HOOKWORM STUDIES IN ETHIOPIA:

INVESTIGATIONS IN GONDAR AND GOJAM REGIONS

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A B S T R A C T

1414 stool samples from schoolchildren of 17 different areas in Gondar Region and 1825 stool samples from schoolchildren of 14 different areas in Gojam Region were examined to show the prevalence of the human hookworm species, *Necator americanus* and *Ancylostoma duodenale*.

The over-all infection rate with *N. americanus* and/or *A. duodenale* was 20.13%. Of the infected individuals 58.9% were males and 41.1% were females. The infection rates in Gondar and Gojam Regions were 22.7% and 18.14%, respectively.

All larvae were identified as *N. americanus* in Gojam Region while 92.52% and 7.48% harboured *N. americanus* and *A. duodenale*, respectively, in Gondar Region.

The incidence of the species varied inversely with the climate/altitude in both regions. The infection rate among male schoolchildren was higher than in females in Gondar Region. In both regions age of schoolchildren was not found to be correlated with infection.

The proportion of children infected by hookworm was highest in the lowlands (below 2000m.) (29.39%) followed by the temperate (2000 - 2500m.) areas (18.46%).

1. INTRODUCTION

Parasitic worms adversely affect the health of humans in many parts of the world. From the standpoint of their prevalence, a large number of reports provide information on the overall prevalence of intestinal helminths in the world but the information varies in representativeness and in techniques of investigation. Experts in their assessment of geographic distribution of intestinal parasitism indicate no uniform distribution; some indicate a decline in certain helminthic infections, others report a static situation and still others show increases of intestinal parasitism in general or in specific parasites (WHO, 1964).

Recent investigations in Africa carried out in Uganda (Kadandi, 1971), Kenya (Kinoti, 1971), Central African Republic (Ricciardi, 1976), Zambia (Hira, 1976) indicate high prevalence rates of intestinal helminths in general, with varying degrees of prevalence for specific parasites.

The prevalence of intestinal helminths in Ethiopia is not well documented. The available reports are based on investigations of certain localities and their results can by no means be considered as representative of the country. However, various authors, among them Lemma et al. (1968 and 1979), Duncan et al. (1970), Kloos et al. (1978) indicate a high prevalence of intestinal parasites in the country.

Helminthic infections as a whole can be viewed as providing an index of a community's progress towards a desirable level of sanitation. Successful management of human pollution problems will eliminate essentially all of the helminths except those of the arthropod-transmitted group, and the elimination of mosquitoes and other arthropod vectors will control the arthropod-transmitted helminths as well. To whatever extent a community falls short of having attained living standards based on these desirable conditions, helminths will have an important bearing on the peoples health. It is also true, that high standards of living depend on high economic productivity, and this inturn requires high levels of physical vigour and health. Thus the helminths are important to whatever extent they detract from the vigour of the community.

In recent years two highly important helminthic diseases - onchocerciasis and schistosomiasis - have been attacked through measures directed against their intermediate hosts. It may now be possible to control these diseases, at least in communities where they constitute major health problems. However, in many of these areas, and in other areas covering a large population of the world, the most prevalent and most important helminths are those of the soil-transmitted group (WHO, 1963).

From the standpoint of their over-all prevalence and the severity of the diseases they cause, the most important soil-transmitted helminths are: *Ascaris lumbricoides* Linnaeus, 1758; *Trichuris trichiura* (Linnaeus, 1771) Stiles, 1901; *Necator americanus* (Stiles, 1902) Stiles, 1903; and *Ancylostoma duodenale* (Dubini, 1843) Creplin, 1843. An additional species, less widely distributed and less restricted to soil transmission, is *Strongyloides stercoralis* (Bavay, 1877) Stiles and Hassall, 1902 (WHO, 1964).

The two hookworms of humans, *Necator americanus* and *Ancylostoma duodenale*, often occur sympatrically over much of the world, including the Indian subcontinent, parts of South America, the Far East and Africa (Hoagland and Schad, 1978). Over three decades ago it was estimated by Stoll (1947) that 457 millions harboured hookworm in a then global population of 2,167 millions. The United Nations estimate for mid-1969 for global population has been put at 3,610 million and there is no evidence that the hookworm incidence has decreased in the interval (Hsieh *et al*. 1971). Applying a similar ratio as Stoll (1947) in mid-1969 there were 760 million people infected by hookworm in the world.

Hookworms affect a large number of people in Ethiopia (Schaller, 1971). However, only few studies have been made (Wang, 1965; McConnell and Armstrong, 1976; Armstrong and Chane, 1975) and these studies are limited in scope. As in many developing countries, Ethiopia suffers from a low standard of living which provides fertile grounds for transmission of hookworms, which may be more common than the available information could reveal. The improvements of highways and rapid shifts of population contribute to the dissemination of the parasites to new areas. This leads to aggravation of the problem in the country at large. Regional surveys can provide sufficient information on the distribution of these helminths and this may pave the way for appropriate intervention measures needed for prevention.

In areas where these two hookworm species occur, they have often been treated as if they were one species, despite the well known principle that two species, if truly sympatric, cannot be ecologically identical. Practical considerations, i.e., the great similarity of the stage used in diagnosis (the egg) and the laboriousness of rearing larvae and distingushing between them, have encouraged lumping these parasites together. Furthermore, except for the fact that A. duodenale is considered the more virulent, the two species are generally thought to provoke the same pathological changes. This obviously has interfered with treatment. Certain drugs (e.g., bephenium hydroxynaphthoate) have a selective action on A. duodenale while others (e.g., tetrachlorethylene) seem to be effective against N. americanus (WHO, 1963). In view of this, combined treatment is employed.

If one properly characterizes the hookworm species in a given region, there is no reason as to why combined treatment should be administered. This will ensure the choice of drug and would be economical.

Ethiopia is situated within the tropics, but most of its people enjoy a temperate climate. Faust and Russell (1964) and Belding (1965) indicate that only N. americanus occurs in Ethiopia, whereas only A. duodenale is specifically mentioned in a few reports, among them, Kubasta (1964), Molineaux (1967), Duncan et al. (1970) as emanating from within the country. These reports are, however, very narrow in scope. Although inadequate, the only piece of recent information that deals with the identification of hookworm species in Ethiopia is that of Armstrong and Chane (1975). They revealed a great preponderance of N. americanus over A. duodenale following administration of single doses of bephenium hydroxynaphthoate to 132 subjects in three localities. However, species determination of the hookworms involved in an infection by examination of worms recovered after treatment has various shortcomings, besides the fact that more precise information in recent years has shown most anthelminthics operate without equal effectiveness against Necator and Ancylostoma. A post-treatment worm count may not be a good indication of the worms present in a given infection.

One of the main problems in Ethiopia, as in most developing nations, is the combination of paucity of data on such factors as climate and moisture with the fact that no adequate analysis of available data have been made to give a picture of the temporal and geographical variations of these factors. However, Daniel (1977) has characterized the country as enjoying different altitudinal ranges - alpine (over 3000m.), temperate (2300-3000m.), subtropical(1500-2300m.), tropical (800-1500m.) and desert (less than 800m.). This has an effect on the prevalence and distribution of helminths.

Various authors, among them, Demayer *et al.* (1955), Pyne *et al.* (1956), Diesfeld (1969), Diesfeld (1970) have indicated the effects of altitude on the distribution of the parasites elsewhere.

