection intensity among the inland group of study localities did not allow for age and sex-related data to be drawn into the analysis with environmental variables, due to the small sample size of study localities. Prevalence and intensity data were therefore pooled for correlations with selected climatic variables. However the intensity of infection could not be successfully associated with any climate-derived variables, perhaps because of the fact that the intensity of hookworm infection depends on the degree of exposure to infection or re-infection and is probably facilitated by the prevailing social conditions. Hence transmission-related variables such as socio-economic status, cultural and behavioural aspects may be better associated with the intensity of infection. Bradley and Chandiwana (1990) also suggested that behavioural factors which influence the degree of exposure to infection or re-infection were likely to play a primary role in determining the intensity of infection in Zimbabwe. Similarly in West Bengal, India, Hominick *et al.* (1987) attributed high worm burdens or high mean faecal egg counts to socio-behavioural factors. In KwaZulu-Natal, no attempt has been made to quantitatively correlate the socio-behavioural transmission-related variables with hookworm data. Contrary to intensity, hookworm prevalence showed a positive association with the temperature-derived variable (MDMIN JAN-mean daily minimum temperature for January) and a negative relationship with the rainfall-derived variable (MNR JAN-mean number of rainy days for January).

High prevalences ($\bar{x}$ = 60.7 %) on the coastal plain (CP) can be attributed to the presence of the well-drained sandy soil type Ab, which combines with suitable rainfall and temperature to provide favourable conditions for the successful transmission of hookworm infection in the area. Moderate prevalences ($\bar{x}$ = 17.3 %) in the inland sandy area (SA) were probably due to the suppressing effect of lower temperatures inland (e.g. 10 °C as given by Schulze, 1982). It is also important to note that the inland sandy soil type Ab has a higher clay content (17.4 %) than the coastal plain soil type Ab (5.6 %), but a much lower clay content than the inland soil types (Fa and Fb) which have 32.7 - 34.4 % clay. Given the relatively high number of rainy days in the inland study area, the sandy soil type there is probably well drained and therefore a better habitat. The low prevalences (4.5 %) in the inland non-sandy areas (IN and IS) may thus be due to the combined effect of clayey soil types and relatively high rainfall, as well as the probable suppressive effect of lower temperatures.

In Liberia, *Ancylostoma duodenale* showed a distribution pattern strikingly similar to that of *N. americanus* in KwaZulu-Natal (Hsieh *et al.*, 1971). *Ancylostoma* was found to be highly endemic only in the coastal areas, vastly reduced in prevalence in the midland area and rare or absent in the hinterland. The higher prevalences of *A. duodenale* were associated with localities where the soil had a high sand content and consequently a low silt and clay content. Only 3 % of 1,058 children were infected with *A. duodenale* in the hinterland where the clay fraction was 19 %. In the lowland area along the Atlantic coast where the clay fraction was about 5 %, 57 % of 466 children were infected (Hsieh *et al.*, 1971).

According to Wilcke (1966), percolating water causes nematodes to move both actively and passively upwards or downwards in sandy soil. Payne (1923) found that in sandy-loam soil *N. americanus* larvae migrated to the surface in large numbers from materials buried at various depths. In red clay soil containing some sand, migration was quite successful but not to the same degree as in the sandy loam, and in relatively pure clay it was unsuccessful. In India, Vinayak *et al.* (1979) observed that the development and survival of hookworm larvae was poor in clay soil but good in sandy soil, and a mixture of sand and clay gave intermediate results.

As suggested in Chapter 4, it seems probable that soil type has a significant bearing on the distribution of hookworm infection, but the precise properties of soil that underpin such observations are still not resolved. Soil variables used in the present analysis showed similar correlations with both hookworm prevalence and nematode loadings. This confirms the view that free-living hookworm larvae and non-parasitic soil-dwelling nematodes are subjected to similar constraints in the soil micro-environment. From the present results it is concluded that the texture of soil (percentage of sand, silt and clay) influences the transmission ecology of hookworm larvae, as it affects drainage, moisture storage, plasticity and aeration. The implication of these findings are discussed below.

Clay soils usually retain more water than sandy soils and this is a consequence of the relationship between texture and moisture. Clay becomes sticky and plastic when wet because it is cohesive (Singer and Munns, 1987) and as a result, clay soils tend to be less well aerated than sandy soils and probably restrict larval migration. This would explain the observed

inverse relationship between clay content and both hookworm prevalences and nematode intensities, as well as the occurrence of low prevalences in the non-sandy areas with high percentages of clay. This is more so because hookworm larvae are obligatory aerobes and migration through the soil probably allows them to move towards the surface or deeper into the soil if conditions (particularly cold temperatures and desiccation) become inimical to their survival. Studies on plant parasitic nematodes in Karala State, India, found that plants in sandy loams suffered more damage than those in clay loams, and concluded that porosity and aeration are essential for nematode activity (Nadal, 1966). Similarly, in New York State, U.S.A., Kable and Mai (1968) observed that plant parasitic nematodes cause severe damage to fruit trees on sandy soils but do little harm to trees on heavier soils. These observations were attributed to a lower rate of movement in clay soil than in sandy soil, and to low permeability of heavy soils to oxygen when almost water-saturated.