The only information that deals with the relationship between altitude (temperature) and the distribution of hookworm in Ethiopia is that of McConnell and Armstrong(1976). According to them, hookworm infections were numerous between Debre Markos and Axum at altitudes between 1300-2100m. They were minimal at the higher altitudes existing between Addis Ababa and Debre Berhan to the northeast and the Blue Nile to the northwest. On the other hand, low infection rates were also encountered at well below 2200m. along the eastern edge of the plateau. Though, they did not try to identify the species, they have also indicated that in general the relationship between altitude and the distribution of hookworm was more marked than with other helminths encountered during the survey.

It is therefore important that one clearly identifies the species of hookworm found in the different altitudinal ranges. The preventive measures to be taken depend on our understanding the extent of the problem in the different regions of the country. A study on the prevalence and identification of hookworms widens the horizon of our understanding and hence helps develop a rational basis for appropriate preventive measures. A country-wide study could have benefited the planners more but such a study requires enormous expenses both in manpower and material resources which are beyond the scope of this study.

This study is therefore limited to Gondar and Gojam Regions and tries to reveal the prevalence and identity of the two human hookworm species, N. americanus and A. duodenale, in relation to altitude, using the modified test-tube filter-paper cultivation (MTFC) method (Hsieh, 1971).

2. LITERATURE REVIEW

2.1. GENERAL

Ancylostoma duodenale and *Necator americanus* are small nematodes found in the upper part of the small intestine and causing hookworm disease. The female *Ancylostoma* is 10-18mm. and the female *Necator* 7-13mm. in length. The males of both are somewhat smaller.

Human beings are infected via the skin or the alimentary canal. The eggs contain immature embryos, which in optimum conditions can complete their development in 24 hrs. The hatched larva (rhabditiform larva) lives freely in the soil and moults twice in the course of 8-10 days and transformed into a filariform larva, the infective stage. Sufficient moisture (muddy water, damp earth), oxygen and warmth (14°C to 37°C, preferably 27°C) are essential for the developmental process. The filariform larva is sensitive to desiccation, direct sunlight and freezing.

The larvae migrate through the skin and enter the lymph and blood systems and are carried to the heart and lungs. The larvae finally reach the upper respiratory system from which they are swallowed. In the intestine the larvae undergo the next moult before becoming sexually mature. Approximately six weeks are required for the complete developmental cycle in the human host (Strong, 1945).

Table 1 summarizes some of the similarities and differences of the two hookworm species of man.

Table 1

Comparison of Life History Characterstics and Ecological Attributes of *A. duodenale* and *N. americanus* (Hoagland and Schad, 1978)

Character of nematode	Species	
	A. duodenale	*N. americanus*
Mean female body weight(g)	2.2×10^{-3}	0.9×10^{-2}
Mean egg size	1.14×10^{-7}	1.31×10^{-7}
Egg out put/female/day	10000-25000	5000-10000
Typical natural life span (yrs.)	1	3-5
Developmental arrest in humans and seasonal egg out put	yes	no
Blood loss/worm/day (ml.)	0.15 - 0.23	0.03
T^oC at which 90% of eggs hatch	15 - 35	20 - 35
T^oC above which eggs do not hatch	45	40

Table 1. Continued.

Character of nematode	Species	
	A. duodenale	*N. americanus*
Resistance of eggs to low oxygen, low temperature, desiccation, chemicals and death in faeces	greater	less
Resistance of larvae to chemicals and desiccation	greater	less
Oral transmission	yes	no
In utero or transmammary transmission	possibly	no
Mean number of worms taken from one host, light infection	11.5	29.5
Mean number of worms taken from one host, heavy infection	193	488
Maximum number of worms recorded from one host	529	2300

Table 1. Continued.

	Species	
	A. duodenale	N. americanus
Distribution: Regions where each species dominates or was dominant	North China, North West India, Middle East, Ivory Coast, Taiwan	South China, South India Indochina, Sub-Saharan Africa, Southern USA, Australia
General geographical and regional preferences	Large cities (Japan), Highlands, Northern climates	Plains, Lowlands, Tropics

2.2. PREVALENCE OF HOOKWORMS

A large number of reports provide information on the overall prevalence of the two hookworm species of man, N. americanus and A. duodenale, in various parts of the world. But it must be recognized that the data do not describe the prevalence in a truly representative manner; they merely give a rough indication of the situation in some countries or parts of countries. Some of these reports indicate a decline, others indicate more or less static situation, often in spite of vigorous efforts to control infection, and in some instances the infection rates are on the increase (WHO, 1964).

Lobel et al. (1968) made a survey of hookworm infection in 525 schoolchildren, white and negro in Alabama and found a prevalence rate of 24.9%. In a recent survey in a rural county of Kentucky, USA, Gloor et al. (1970) found hookworm to be the most common imtestinal helminth, in studies made among students. Another survey in Texas showed 33% of 8000 faecal specimens positive for hookworm (WHO, 1964).

Hookworm disease is an important public health problem in nearly all Latin American countries (Bloch and Rivera, 1977). High prevalence rates have been reported in Brazil (Vinha and De Souza Martins, 1967); Southern Paraguay (Canese et al. 1975, Canese and Canese, 1976); and Sao Paulo (Correa et al. 1979). Similar prevalence rates have also been reported by Bloch and Ruiz (1966) and Linero and Merazo (1967) in El

Salvador and Venezuela, respectively.

Although hookworm infection used to constitute a disease problem in the mines in Europe, it is evidently no longer important except in parts of Portugal and Italy. Mangiafico (1968) has made a statistical study of A. duodenale infection in the province of Pavia, Italy, and has noted a progressive diminution of the disease to the point where it has almost disappeared. However, De Carneri and Biasin (1973) found 153 persons with hookworm eggs in their faeces out of 959 agricultural workers and their families examined in Lusia, Italy. De Carneri (1974) has indicated a high prevalence of hookworm in Northern Portugal.

Various reports, among them, Nazarian (1973), Ebrahimzadeh (1976) and Rudenko (1980) give the general impression that in parts of Yemen, Iran and Lebanon hookworm infection is virtually absent but that foci of high prevalence may occur.

In Egypt, the over-all rate of infection in the total rural population was estimated at about 10%, during a survey in the years preceeding the late thirties, the majority of the people being farm labourers (Scott, 1937). In West Pakistan, the highest hookworm infection rate in all the villages surveyed was 36% and in the East the hookworm rate among a group of selected patients was 28% (Aziz and Siddiqui, 1968).

Various authors, among them, Raghavan (1967), Vedyarthi (1968) and Nawalinski et al. (1978) have reported the prevalence of hookworms in different parts of India. In different parts of Indonesia *Ascaris lumbricoides*, hookworms and *Trichuris trichiura* are the most common helminths encountered (Carney *et al*. 1974). Carney *et al*.(1974) in a survey conducted in a sparsely populated area in West Sumatra, given over largely to the cultivation of rice, using 364 people in four villages they found a 66% infection rate for hookworm. In Hawaii, Desowitz and Wiebenga (1975) examined 275 locally-born school-children and 115 foreign-born, most of them from the Philippines or Samoa in Oahu. In general, there was a high prevalence of hookworm infection in the immigrant children. Later Carney *et al*. (1980) examined intestinal parasites of man in Northern Philippines and found 71% incidence for hookworm.

In Korea, according to Rho *et al*. (1968), 11.6% of 155 urban dwellers and 41.6% of 693 persons in rural areas had hookworms in their faeces after treatment with bephenium hydroxynaphthoate. Later Choi *et al*. (1973) reported a prevalence rate of 23.8% in the northern part of South Korea.

In Taiwan it is stated that hookworm infection is highly endemic in most rural and sub-urban districts. Hookworm infection is most prevalent among adults in all localities investigated (Hsieh *et al*. 1965; Hsieh, 1970). Bargner *et al*. (1973) in a parasitological survey of the Yami aborigines on Orchid Island, Taiwan, found a prevalence rate of 33.7% for hookwprm.

In Japan, hookworm and *Ascaris* are the most prevalent helminths encountered. Ishii (1972), using two different methods, estimated the prevalence of hookworm infection in a region of South Japan and obtained infection rates of 31% and 25%. Hookworm infection is rare in city dwellers but still common among agricultural workers.

Hookworms are wide-spread in Africa. Reports indicate high prevalence rates of hookworm infection in Congo (WHO, 1963; Ripert and Carteret, 1969); Liberia (Franz *et al.* 1964; Hsieh *et al.* 1972), Zimbabwe (Gelfand and Warburton, 1967), Uganda (Banwell *et al.* 1967), Ghana (Bakker, 1969), Kenya (Miller, 1970; Pamba, 1980), Togo (Ricciardi, 1974), Tanzania (Rep and Shaba, 1976), Zimbabwe (MacCabe and Goldsmid, 1976), Zaire Republic (Colaert *et al.* 1978) and Nigeria (Oyerindi, 1978; Nwosu and Anya, 1980).

Ancylostomiasis affects a large number of people in Ethiopia. Various reports indicate various prevalence rates of the hookworms in different parts of the country. In Gondar hookworm infection among schoolchildren was 2.1% (Wang, 1965), 12.2% and 13% (Chang, 1962), 15% and 46.7% (Hiatt, 1976) and 77% (McConnell and Armstrong, 1976).

Studies made in different parts of Gojam show a high prevalence of parasitic infections. 6%, 4%, 14%, 69%, 85% 95%, 88%, 57%, 79%, 56%, 98%, and 95% infection rates due to hookworm were found in Yeteman, Wegel, Lumame, Amanuel, Dembecha, Mankusa, Bure, Dangla, Wetet Abay, Merawi, Jiga and Fenote Selam schoolchildren, respectively (McConnell and Armstrong, 1976).

The rates in the province of Harar from hospital cases were 19.6% (Blahos and Kubasta, 1963), 28% (Kubasta, 1964), 2.7% (Lo et al. 1973) and 4.9% (Lainovic, 1974). In localities in the Lower Awash, Lemma (1969) found the following infection rates: Dubti 11%, Assayta 4%, Banga 3%, Awash station 5% and Melka Konture 2.2%. The highest rate reported was 16.6% from Koka. From Adwa 3.7% infection rate was reported by Buck (1965); and Torrey (1966) reported 1.5% infection rate among the Say Say people in Welega.

McConnell and Armstrong (1976) in their survey of intestinal parasites have reported the following prevalence rates for hookworm: Gebre Gurecha 17%, Filklik 91%, Meshenti 55%, Zerima 77%, Adi Arkai 20%, Maitseberi 29%, Inda Baguna 45%, Selekleka 24%, Wukro 9%, Mekele 9%, Mukufuto 7%, Kombolcha 6%, Karakore 21%, Efeson 26%, Jewha 26%, Debre Sina 11%, Sela Dingay 3%, Debre Berhan 3%, Ankober 14%, Sheno 3% and Lege Tafo 3%.

2.3. FACTORS INFLUENCING TRANSMISSION

The effects of altitude in the distribution of these helminths have been considered by many authors.

Demayer et al. (1955) state that there is no striking difference in infections of helminths at higher altitude. The same opinion is expressed by Pyne et al. (1956). On the other hand Svensson (1956) suggests that high altitude and dryness prevent the development of immature helminths to the infectious stage outside the host. Watson et al. (1956) indicate that increasing altitude caused a diminishing incidence of helminthic infection except for *Ascaris lumbricoides* and *Hymenolepis nana* and Wang (1965) found a significant difference between Ethiopian children living in highlands and those in lowlands (68.8% and 95%, respectively). Diesfeld (1969) states that incidence of hookworm in Kenya decreases with rising altitude. Kelly and Garavusi (1974), however, indicate a high incidence of hookworm infection in mountainous regions of Papua New Guinea. Ebrahimzadeh (1974) found hookworm to be predominant in areas of Iran with warm, humid and oceanic climate. He examined 1042 stool samples in Iran from Roudzar and Shahy in the north (warm and very humid oceanic climate), from Kharramabad in the west (dry and temperate) and from Ahwaz in the south (dry and very hot, but often with cold nights). Hookworm was predominant in the north.

Diesfeld (1970) has analysed the conditions which are associated with a high prevalence of hookworm infection in Kenya and other parts of East Africa, including Ethiopia and Uganda. Data show that spread occurs most readily in a low-lying moist area. In one area, at a height of 1100 to 1400m.

above sea level, the infection rate of the population was 50 - 80% falling to 10% in nearby upland regions. Briefly, Diesfeld (1970) indicated that a warm, moist climate is suitable for the propagation of Ancylostoma, whereas a cool, dry climate is harmful. McConnell and Armstrong (1976) indicated that in general, the relationship between altitude and the distribution of hookworm was more marked than with other helminths encountered during a survey in Ethiopia.

Hookworms differ in their tolerance of high and low temperatures, and it is this range of optimum temperatures which may account for the more northerly distribution of A. duodenale. This species can stand low temperatures approaching 0°C, whereas N. americanus is killed by brief periods of chilling at temperatures well above freezing (WHO, 1964). Matsusaki (1963) indicated that the eggs of A. duodenale are the more resistant to low temperatures. In his study, they were not affected by exposures for 3-23 hours at temperatures higher than 5°C, for one day at temperatures above 8°C, for 2-3 days at temperatures above 16°C, nor for 6-10 days at temperatures above 20°C. Kim (1969) carried out experiments to determine the survival of eggs and infective larvae of A. duodenale in different conditions in Korea and found out that the eggs in the stool completely lost their viability after 30 days at 28°C, 22 days at 5°C and 12 hours at -10°C, respectively. On the other hand, Necator can develop at temperatures above the range of tolerance for Ancylostoma.

Experts indicate that the optimum temperatures for development are 28°C - 32°C for *Necator* and, for *Ancylostoma*, 5°C - 8°C lower than this (WHO, 1963). Once the infective stage has been reached, both species are relatively resistant to temperature extremes.

Seasonality in human hookworms has been the subject of many investigators. Sturrock (1967) made monthly counts of hookworm eggs in schoolchildren for twelve months. The pattern differed in individual children, but transmission appeared to be limited to a period between the short and the long rains. An experiment with hookworm infective larvae also indicated that climatic conditions were unfavorable for transmission after heavy and prolonged rains. According to Vinayak *et al.* (1979), the rainy season was found to be the most favorable season for the development and survival of hookworm larvae, whereas the least favorable were the Winter months. During colder months, the eggs remained dormant for a long period. Larvae reaching the surface of the soil during Summer months were killed by direct sunlight. Nwosu and Anya (1980) indicated that there was a marked increase in the over-all hookworm burden following the onset of the rainy season, and then a gradual decrease in infection during the dry season.