Sand particles are comparatively large and hence have much lower surface area than equal masses of clay or silt. Unless few are present, sand particles increase the size of interstitial spaces, facilitating the movement of air and drainage (Foth and Turk, 1972). Such conditions probably provide sufficient oxygen and a suitable medium for larval survival and migration. Following May (1958), the physiology of hookworm larvae is such that they can survive only if their bodies are covered by a water film, probably because they absorb oxygen from the water film. If this were the case, the water film would absorb more oxygen from the soil air as larvae remove more oxygen from the aqueous solution. The depth of the soil water layer between the nematode cuticle and the air-water interface is, therefore, vital. Wallace (1963) stated that the concentration of oxygen in the soil air is controlled by the rate at which oxygen

is consumed and is replaced by diffusion from the atmosphere. According to Singer and Munns (1987), coarse sand influences soil properties because it occupies more space, but it does not contribute space for air or water in the soil because it has no pores. In addition, coarse textured sands have high permeability and drain more quickly than fine sands, and thus tend to produce drier conditions. This probably explains low percentages of coarse sand in areas of high hookworm prevalence and nematode intensities, and the observed inverse relationships between coarse sand percentage and the two nematode variables (Tables 5.3 and 5.4). Study localities with low coarse sand fractions had high proportions of medium and fine sand which thus showed positive relationships with hookworm prevalence and nematode loadings.

One disadvantage of sand is, as mentioned earlier, that solar radiation penetrates the soil surface such that direct sunlight can cause desiccation of free-living stages just below the surface. Udonsi and Utata (1987) observed that infective larvae (L_3) of *Necator americanus* live longer in shade and suggested that light acts as a stimulus that may increase larval activity, thus increasing lipid depletion. This is more so as the physiological condition of larvae is determined by their activity and lipid content: "Hence desiccation-revival cycles, occasioned by diurnal fluctuations in soil surface moisture, would lower the longevity of larvae on account of irresistible but continuous desiccation coupled with continuous depletion of unreplaceable lipid reserves". (Udonsi, 1984). Thus soil covered by vegetation is more favourable to the transmission of hookworm as it protects the larvae and also prevents rapid temperature or moisture fluctuations within the soil microenvironment (Crofton, 1966).

The lack of any significant influence from organic matter or pH in the present study does not rule out the possibility that these soil variables may affect the transmission of hookworm infection. In Liberia, no correlation was found between prevalence of *A. duodenale* and soil acidity or organic matter content (Hsieh *et al.*, 1971). However, Foth (1984) stated that among various factors affecting soil organisms, the influence of organic matter was one of the most important, especially under humid conditions, because organic matter was a source of food and energy for the majority of these organisms. However, light sandy soils are frequently well aerated which restricts accumulation of organic matter, whereas heavier textured soils are often poorly aerated (Foth, 1984). Thus in heavy clay which may contain adequate organic material, there are few if any nematodes because the air and water spaces are greatly reduced (Crofton, 1966). This may explain the relatively low organic matter content on the coastal plain and in the inland sandy area compared to the non-sandy areas. This could also mean that the faecal micro-fauna or the organic material at the point of defaecation are probably sufficient for the development of L_1 and L_2 larvae into the filariform L_3 larvae, especially because the feeding free-living larval stages (L_1 and L_2) are short lived and not very motile.

The pH of the soil is of particular importance in influencing the activities and relative abundance of different groups of soil organisms (Foth, 1984). However, the present results do not allow any generalisations to be made. Soil pH may be unimportant in the transmission of hookworm infection in KwaZulu-Natal but there are not enough observations as yet to support such a view. The scarcity of data on the effects of pH on hookworm transmission emphasizes the need for further investigations.

CHAPTER 6

General discussion

This study has confirmed the findings of Maurihungirire (1993) and Appleton and Gouws (1996), that the distribution of hookworm transmission in KwaZulu-Natal is confined to the coastal plain. This is part of one of the largest coastal plains in Africa, stretching down the east coast from Somalia to Moçambique and extending to the northern part of KwaZulu-Natal, becoming progressively narrower southwards (King 1963; Bruton and Cooper, 1980). The observed pattern of high and low hookworm prevalence in KwaZulu-Natal is closely associated with this area. The highest hookworm prevalences ($\bar{X}$ = 45 %) were found in the northern part of the province where the coastal plain is widest (±74 km), and prevalence decreased ($\bar{X}$ = 6 %) southwards as the coastal plain narrows (31-0 km) in this direction. Similar observations were made in a study on the distribution of frog (anuran) species along the eastern coast of KwaZulu-Natal (Stuckenberg, 1969). A significant correlation was found between number of species and the width of the coastal plain, i.e. a decrease in number of anuran species was associated with a decrease in width of the coastal plain from ± 75 km at 27° S to 0 km at 32° S.

In the present study, there was also a westwards decrease in hookworm prevalence that is attributed to the increasing altitude from the coast to the interior. This rise in topography becomes progressively greater southwards, and at the same time approaches the coast more and more (Stuckenberg, 1969; Christopher, 1982). This could also explain the absence of

hookworm infection in the former Transkei (now part of Eastern Cape Province) where the landscape is broken or dissected, creating a varied and often rugged topography.

There is in fact an inverse relationship between hookworm prevalence and altitude. This can be attributed to ecological conditions related to altitude (i.e. rainfall and temperature) which are thought to play an important role in transmission (Telda and Jamesh 1985; Xu *et al.*, 1995). Temperature appears to exert a dominant influence on the extent of hookworm transmission in KwaZulu-Natal since a strong positive correlation was found between prevalence and a temperature-derived variable (minimum daily temperature for January-MD min Jan). Maurihungirire and Appleton (unpublished data) also found a significant correlation between hookworm infection and temperature from north to south along the coastal plain of KwaZulu-Natal. Rainfall too is important, as the number of rainy days for January (MD min Jan) was second to temperature in importance in the present analysis. Hookworm eggs and larvae need temperature for development and rainfall in the form soil moisture for survival. However, the amount of moisture necessary to maintain optimum conditions for hookworm transmission depends on the nature of the soil, because soils with the same water content need not be similarly "wet" (Smith, 1990).

The occurrence of high hookworm prevalence on the coastal plain (≤ 150 m above sea level) was significantly associated with sandy soil types broadly referred to as grey regic sands, in combination with warm temperatures and moderately high rainfall. Low prevalences above 150 m were associated with weakly developed soils, red or black clays and duplex soils, as well as with lower inland temperatures. It seems probable therefore that the distribution of

hookworm depends on the interaction of temperature, rainfall and soil type of the environment in which it is found.

In the altitudinal transect by Appleton and Gouws (1996) in KwaZulu-Natal, inland study sites (away from the coastal plain) had a mean hookworm prevalence of 3.4 % (n = 2), the lowest being 0.0 %, and prevalence showed a negative correlation with rainfall-derived variables. The present study found moderate prevalences ($\bar{x}$ = 17. 3 %, n = 3) in the inland sandy soils and low prevalences ($\bar{x}$ = 5.3 %, n = 2) in the surrounding non-sandy soils. The number of rainy days (one of the rainfall-derived variables used in the analysis) was found to be relatively high in the inland study area, but hookworm prevalence showed a negative correlation with this variable.

With rainfall being important in the transmission of hookworm infection, the above observations could be an indication of the importance of the soil-water relationship, i.e. the relationship between soil type and the amount or frequency of rainfall. For example in the inland sandy area, the sandy soil is well aerated and drains rapidly following rainfall and thus permits unhindered movement by hookworm larvae. In the surrounding non-sandy area however, the clay soil is poorly drained or aerated and packs tightly following rainfall and is thus unsuitable for hookworm larvae. According to Levine and Todd (1975), soil moisture results from the combined effect of precipitation and the nature of the soil, and it determines the degree of hydration of nematode eggs or larvae, which lose water readily when soils dry out. Steep slopes can also influence the soil-water relationship by its flushing effect on nematode eggs and larvae especially after heavy rains. This is likely to result in the eggs being

carried away with the surface run-off from sites of defaecation and deposited at high densities in the top soil elsewhere, particularly at the bottom of the slope (Appleton and Gouws, 1996). Equally important in this regard is the distance of the water table.

In addition to the above-mentioned factors, the pre-infective stages of hookworm eggs (L_1 and L_2 larvae) require warmth for development to the infective filariform (L_3) stage (larvae). In this way, the lower temperatures inland probably suppress transmission in both areas regardless of soil type. But this thermal effect is probably greater in the non-sandy areas where it is combined with the fact that the clay soils do not serve as good media for development of hookworm larvae. In the area of sandy soils, moderate hookworm prevalences were probably due solely to the effect of low temperatures as sandy soils provide suitable medium for transmission. Particularly important are the fine and medium sand fractions found in high proportions on the coastal plain and in the inland sandy area. These sand fractions also showed positive correlations with prevalence of hookworm infection in the present study. The water retaining properties of finely-textured (aeolian) sand slows down the drying process (Evans *et al.*, 1990), which is to the advantage of free-living larval stages.

Importantly, relatively high prevalences of two other common soil-transmitted nematodes *Ascaris lumbricoides* and *Trichuris trichiura,* were recorded at all inland study localties irrespective of soil type or climatic conditions (Appendix D). This may be due to the thick-shelled eggs of the two species which probably allow for better survival under harsh environmental conditions than the thin-shelled eggs of *N. americanus* (WHO, 1964). With respect to *Strongyloides stercoralis* infection in KwaZulu-Natal, Maurihungirire (1993) found

that it occurred sympatrically with hookworm infection and had a similar distribution. However, due to inadequate data from the previous and present surveys, I was unable to confirm these findings. The parasite *S. stercoralis*, represents a challenge for laboratory diagnosis. The concentration methods commonly used for other parasites are not suitable because rhabditiform *S. stercoralis* larvae are difficult to identify (Speare, 1989). Specific methods include Baermann filtration and the coproculture technique. However, Marti (1997) cautions that one should also be aware of the fact that a negative faecal sample does not exclude an infection. Thus accurate estimates of *S. stercoralis* prevalence are not easy because of technical difficulties in the quantitative enumeration of larvae in stool.