In relation to the consistency of the soil, it has been observed that there is an inverse relationship between prevalence of hookworm infection and density of the soil. In heavy soils prevalence is low, and it is generally considered that sandy soils are most suitable for hookworm larvae (WHO,1964). The same opinion is held by Vinayak *et al.* (1979), where sandy soil was found to be an excellent medium for development and survival of larvae. Clay was the worst soil for the survival of the hookworm larvae. A mixture of clay and sand gave results between the two extremes.

Vieira and Fraga De Azevedo (1966) also report that hookworms (*N. americanus*) were most common in soils which contained more organic material.

2.4. PATHOGENESIS

There is a considerable body of knowledge to indicate that in certain parts of the world a very large number of people are actually suffering from some degree of anemia produced by hookworms and that this has notably retarded their capacity to work and to learn. Roche and Layrisse (1966) consider that hookworm anemia is closely related to the iron intake of the subjects in whom it occurs. Whether anemia does develop depends on a balance between the iron utilized by the body for haemoglobin production and that which is lost via the hookworm. A significant correlation between worm burden and anemia has also been found by White *et al.* (1957) and by Farid and Miale (1962) and evidence (Martinez-Torres *et al*, 1967; Gajawani and Trivedi, 1969) suggest that in hookworm infection the principal cause of anemia is loss of iron from the intestine brought about by bleeding.

Blood loses per worm have been variously estimated (Roche and Layrisse, 1966; Mahmood, 1966; Farid *et al.* 1966; Bloch and Ruiz, 1966; Farid *et al.* 1970). Though estimates generally range higher in terms of losses per worm in light infections than in heavier ones, it is believed that blood loss per worm per day is 0.005 - 0.1ml. for *N. americanus* and 0.015 - 0.34ml. for *A. duodenale* (Komiya and Yasuraoka, 1966; Roche and Layrisse, 1966; Hoagland and Schad, 1978).

The effect of hookworm infection on the intestinal mucosa has been the subject of several studies. Rai *et al.* (1968) found some correlation between abdominal abnormality and hookworm infection. According to Halsted *et al.* (1969) and Tandon *et al.* (1969), hookworm infection was not a cause of malabsorption. However, Burman *et al.* (1970) suggest biochemical evidence for malabsorption, obtained in six patients, all of whom had high egg counts and low haemoglobin levels. Defective absorption of folic acid has been reported in hookworm infection (Areekul *et al.* 1975) and this could be a manifestation of more generalized intestinal malabsorption.

Both species cause skin reactions that are more or less severe depending on the intensity and frequency of exposure. Repeated infections with *Necator* may cause the typical picture of creeping eruption (WHO, 1964). Pulmonary reactions to *Necator* are apparently mild. Pulmonary reactions to *Ancylostoma* have been noted, especially in Japan, where the condition is referred to as 'Wakana disease' (Muller, 1975).

2.5. DIAGNOSIS

The development of infective larvae in faecal cultures was frequently used for hookworm diagnosis. However, not until Harada and Mori (1951) produced a test-tube type culture, was it restored as an easy laboratory method. Sasa et al. (1958) demonstrated the value of the test-tube culture in epidemiological surveys for hookworm, as well as for Strongyloides stercoralis. This was due not only to the simplicity of diagnosis through finding infective larvae in test-tube type cultures, but the feasibility of rapid differentiation of the third larval stages of Necator and Ancylostoma and Strongyloides. Stoll and Hsieh (1961) employed the technique with dilution egg counting in attempting to obtain a preliminary assessment of the relative amount of N. americanus and A. duodenale in West Africa. Maruashvili et al. (1966) modified the test-tube method of cultivation of hookworm larvae, and obtained large yields of larvae by using glass jars and wide strips of filter paper. Lobel et al. (1968) indicated that culture for hookworm larvae was a more sensitive diagnostic method than either salt flotation or formol-ether concentration.

A number of other authors, Franz et al. (1964) in Liberia, Hsieh et al. (1965) in Taiwan, Hsieh et al. (1972) in Liberia, Choi et al. (1973) in Korea, Chacon et al. (1975) in Mexico, Canese and Canese (1976) in Paraguay, Marzochi and Chieffi (1978) in Londrina, Brazil, Colaert *et al.* (1978) in Kinshasa, Oyerinde (1978) in Lagos, and Correa *et al.* (1979) in Greater Sao Paulo, Brazil, have utilized the Harada Mori method to study the identity and distribution of the two human hookworm species.

What makes the test-tube culture a workable device is related to the biology of the free-living stages of hookworm. When a strip pf filter paper is smeared lightly with faeces containing hookworm eggs, and the strip placed so that it dips into a small amount of water in the bottom of a large test-tube, the filter paper acts as a wick and the smear become saturated. Evaporation is prevented and high humidity is produced by covering the top of the test-tube tightly with cellophane. A zoogloeal growth soon appears on the moist surface in which hookworm larvae hatch and develop. After a few days at favorable temperature these become mature free-living forms and reach the non-feeding third stage. The infective larvae tend to leave the surface of growth and gravitate to the water and, being poor swimmers, they become trapped at the bottom of the test-tube. The infective larvae in the sediment at the bottom can be withdrawn easily for examination, and can be morphologically differentiated at a relatively low power under the microscope. Both hookworm species behave the same way.

However, infective *Necator* larvae gravitated to the water less frequently than *Ancylostoma* larvae and further study of this difference in the reaction of infective larvae under test-tube culture conditions has led Hsieh (1971) to make a significant modification in the technique.

In order to secure optimum yield of the infective larvae produced by both species, examination of more than the bottom water of an undisturbed culture is required. Those larvae that did not migrate to the bottom water by the end of the culture period need to be included. It was found that by gently filling the tube with water, so as to immerse the entire vertical surface of the filter paper, allowed any reluctant larvae to be washed off the surface and sink to the bottom. After an interval they settle, and the full culture yield is obtainable in the sediment. Introduction of this obligate immersion as one in a set of procedural steps for standardized qualitative results is termed 'Modified Test-tube Filter-paper Cultivation' and designated MTFC (Hsieh, 1971).

2.6. TREATMENT

Many attempts have been made to control hookworm infection and a number of drugs have been utilized for the treatment of ancylostomiasis and necatoriasis.

Various authors, among them, Botero and Perez (1970) and Chitrathorn *et al.* (1972), have reported phenylene di-isothiocyanate to be effective against hookworm infections. Other researchers, Goldsmid and Saunders (1972), Holz *et al.* (1972) and Vakie *et al.* (1972), have indicated the efficacy of levamisole, mebendazole and pyrantel embonate in the treatment of hookworms.

Hsieh (1970) has reviewed the results of ten years work on the treatment of hookworm infection with different drugs in Liberia and Taiwan. Tetrachlorethylene, bephenium hydroxynaphthoate, bromonaphthol and phenylene di-isothiocyanate were the most effective, and except with bephenium, higher cure rates were obtained in *Necator* infections.

At the present time there are three products of choice for the treatment of hookworm infection: tetrachlorethylene, bephenium hydroxynaphthoate, and pyrantel embonate; with the former two more frequently used.