The occurrence of moderate hookworm prevalences in the inland sandy area where unfavourable climatic conditions (particularly lower temperatures) occur, presents narrow limits for development and survival of free-living larval stages. This suggests that there are probably sites within these areas which do provide favourable conditions for transmission which is most likely to take place in shady, moist, sandy and warm microenvironments. In the central Namib desert, Namibia (too hot during the day and too cold at night), Appleton and Brain (1990) observed that shaded soil provided a much more stable thermal environment than unshaded soil. In fact high hookworm prevalences have been reported in Namibia, where the climate is tropical and arid (unfavourable for larval development), but the soil is sandy and consists of the fine aeolian Kalahari sand (Evans and Joubert, 1989; Evans *et al.*, 1990). In the above survey most study localities were situated in the vicinity of Okavango River which probably provides ideal microenvironments for transmission. Alternatively, shallow depressions or pans were suggested as possible transmission sites, where shade is provided by

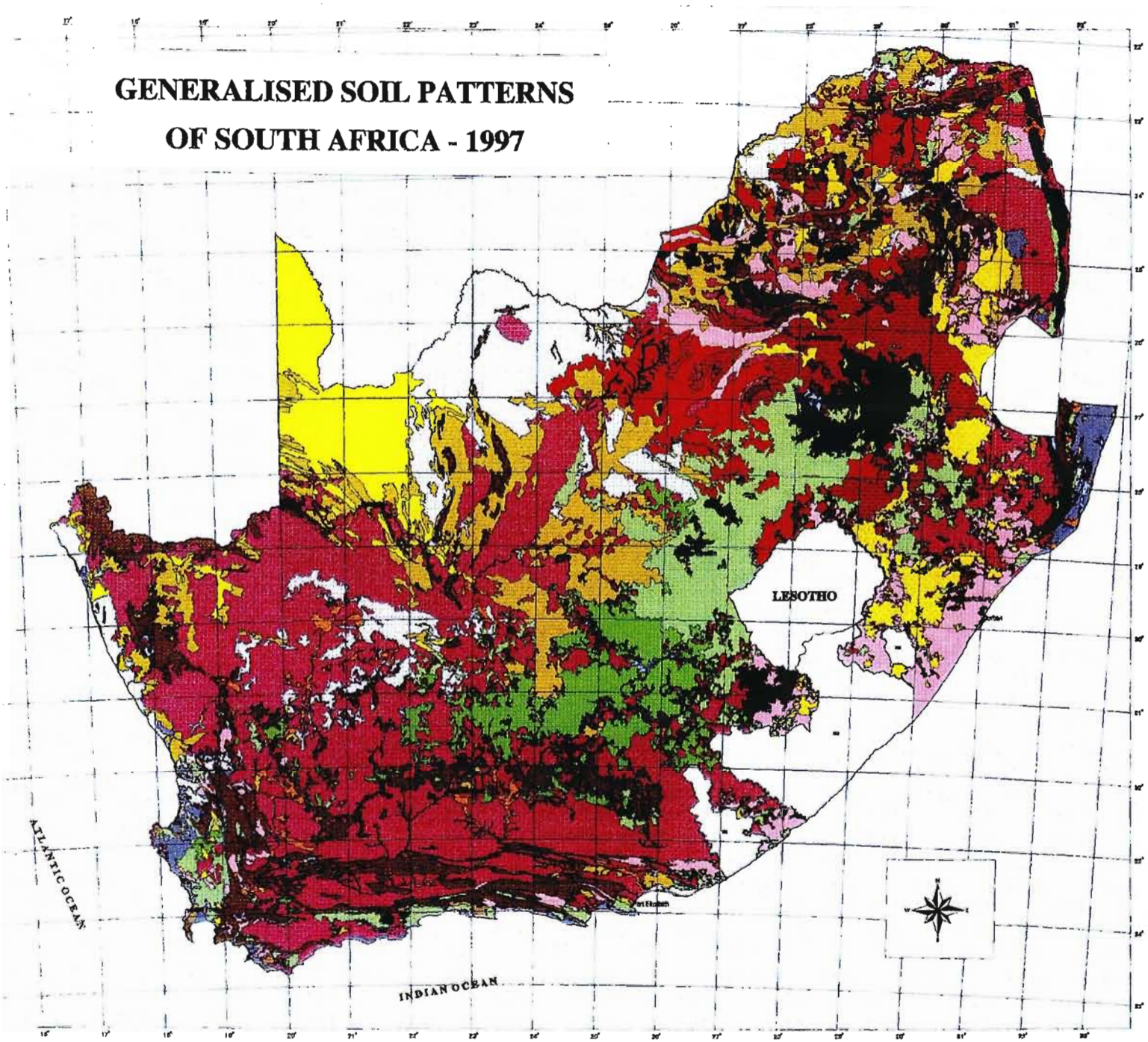
mixed vegetation which thrives in the better water-retaining areas (Evans *et al.*, 1990). Inevitably, situations which offer privacy adjacent to these water points, become polluted by habitual promiscuous defaecation (Evans *et al.*, 1990). Udonsi (1988) also suggested that faecally contaminated river banks may form additional hookworm transmission foci in areas where various water-related activities are carried out by a human host population.

While the cold and wet winter climate in the Western Cape will retard the development of hookworm eggs and kill developing larvae, the warm and dry summer climate should desiccate the eggs and free-living stages (Maurihungirire, 1993). The above points raise the possibility that hookworm could establish itself in other sandy areas of South Africa (see Figure 15), where climate (combination of temperature and moisture) is not suitable for hookworm transmission though the sandy soils could be, especially in microenvironments around water bodies. Surveys should be made in such areas to assess the status of hookworm and to follow up or trace positive individuals to their areas of residence for identification of actual transmission foci. Based on the present results there is also a need to take a closer look at the soil structure, i.e. nature/shape in relation to the width or diameter of the larvae.

In the northern part of KwaZulu-Natal where high hookworm prevalences are confined to the coastal plain (Schutte *et al.*, 1981), moderate prevalences (± 20 %) were found in some localities on the Lebombo Mountains (> 300 m asl) to the west of the Pongolo floodplain. This can be attributed to ideal environmental conditions along the floodplain (50-100 m asl),

Figure 15. Generalised soil map of South Africa showing soil type ED (grey regic sand) on the coastal plain of KwaZulu-Natal and patches of it along the coastal areas in the Eastern and Western Cape, as well as red dune sand (AD) in the northern Cape.

GENERALISED SOIL PATTERNS OF SOUTH AFRICA - 1997



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LEGEND

RED-YELLOW WELL DRAINED SOILS LACKING A STRONG TEXTURE CONTRAST

- AA — Red and yellow soils with a humic horizon
- AB — Red and yellow, massive or weak structured soils with low to medium base status
- AC — Red, massive or weak structured soils with high base status
- AD — Red, excessively drained sandy soils with high base status. Dunes present

SOILS WITHIN A PLINTHIC CATENA

- BA — Red, yellow and greyish soils with low to medium base status
- BB — Red, yellow and greyish soils with high base status

SOILS WITH A STRONG TEXTURE CONTRAST

- CA — Soils with a marked clay accumulation, strong structure and a non-reddish colour. In addition one or more vertic, melanic and plinthic soils could be present
- CB — Soils with a marked clay accumulation, strong structure and a reddish colour

SOILS WITH A HIGH CLAY CONTENT

- DB — Black and red, strongly structured clayey soils with high base status

SOILS WITH LIMITED PEDOLOGICAL DEVELOPMENT

- EA — Soils with minimal development, usually shallow on hard or weathering rock, with or without intermittent diverse soils. Lime rare or absent in the landscape
- EB — Soils with minimal development, usually shallow on hard or weathering rock, with or without intermittent diverse soils. Lime generally present in part or most of the landscape
- EC — Red and yellow, sandy well drained soils with high base status
- ED — Greyish, sandy, excessively drained soils
- EE — Soils with negligible to weak profile development usually occurring on recent flood plains

PODZOLIC SOILS

- FA — Soils with a sandy texture, leached and with subsurface accumulation of organic matter, iron and aluminium oxides, either deep or on hard or weathering rock

ROCKY AREAS

- R — Rock with limited soils

- NM — Not mapped
- Water bodies

Based on the Land Type Series 1972 - 1997.

Refer to this map as:
Land Type Survey Staff, 1997
Generalised Soil Patterns of South Africa
ISCW, Pretoria.

SCALE 1 : 2 800 000

100 0 100 200 km

Albers equal-area projection
Standard parallels 18° 0'0"S and 32° 0'0"S

i.e., shady, moist, sandy soils at the edge of water bodies (e.g. rivers, streams and pans), as well as warmth, since the altitude is uniformly low in this area. Similar conditions may exist in riverine communities elsewhere in KwaZulu-Natal, especially in areas characterized by major river systems (river valleys) that flow from the interior landmass to the coast. In Kenya, Koniti (1971) found that helminth infections were highest on the Kano plain but hookworm infection was most prevalent on the shores of Lake Victoria. In Zambia, hookworm prevalence varied from 12 % in the Eastern Province to 68 % in the Western province, but peak prevalences (71 %) occurred in the Zambesi river valley (Wenlock, 1966). Similarly, in Malawi high hookworm infections were found amongst people living on the shores of Lake Malawi or on the banks of the Shire River (May, 1958). May (1958) also reported that in Madagascar hookworm infection occurred in the lowlands, but major foci were found near the Antalaha and Manajary rivers. Lowlands are also areas where people are likely to find suitable arable land due to hospitable climate, as a result more people are found in these areas than in the highlands.

Futhermore, in a climate which for most of the year has temperatures which are too low to permit development of larvae, an important aspect is whether the rain falls during the unfavourably cold season or during the summer when the temperature factor is favourable. However, in most countries where hookworm disease exists, the rainy season occurs during the period when temperature is favourable (May, 1958). As mentioned in Chapter 4, KwaZulu-Natal experiences seasonal fluctuations in temperature but rain falls throughout the year, though there is usually a summer maximum. Climatic variables (temperature and rainfall) that showed significant correlations with hookworm prevalence in the present study,

both related to mid-summer. This raises the possibility of seasonal transmission, i.e. the favourable time appears to be during the summer period when warmth coincides with sufficient moisture. However, for conclusive observations to be made more work needs to be done on this aspect of hookworm epidemiology.

In addition to factors examined in relation to hookworm infection in the present study, there are probably certain agricultural activities that favour hookworm transmission (see Chapter 2). Even though in the present study no particular attention was given to this aspect, the presence of sugarcane plantations in areas of high hookworm prevalence was noted, for example at Sizani and Sakhesethu on the coastal plain. Interestingly enough, the sugarcane plantations were also a feature common to the inland sandy areas (Mushane and Tshelabantu) where moderate prevalences were found. This may be due to the sandy nature of soil in these areas or to the fact that tilled or friable soils contain increased amount of air (Foth, 1984; Wallace, 1963) which favour larval development and survival. In the Lenkoran area of Azerbaidzhan, ancylostomiosis (predominantly *N. americanus*) was more frequent in a lowland than in a foothill settlement (Leikina, 1959). In the former almost every farm was a microfocus of infection while in the latter only occasional farms were affected

In Tanzania, Sturrock (1966) emphasized the need to investigate agricultural or farming communities for hookworm infection. Pampliglione and Hadjer (1956) found that hookworm infection was very high in villages in eastern Nigeria, where the main occupation is agriculture. In north Queensland, Australia, high hookworm infections were found in all sugarcane growing districts (Lambert, 1921). Similarly in China, Yu and Shen (1990) observed that the

intensity of hookworm transmission was closely related to agricultural activities involving crops such as sugarcane among others. The Burma valley and Vumba farming areas on the border between Zimbabwe and Moçambique had prevalences between 62 % and 72 % (Bradley *et al.*, 1993). However, in KwaZulu-Natal as already mentioned, no attempt has been made to investigate the status of hookworm in farming communities (especially in sugarcane areas) or with other aspects of human ecology, e.g. socio-cultural behaviour, socio-economic status, and of personal and environmental sanitation.