Lim *et al.* (1975) reported bephenium to be more active than pyrantel pamoate against *N. americanus* infections. However, Zaman and Loh (1974), Chinery *et al.* (1973) and Haddock (1970) reported a poor therapeutic response to bephenium in patients with *N. americanus*.

Bephenium hydroxynaphthoate is more effective against *A. duodenale* than *N. americanus* (WHO, 1963). Pyrantel pamoate was also found to be as highly effective as bephenium in patients with *A. duodenale*.

Necator americanus is more susceptebile to tetrachlor-ethylene (Oliveira, 1970; Balmer *et al.* 1970; Senewiratine *et al.* 1975).

In view of the fact that bephenium appears to have a more selective action on *A. duodenale*, while tetrachlor-ethylene seems to be effective against *N. americanus*, combined treatment is employed (WHO, 1963; Nooman, 1971).

2.7. PREVENTION AND CONTROL

Many writers inform us about the numerous methods of prevention and control of hookworm infection. According to WHO (1981), control of hookworm infection and anemia involves four approaches ; the sanitary disposal of faeces, health education, chemotherapy, and correction of the anemia.

The provision of latrines and education in their proper use are crucial to the control of hookworm infections. The wearing of protective footwear is an essential complementary measure.

3. MATERIALS AND METHODS

3.1. DESCRIPTION OF STUDY REGIONS

The study areas in this project were Gondar and Gojam Regions. The two regions were selected because:

- they represent the defined altitudinal areas (highland, temperate and lowland areas);
- available reports indicate a relatively higher hookworm infection rates in the two regions; and
- study in these regions was found to be economical both in material and financial expenses.

3.1.1. GONDAR REGION

Gondar Region (Fig. 1.) is located in the North Western part of Ethiopia bordering Gojam Region to the South, Welo Region to the east, Tigrai Region to the north and Eritrean Region and the Sudan to the northwest and west, respectively. The area is 74,200 sq. km. and the population is estimated to be about two million, with average population density of 28.4/ sq. km. The northern part of the region comprises the Semien Mountains and Ras Dashen, the highest mountain in Ethiopia with a peak of 4575m. There are fertile lowland areas in between the mountains. In the northwest there is much sloping down towards the plains along the Sudan border. The most western end of the highland plateau merges into the lowlands of Metema. To the south, the border of the area is Lake Tana (Min. Public Health, 1977).

Frequent ground-frost occurs in the high regions. Average annual rainfall is 1700mm. at high altitudes and about 1250mm. in lower regions. Rainy seasons are February to April and June to September. Average annual range of temperature is from 16°C to 30°C (Daniel, 1977).

Teff, wheat, barely, sorghum, maize and some millet are cultivated. Pulses and oil seeds are also grown. There are potential cotton-growing areas.

3.1.2. GOJAM REGION

Gojam Region (Fig. 1.) is bounded on the north by Lake Tana and Gondar Region, on the east by Welo Region, on the south by Welega and Shewa Regions and on the west by the Sudan. The Blue Nile River traces the border of the region to the Sudan frontier. The eastern part of the region is mountainous terrain, sloping gradually toward the scrub lands near the Sudan border. The region has an area of 61,600 sq. km. and a population of about two million with average population density of 34/sq. km (Min. Public Health, 1977).

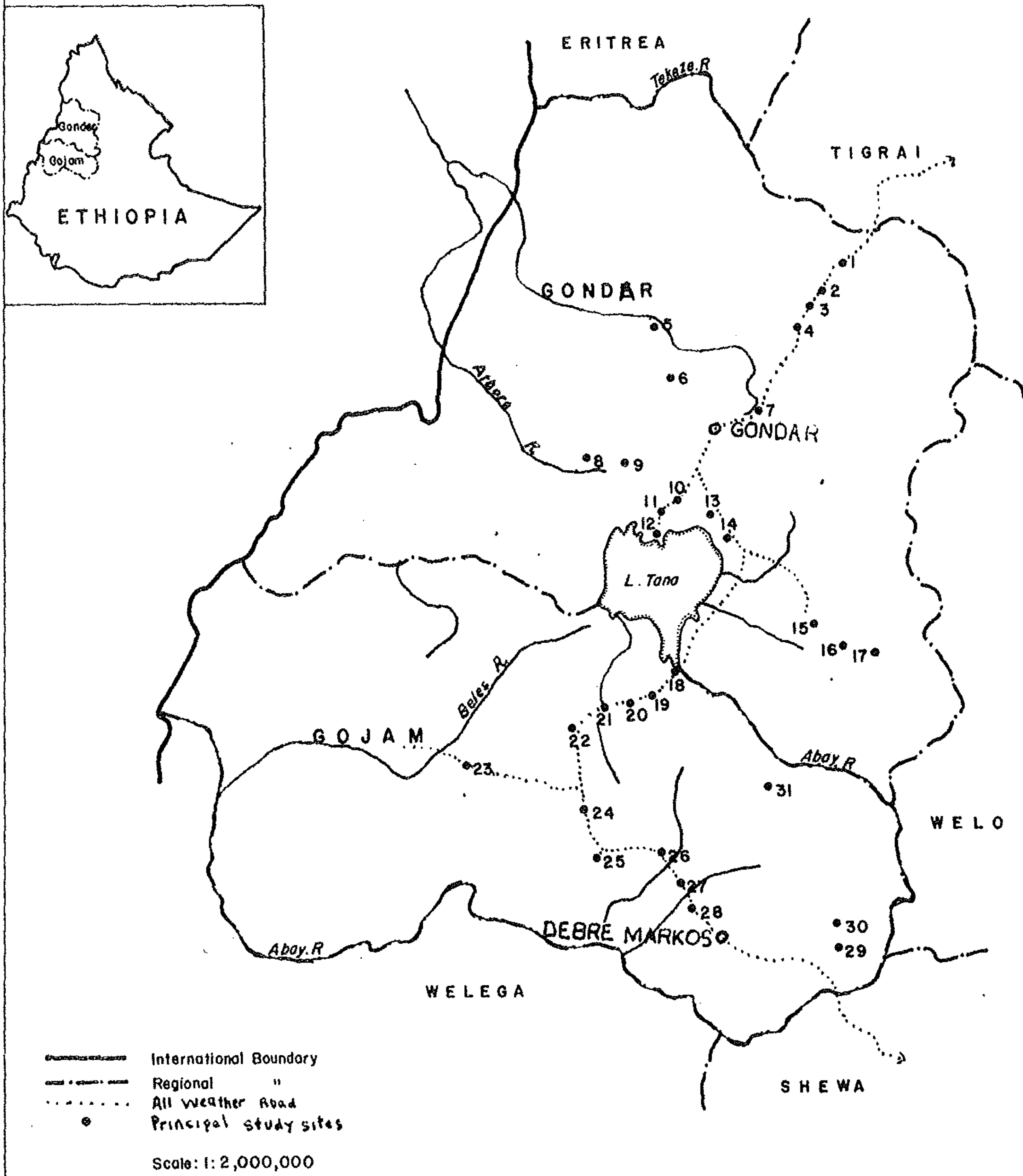
There are wide temperature variations - cold, especially at night, in highlands and very hot in the valleys. Average annual temperature ranges from 16°C to 30°C. At altitudes of 3,000 to 4,000m. the temperature is quite cold. In the mountainous regions, frequent ground-frost is observed in December and January. The rainy seasons are from March to

April and June to September. Average annual rainfall is between 1200 and 1500mm (Daniel, 1977).

The region is fertile. The principal grains are teff, barely, wheat, maize, sorghum, and millet. Potatoes and other vegetables are also grown. Pulses and oil seeds are cultivated in some areas and there are potential cotton producing areas.

In both regions, the population is almost wholly static. It is estimated that over 90% of the working population is engaged in agricultural practices. Everywhere, with few exceptions, the inhabitants live a life which revolves around the annual cycle of planting, growing and harvesting. Since there is no essential difference in the sanitation of the people living in urban areas and of those living in rural areas, the environmental conditions which are responsible for the dissemination of helminths are present. Urban dwellers for the most part live in crowded situations, with activities primarily centered around the houses, while those in the rural villages live in widely disseminated areas and often travel, to work or to school. Water supply, sewage and refuse disposal are poorly organized. Some poorly-constructed pit-latrines are available in urban areas, but are seldom used. People defecate in the fields, in bushes near the houses, in the ditches or in secondary canals. Shoes are not worn in rural areas, even by the better-educated leaders of the community, while engaged in work.

Fig. 1. Gonder and Gojam regions along with the prinicipal study sites



Both regions exemplify temperature-altitude -potential evapotranspiration relationships as classified by Daniel (1977) for Ethiopia and accordingly, in the lowlands temperatures and water losses are high and rainfall amounts are low. In general, the reverse is true of highlands.

3.2. SELECTION OF SPECIFIC STUDY AREAS AND SAMPLE POPULATION

Student populations from elementary schools were selected to represent the community they live in for various reasons.

a. They share the same environment as the other sections of the community.

b. Since they live near the schools, they can provide relatively reliable local information.

c. They are likely to accept the inconveniences of providing stool and other investigations.

d. With the exception of age, they reflect community practices.

Elementary schools were taken for comparisons; because elementary schools were relatively wide-spread in rural and urban areas of the regions, as compared to secondary and middle schools.

After an initial survey had been conducted to determine the altitude, all schools in the two regions were classified into highland, temperate and lowland schools. All places below 2000m. were considered lowlands, 2000-2500m. are designated as temperate and above 2500m. are considered highlands (Min. Public Health, 1977). Schools were selected on the basis of the pre-determined criterion which required that schools vary in altitude but to be similar in all other respects, as far as possible. Thus the method of selection was stratified cluster sampling. This method of sampling has the benefit in terms of precision (less variance) and is economical. The advantages of stratified sampling are extensively discussed in the literature (Kish, 1961).

Accordingly, seventeen schools representing 6,000 students from Gondar Region and fourteen schools representing 7,500 students from Gojam Region, a total of thirty-one schools (Fig. 1.) representing 13,500 students were selected as principal study sites. The lowest point considered in Gondar Region has an altitude of 1200m. and the highest 3150m. above sea level. Similarly in Gojam, the lowest site considered had an altitude of 1650m. and the highest 2550m. (Table 2.). Areas out of these limits could not be dealt with because of various problems.

Mostly students of grades three to six were taken to represent elementary school students, whenever possible lower grades were excluded.

After the purpose of the work had been explained to the principals and to those directly concerned, students who fulfilled the following criteria were listed as prospective donors of faecal material:

a. lived in the area continuously for five or more years;

b. never had treatment for any intestinal ailment during the last three months; and

c. attended classes at the time of study.

From the lists containing the names of prospective donors, a total of 3239 students, 1414 from Gondar Region and 1825 from Gojam Region, were selected, using systematic sampling with a random start, to constitute the sample population for all thirt-one areas studied (Table 2.).

This work was carried out in two phases. The first part of the work comprised the study in Gondar Region and took place in October and November, 1982. The second phase of the work involved the areas of Gojam Region and was accomplished in February and March, 1983. On all occasions similar methods were employed.

3.3. SPECIMEN COLLECTION

After lists of donors had been drawn up, a container was numbered for each student and distributed. Prospective donors were asked to furnish not less than 10g. of faeces. Whenever possible, the containers were handed to students in the morning and collected within a few hours. The specimens were transported to the Gondar College of Medical Sciences Hospital Laboratory on the same day of collection whenever possible, or within a couple of days, by car.

Table 2

Number of Study Subjects by Area and Altitude

Region	Area (School)	Sample size	Altitude (m.)	Remark
Gondar	Nefas Mewcha	61	3150	Highland
	Kossoye	61	3000	"
	Kimer Dingaye	50 } 336	3000	"
	Debarq	100	2950	"
	Gassay	64	2850	"
	Aykel	64	2350	Temperate
	Dib Bahir	70	2200	"
	Tikil Dingaye	101	2100	"
	Enfraz	50 } 506	2100	"
	Maksegnet	48	2050	"
	Chuahit	72	2050	"
	Mizaba	101	2050	"
	Kola Diba	87	1950	Lowland
	Gorgora	100	1850	"
	Adi Arkai	120 } 572	1700	"
	Zarema	145	1200	"
	Sanja	120	1200	"
	Total	1414		
Gojam	Telema	100 } 200	2550	Highland
	Betchena	100	2550	"
	Mota	100	2450	Temperate
	Amanuel	70	2400	"
	Tilili	150	2400	"
	Dangela	150 } 870	2150	"
	Dembecha	100	2100	"
	Merawi	150	2050	"
	Bure	150	2050	"
	Dur Bete	150	2000	Lowland
	Wetet Abay	150	1900	"
	Fenote Selam	205 } 755	1900	"
	Bahir Dar	100	1800	"
	Chagni	150	1650	"
	Total	1825		
	Over-all total	3239		

In the laboratory, the containers were arranged in numerical order. A small amount of each stool (about two grams) from each sample was processed to indicate the presence of hookworm ova by the method of Ritchie (1948).

In Ritchie's (1948) method, about two grams of each stool sample was preserved in a numbered bottle using 7.5% formalin. The specimen was then thoroughly stirred, and depending on the size and density of the specimen, sufficient quantity was strained through double layered cotton gauze into a numbered 15ml. centrifuge tube to give about 0.5 to 1 ml. of sediment. Tap water was then added and mixed thoroughly and centrifuged at 2000 rpm. for one minute. The supernatant was decanted and about 10 ml. of buffer was added to sediment and allowed to stand for 2-3 minutes. After adding about 3 ml. of ether and shaking, the specimen was centrifuged for two minutes at 2000 rpm. The supernatant was then decanted and the whole sediment transferred to a slide, covered with a wide cover glass and examined for hookworm ova. Positive stool samples were thus identified for culturing, which in turn took place immediately.

3.4. CULTIVATION OF LARVAE

Cultivation of the filariform larvae involved the utilization of the modified test-tube filter-paper cultivation (MTFC) method as developed by Hsieh (1971).

Test-tubes (18mm. X 180mm.) were taken and adhesive tape was put on the upper portion of each about five centimeters from the top. To each tube about 10 ml. of distilled water was added and the tubes were placed in test-tube racks. Old newspapers were spread on the laboratory bench top. Lengthwise crease was made in filter paper strips and laid on the old newspapers. With wooden applicators about 0.1 g. of faeces was smeared from a positive stool sample on a filter paper leaving about 4 cm. at the left end of the filter paper unsmeared. The faecal smear was spread as thin as possible. Undigested fibres or seeds were removed from the smears. The smeared strip was inserted into the test tube, by the side of the unsmeared portion, and pushed into the water to nearly reach the bottom of the tube, and it was ensured that the faecal smear did not touch the water. The top of the test-tube was covered with a piece of cellophane and fixed in place tightly with a rubber band. Several minute holes were made in the cellophane sheet with a dissecting needle. A serial number corresponding to the number of the stool sample under culture was recorded on the adhesive tape. The test-tube was then kept at 24 - 29°C for at least seven days.