In conclusion, it is clear that hookworm infection in KwaZulu-Natal is limited in its distribution by a preference for environmental conditions offered by the coastal plain, and that as one moves away from this area or as the coastal plain narrows, hookworm infection and intensity decreases. However, it is important to note that local environments may be different from those of the broader surrounding countryside, and that climatic, topographic and edaphic variations can produce an area suitable for hookworm transmission within a larger area that is not. Thus there is still a need for a close look at the role played by soil type and both rainfall and temperature in the transmission of hookworm infection at the community and household levels (micro-epidemiology). Emphasis should be on developing sufficient understanding of the dynamics of transmission to allow for a much more focused control effort. In other words, the identification of the exact location, densities and survival of the different stages of the parasite in the environment and of human behaviour in relation to the dissemination and acquisition of the infection (Nelson, 1990).

6.1.1 Future considerations

Global warming and the "greenhouse effect" may alter the present distribution pattern of hookworm infection. The greenhouse effect results from build up in the atmosphere of gases (carbon dioxide and methane) which absorb heat, i.e. long wavelength infrared radiation, and re - radiate it back to the surface of the earth. This is predicted to alter the climate across the surface of the earth (Abrahamson, 1989; Lugget, 1990; Cooper, 1995). Abrahamson (1989) also predicted that this change will impact on ecology and the environment, and could also be characterized by widespread species extinction. According to Cooper (1995) coastal areas of KwaZulu-Natal will be hard hit, as the rise in sea level will innudate and affect a host of ecological systems. The KwaZulu-Natal coastal plain could be submerged or become part of the continental shelf once again, and the hookworm problem could disappear with it or move inland to the sandy soils if temperature belts shifts. Since changes in global, regional and local temperatures induced by global warming, e.g. temperature increases in areas which presently experience low temperatures, could have a significant effect on the global, regional and local distribution of hookworm infection. As far-fetched or exaggerated as this may seem, the prolonged warm summer weather in Britain in 1964 provided favourable conditions for hookworm transmission (Buckley and Pester, 1965).

Additionally, the current influx of people to South Africa from neighbouring and other African countries especially from areas where *Necator americanus* and *Ancylostoma duodenale* co-exist, could lead to the establishment of the latter species in areas free of the former due to excessively harsh environmental conditions. In fact Matsusaki (1963) showed that *A.*

duodenale eggs and larvae are more tolerant of lower temperatures and desiccation than those of *N. americanus*. It has also been suggested that hypobiosis allows *A. duodenale* to establish itself over a large area where it would otherwise not survive due to inhospitable conditions (see Chapter 2).

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APPENDIX A

Modified Harada-Mori method for identification of hookworm and threadworm larvae (from Sasa *et al.*, 1958)

1. Using a wooden applicator stick, weigh out 0.1-0.5 g of faeces onto a strip (10 x 2.5 cm) of Whatman No.1 filter paper, and smear it evenly over the middle third of the strip.

2. Pipette 5 mℓ of tap water into the bottom of the test tube.

3. Then insert the strip into the test tube with the faecal smear facing away from the lumen of the tube and the bottom end of the strip standing about 1 cm into the water.

4. Close the tube with the rubber bung to prevent desiccation.

5. Repeat this for each faecal sample.

6. Then place the tubes into a rack, cover with a black plastic bag and keep at room temperature (22-25 °C) for 10 days.

7.After 10 days use a Pasteur pipette to remove the sample of water from the bottom of the test tube and place a few drops onto a microscope slide.

8. The slide containing larvae can be heated on a magnetic-stirrer / hot plate.

9. The specimens should only be heated enough to kill and relax the larvae otherwise the internal structures may be destroyed if over-heated.

10. Identify the species of larvae present using a compound microscope with 10x and 40x objectives.

APPENDIX B

Modified formal-ether concentration method (from Allen and Ridley, 1970; Cheesebrough, 1981)

1. Weigh out between 0.5 and 1.0 g of faeces into a conical 15 mℓ polypropylene tube.

2. Add 7 mℓ formalin into the tube.

3. Using a wooden applicator stick, thoroughly mix the stool sample and filter it through a double layer of wettted wound gauze into a waxed paper cup (Monocups). Wetting the gauze prevents the filtrate from being absorbed by the gauze and reduces the chance of eggs sticking to it.

4. Add a small volume of 10 % formalin to the Monocup, rinse out all remaining sample by pouring it through the gauze.

5. Decant the filtrate into a clean 15 mℓ test tube, rinse the cup with a small amount of 10 % formalin to collect any remaining filtrate and pour this into the test tube.