At the end of seven days, the cellophane cover was removed and discarded with caution. Distilled water was added to the test-tube until the water level reached above the top of the filter paper strip. After about five hours, the filter paper strip was removed with forceps and discarded. The supernatant was then drawn off using pipettes until the water level reached about 25 mm. from the bottom. The test-tube was shaken and the suspension rapidly transferred to numbered 15 ml. centrifuge tubes and the larvae concentrated by centrifugation for two minutes. The supernatant was carefully pipetted off from the surface down, leaving about 3 ml. of water remaining at the conical bottom.

A drop of the Dobell and O'Connor's solution was added to the water in the centrifuge tube. Immediately the whole sediment was transferred as a drop to a slide, covered with a wide cover-glass and examined for the presence of larvae under low magnification.

3.5. IDENTIFICATION OF LARVAE FROM CULTURE

The following key was utilized to differentiate larvae of A. duodenale and N. americanus (WHO, 1963; Hsieh, 1971; WHO, 1981).

a. Oesophagous about one-fourth the length of body of a sheathed larva. Body length about 500 - 600 μ , and sheath length about 660 μ; sheath conspicuously striated, more clearly observed around the tail portion of the body; mouth 'spears' appear dark, anterior end of body (not the sheath) rounded, like the small end of a hen's egg; anterior portion of intestine as wide as oesophageal bulb; tail end sharply pointed (Fig. 2.)............................. N. americanus

b. Oesophagous about one-fourth the length of body of a sheathed larva. Body length about 600 - 700 μ , and sheath length about 720 μ ; sheath less clearly striated, mouth 'spears' less conspicuous; anterior end of body (not the sheath) blunt; [illegible], intestine narrower in diameter than the oesophageal bulb; tail end blunted (Fig. 2.) .... A. duodenale

c. Occasionally infective larvae were found exsheathed. The presence of dark 'mouth-spears' and the narrowly-rounded anterior end of Necator differentiated it from Ancylostoma.

For differentiation of Ancylostoma and Necator the magnification of the microscope used was X 100 (10 X 10 mm.) and occasionally the high power objective was also used.

3.6. METHOD OF DATA ANALYSIS

a. The data was classified and presented in tables and figures.

b. The determinants of the distribution of parasites in this study were climate/altitude, sex and age. Initially simple linear correlation was estimated to see the degree of association between the parasites under study and the above determinants. The ones with significant results were identified and whenever possible a multiple regression equation, involving more than one determinant, was calculated.

c. Statistical tests were employed to see which of the parasites was more prevalent in each of the regions. Moreover, a comparative study was made between the two regions on the prevalence of a parasite. The method of comparison was

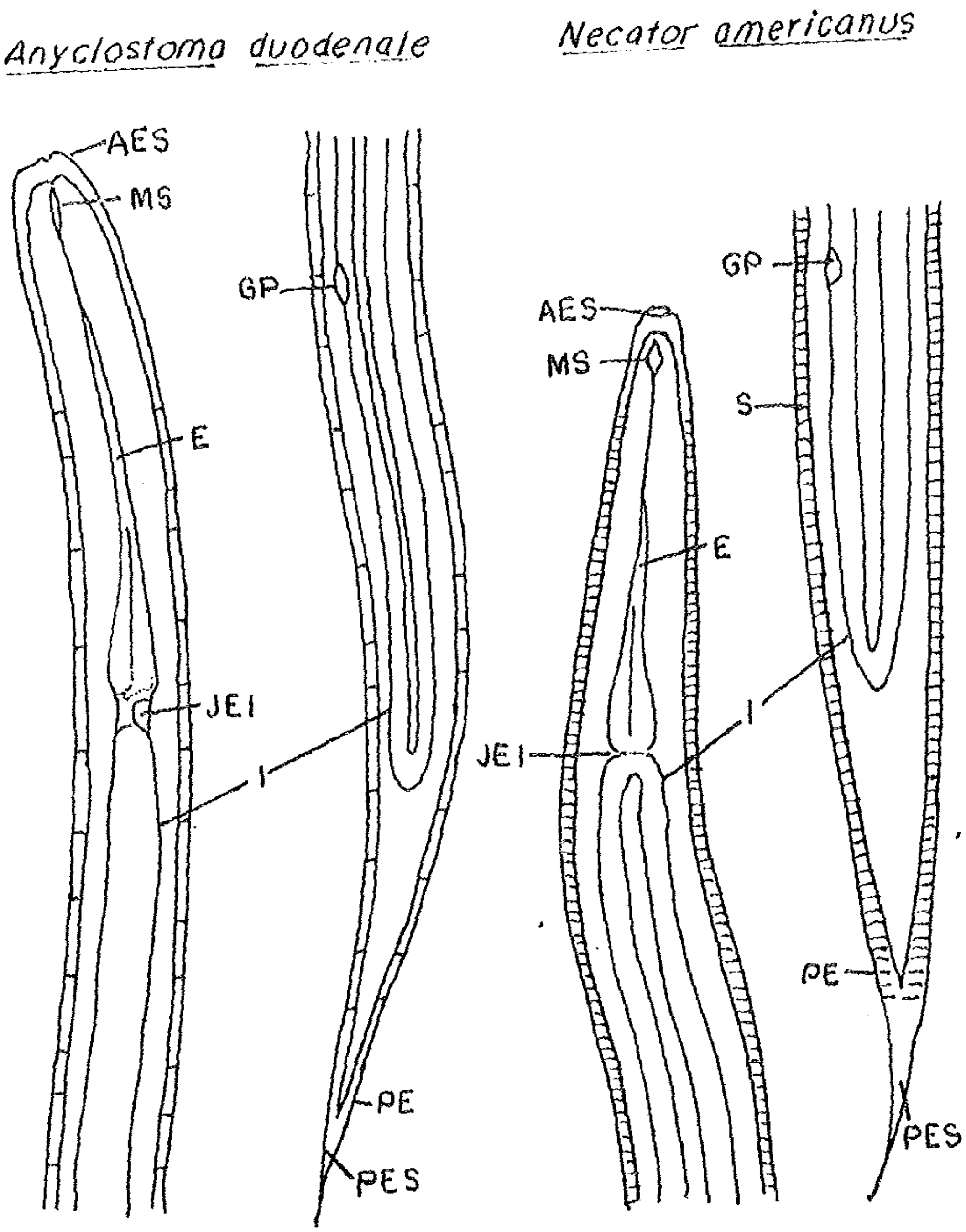


Fig. 2. Comparative morphological features useful in distinguishing infective larvae of N. americanus and A. duodenale (Hsieh, 1971).

the standardized normal (Z) statistic.

d. Variation of the parasites by climate/altitude in each of the regions was analysed using the Z statistic.