6. Add 4 mℓ of diethylether and cover the mouth of the test tube with a square piece of Cling Wrap.

7. Using a thumb (with non-sterile surgical gloves on) close the test tube, shake it vigorously for 60 sec., releasing the gas build-up very carefully when necessary during the process.

8. Remove the Cling Wrap and centrifuge at 2000 r.p.m. for 2 min.

9. Once centrifuged, the tube's contents are layered: at the buttom of a tube is a pellet containing the parasites' eggs (sometimes larvae) and cysts; above it a layer of formalin, above that, a plug of debris and on top, the ether.

10. Using a wooden applicator stick, loosen the plug of debris and turn the tube upside down, pouring off all the supernatant and leaving the deposit behind then add a drop of diluted iodine as a stain.

11. Using a Pasteur pipette and rubber teat draw up all the deposit) and place it on a microscope slide for examination (using 10x objective to scan the slide and 40x objective to see detail).

APPENDIX C

Modified Baermann apparatus for separating nematodes from soil or faeces

(from Cheesbrough, 1981)

1. Use a plastic funnel (13 cm inner diameter) with a piece of rubber tubing (14 cm long) attached to the stem and closed by a Hoffman Clip.

2. Clamp the apparatus to a retort stand.

3. Place a round-bottom glass test-tube (15 x 2 cm inner diameter) beneath the tubing to collect the filtrate.

4. Place two layers of pure cotton (wound) gauze swabs on the strainer to prevent filtering particles of soil debris from falling into the test tube.

5. Place a 100 g of soil sample (well broken up) on the strainer and cover with parts of the gauze.

6. Pour 200 mℓ warm water (45 °C) into the funnel.

7. Leave to stand overnight (24 hrs); the larvae will migrate downwards and settle at the bottom of the tube.

8.Open the tube by unfastening the clip and collect 20 mℓ of the filtrate.

9. Pour warm water (45 °C) into the funnel to wash out nematodes remaining in the soil, wait for 30 min. and collect the larvae.

10. Transfer the contents of the tube onto a glass Petri dish and carefully count the larvae under a dissecting microscope.

APPENDIX D

The prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* in the inland group of study localities

Group of study localities	*Ascaris lumbricoides* (%)	*Trichuris trichiura* (%)
Inland north (IN)	42	39
Inland sandy area (SA)	31	55
Inland south (IS)	20	46

APPENDIX E

Soil data

Study localities (n=10 replicates in each case)	% Clay	% Silt	% Coarse sand	% Medium sand	% Fine sand	Total	pH	Organic Matter content
Sakhesethu (CP)								
1	6.3	7.0	4.2	39.2	44.4	101.4	6.9	1.4
2	5.6	2.4	1.1	38.7	51.4	99.2	5.3	1.2
3	4.4	7.3	3.7	36.2	47.5	99.1	7.9	2.2
4	5.5	3.9	3.2	37.7	50.6	100.9	5.6	3.4
5	4.9	5.9	2.1	46.6	39.5	98.9	4.9	0.7
6	4.1	5.2	4.4	50.7	34.8	99.1	6.4	2.4
7	5.1	6.7	2.7	35.3	49.3	98.9	5.8	2.3
8	4.3	6.2	3.4	46.0	41.1	101,0	4.7	1.1
9	4.4	5.2	1.7	37.5	49.1	97.9	5.4	2.8
10	5.8	7.4	2.5	38.5	45.1	99.3	5.6	1.3
Sizani (CP)								
1	6.2	5.4	2.1	40.9	44.5	99.1	4.8	0.7
2	6.5	7.8	1.8	36.9	47.9	100.9	5.9	0.3
3	5.2	4.1	4.7	50.3	36.5	100.8	5.6	1.2
4	5.8	7.7	1.9	39.9	43.35	98.6	6.9	0.9
5	7.2	6.9	2.6	50.8	32.0	99.5	6.7	2.1
6	5.2	4.4	1.8	40.5	47.1	98.9	4.7	0.7
7	4.7	3.5	1.4	43.8	47.4	100.7	6.2	0.5
8	5.5	4.3	2.5	47.3	40.0	99.54	5.3	0.3
9	4.8	5.7	1.7	50. 9	37.0	100.1	6.5	1.4
10	5.7	6.7	4.3	42.2	40.2	99.0	4.5	4.5
Thimuni (IN)								
1	34.3	9.4	25.7	14.7	16.7	100.7	4.9	1.6
2	33.2	5.6	23.1	16.9	19.9	99.3	5.3	2.5
3	32.2	8.9	22.4	13.7	21.4	98.4	5.3	0.6
4	30.3	6.1	25.4	15.9	19.2	96.9	6.3	1.6
5	34.1	8.6	22.3	17.1	18.8	100.8	4.7	1.1
6	31.9	9.6	19.5	14.9	22.0	97.9	4.0	2.5
7	29.5	6.3	20.8	16.9	25.9	98.9	4.2	1.6
8	30.0	8.4	21.1	14.3	26.5	100.3	5.9	1.0
9	33.2	3.2	21.6	17.3	23.1	98.9	4.2	0.8
10	29.9	5.1	19.4	18.0	26.5	98.8	7.7	0.6
Empungeni (IN)								
1	31.6	9.17	19.1	14.65	22.3	96.8	6.9	7.1
2	35.4	3.89	18.4	17.31	23.9	98.8	7.9	8.9
3	30.1	5.38	20.5	16.7	24.5	97.5	7.5	6.7
4	33.5	5.24	17.9	15.8	26.3	98.8	7.7	6.9
5	32.8	8.58	14.4	15.7	26.4	97.6	7.9	8.2
6	35.2	4.33	18.7	15.4	26.2	99.7	7.69	2.7
7	33.6	5.47	21.7	14.1	25.3	100.6	7.5	6.9
8	36.4	4.1	19.4	15.7	23.8	99.3	7.2	4.4
9	31.27	6.2	20.5	19.2	23.3	100.4	7.8	4.5
10	34.3	3.6	19.3	17.7	25.0	99.9	6.9	11.8

Appendix E continued

Nyamazane (SA)	% Clay	% Silt	% Coarse sand	% Medium sand	% Fine sand	Total	pH	Organic matter
1	20.8	12.5	16.3	16.4	32.9	98.7	5.9	3.7
2	18.9	13.5	14.6	19.5	33.9	100.2	4.8	4.7
3	20.2	9.8	13.8	17.3	36.2	97.2	5.5	3.3
4	22.7	12.6	14.3	18.7	30.5	98.7	4.1	4.9
5	25.4	8.8	14.2	16.9	33.6	98.8	4.2	5.6
6	23.9	9.6	14.5	18.4	31.7	98.1	5.8	4.2
7	20.5	9.9	13.9	19.5	35.8	99.6	4.4	4.6
8	19.4	8.9	16.7	18.1	35.5	98.4	4.3	5.0
9	18.9	10.5	18.5	16.9	33.8	98.6	4.5	4.3
10	21.5	8.5	18.3	16.8	35.7	100,5	5.9	3.2
Mushane (SA)								
1	16.8	13.9	18.2	22.5	26.7	98.9	5.5	2.2
2	18.7	17.2	18.9	15.4	28.9	99.2	5.1	2.3
3	10.5	6.8	28.1	20.2	34.5	99.8	4.7	0.9
4	11.5	9.3	17.1	24.7	37.5	100.1	4.4	1.1
5	16.8	7.5	23.9	15.0	36.2	99.6	5.4	1.5
6	17.9	9.36	19.1	22.8	30.3	99.4	4.6	2.9
7	19.5	11.9	13.9	17.8	37.4	100.5	4.6	4.2
8	18.9	12.9	21.3	18.2	29.5	98.3	5.9	1.6
9	11.6	7.3	27.1	19.3	35.4	100.7	6.5	3.8
10	20.7	11.9	21.6	16.4	28.4	98.9	6.1	1.2
Tshelabantu (SA)								
1	12.6	9.9	6.8	13.1	56.8	99.2	6.2	0.9
2	11.1	14.8	4.4	13.1	55.6	98.9	6.5	0.7
3	10.3	9.8	8.3	16.3	55.9	100.5	6.2	1.6
4	13.8	8.6	8.3	14.3	53.3	98.3	6.9	1.4
5	11.8	6.3	10.5	21.1	50.25	99.8	6.7	0.8
6	9.8	2.6	11.3	20.2	53.9	97.7	7.6	0.9
7	17.8	3.4	7.2	7.2	16.5	101.2	6.9	0.9
8	10.3	6.5	11.5	11.5	14.5	99.6	6.7	0.6
9	11.3	7.4	16.6	16.6	20.5	98.4	7.5	1.4
10	18.4	8.8	11.8	11.8	14.9	99.5	5.8	0.9
Mary Gray (IS)								
1	34.4	5.1	19.7	13.8	24.9	97.9	5.1	3.7
2	30.6	6.9	19.4	14.7	27.3	98.9	5.2	1.9
3	33.6	4.2	16.5	17.9	27.4	99.6	5.5	6.8
4	32.4	5.2	20.1	16.4	25.9	99.9	6.9	3.8
5	26.8	6.9	19.5	21.4	24.4	99.0	8.1	2.3
6	31.5	3.3	22.3	19.7	24.4	101.0	4.3	3.5
7	32.0	6.2	20.9	17.1	24.6	100.8	5.5	1.4
8	31.0	3.6	20.0	17.2	28.7	100.5	5.8	4.6
9	33.6	9.4	13.5	15.3	27.4	99.1	5.9	3.6
10	27.8	3.3	21.5	16.3	29.5	98.1	5.5	2.0

Appendix E continued

Nsuze Gcwensa (IS)	% Clay	% Silt	% Coarse sand	% Medium sand	% Fine sand	Total	pH	Organic Matter
1	36.4	16.7	10.4	12.8	21.9	98.2	4.5	3.8
2	33.2	14.3	19.9	13.7	19.5	100.5	5.2	2.1
3	35.9	8.9	14.7	18.4	21.2	99.1	6.8	4.1
4	38.4	7.9	10.9	18.4	22.7	98.3	4.5	3.2
5	38.7	12.2	10.3	18.7	21.5	101.4	5.3	4.0
6	35.9	10.9	12.1	18.7	22.5	99.9	5.5	4.5
7	33.8	15.2	14.9	16.7	20.0	100.5	4.5	3.0
8	36.8	17.1	11.4	15.2	19.3	99.8	5.4	2.9
9	34.7	12.5	14.3	16.8	21.9	100.1	4.5	2.5
10	35.8	9.6	12.5	18.7	23.8	100.6	4.6	4.1

Key:

CP - study localities on the coastal plain
SA - study localities in the inland sandy area
IN - study localities in the inland non-sandy area north of SA
IS - study localities in the inland non-sandy area south of SA