4. R E S U L T S

4.1. PROPORTIONS OF PUPILS INFECTED AND HOOKWORM SPECIES

Altogether 3239 (1827 males and 1412 females) schoolchildren were examined in 31 schools. Of these 20.13% were infected with hookworm. In 13 of these schools, 20% or more of those examined were positive and in another 13 schools, the infection rate ranged from 1,33% to 19.33%. Schoolchildren examined in the remaining five schools were negative. Of the total hookworm-infected individuals 58.9% were males and 41.1% were females.

When considering the two regions independently, of 1414 (739 males and 675 females) schoolchildren examined in Gondar Region 22.7% were found positive (Table 3). In Gojam Region, out of 1825 schoolchildren (1088 males and 737 females) 18.14% were positive (Table 4).

Necator americanus was found in a very high proportion. From the total of 652 hookworm infected cases 613 (94.02%) (364 males and 249 females) harboured *N. americanus*. Though in 15 cases eggs did not hatch and the larvae could not be identified, *Ancylostoma duodenale* was found in very low proportion. From the total positives, the species was identified in only 24 cases (3.68%). It also occured as a double infection with *N. americanus* in an additional six cases.

Table 3

Hookworm Preualence Without Regard

To Species In Gondar Region

Area Of Study	Elevation (m)	No. Of Individuals Examined	Age Range	Sex (M) (F)	No. Of Individuals Positive			% Positive		
					M	F	M	M	F	T
Nefas Mewcha	3150	61	11-26	39/22	-	-	-	-	-	-
Kossoye	3000	61	8-16	47/14	-	-	-	-	-	-
Kimer Dingaye	3000	50	7-18	30/20	-	-	-	-	-	-
Debarq	2950	100	8-20	54/46	4	2	6	7.4	4.3	6.0
Gassay	2850	64	10-18	50/14	2	-	2	4.0	-	3.1
Aykel	2350	64	8-12	33/21	11	3	14	33.3	9.7	21.9
Dib Bahir	2200	70	7-30	40/30	10	5	15	25.0	16.7	21.4
Tikil Dinagaye	2100	101	8-23	49/52	19	23	42	38.8	44.2	41.6
Enfraz	2100	50	7-30	27/23	-	5	5	-	21.7	10.0
Maksegnt	2050	48	10-23	24/24	1	-	1	4.2	-	2.1
Chuahit	2050	72	10-30	40/32	7	4	11	17.5	12.5	15.3
Mizaba	2050	101	7-37	26/75	13	24	37	50.0	32.0	36.6
Kola Deba	1950	87	8-23	34/43	6	-	6	17.6	-	6.9
Gorgora	1850	100	8-33	47/53	2	6	8	4.3	11.3	8.0
Adi Arkai	1700	120	8-31	55/65	16	31	47	29.1	47.6	39.2
Zarema	1200	145	8-32	81/64	24	23	47	29.6	35.9	32.4
Sanja	1200	120	6-20	63/57	48	32	80	76.2	56.1	66.7
Total		1414		739/675	163	158	321	22.1	23.4	22.7

Table 4

Hookworm Prevalence Without Regard To Species In Gojam Region

Area Of Study	Elevation (m)	No. of Invividuals Examined	Age Range	Sex (M/F)	No. of Individuals Positive			% Positive		
					M	F	T	M	F	T
Telama	2550	100	7-25	49/51	-	-	-	-	-	-
Betechena	2550	100	7-18	47/53	-	-	-	-	-	-
Mota	2450	100	7-19	51/49	-	4	4	-	8.2	4.0
Amanuel	2400	70	10-18	52/18	6	4	10	11.5	22.2	5.7
Tilili	2400	150	9-19	94/56	2	-	2	2.1	-	1.3
Dangela	2150	150	10-28	81/69	18	15	33	22.2	21.7	22.0
Dembecha	2100	100	6-12	63/37	12	4	16	19.1	10.8	16.0
Bure	2100	150	8-14	74/76	13	16	29	17.6	21.1	19.3
Merawi	2050	150	10-18	109/41	30	5	35	27.5	12.2	23.3
Dur Bete	2000	150	8-16	81/69	25	13	38	30.9	18.8	25.3
Wetet Abay	1900	150	7-19	105/45	38	6	44	36.2	13.3	29.3
Fenote Selam	1900	205	8-24	130/75	43	21	64	33.1	28.	31.2
Bahir Dar	1800	100	8-20	50/50	8	4	12	16.0	8	12.0
Chagni	1650	150	7-15	102/48	26	18	44	25.5	37.5	29.3
Total		1825		1088/737	211	110	331	20.3	14.9	18.1

All larvae were identified as *N. americanus* in Gojam Region while 92.52% (297) hookworm-infected cases harboured *N. americanus* in Gondar Region. In contrast, 7.48% (24) cases were found to harbour *A. duodenale* in Gondar Region. From the total sampled populations in Gondar Region, 297 (21.0%) harboured *N. americanus* (Table 5), while *A. duodenale* was recovered from 1.7% (Table 6). In Gojam Region 17.3% of the total sampled population had *N. americanus* (Table 7).

4.2. SPECIES Vs. CLIMATE/ALTITUDE, AGE AND SEX

In an attempt to identify the determinants of prevalence of the species multiple linear regression analysis was performed. The regression equations were estimated to be:

$$Y = 0.7313 - 0.00041X_1 + 0.2485X_2 \quad , \quad R^2 = 0.54$$
$$(0.000065) \quad (0.0454)$$

(*Necator americanus* in Gondar Region)

$$Y = 0.2346 - 0.000286X_1 + 0.0335X_2 \quad , \quad R^2 = 0.76$$
$$(0.000062) \quad (0.0262)$$

(*Necator americanus* in Gojam Region)

Where;

Y = Proportion of sampled cases with positive results

X_1 = Elevation in metres

X_2 = Sex ratio (male/female)

(Values in parenthesis are standard errors. Level of significance is 95%)

Table 5

Prevalence of Necator Americanus (Na)

In Gondar Region

Area Of Study	Elevation (m)	No. of Individuals Examined	Age Range	Sex (M/F)	No. of Individuals With Na			% With Na		
					M	F	T	M	F	T
Nefas Mecha	3150	61	11-26	39/22	-	-	-	-	-	-
Kossoye	3000	61	8-16	47/14	-	-	-	-	-	-
Kimer Dingaye	3000	50	7-18	30/20	-	-	-	-	-	-
Debarq	2950	100	8-20	54/46	4	2	6	7.4	4.3	6.0
Gassay	2850	64	10-18	50/14	2	-	2	4.0	-	3.1
Aykel	2350	64	8-12	33/21	11	3	14	33.3	14.3	21.9
Dib Bahir	2200	70	7-30	40/30	10	2	12	25.0	6.7	17.1
Tikil Dingaye	2100	101	8-23	49/52	19	23	42	38.8	44.4	41.6
Enfraz	2100	50	7-30	27/23	-	4	4	-	17.4	8.0
Maksegnt	2050	48	10-23	24/24	1	-	1	4.2	-	2.1
Chuahit	2050	72	10-30	40/32	7	4	11	17.5	12.5	15.3
Mizaba	2050	101	7-37	26/75	10	18	28	38.5	24.0	27.7
Kola Deba	1950	77	8-23	34/43	6	-	6	17.6	-	6.9
Gorgera	1850	100	8-33	47/53	2	6	8	4.3	11.3	8.0
Adi Arkai	1700	120	8-31	55/65	12	30	42	21.8	46.2	35.0
Zarema	1200	145	8-32	81/64	24	23	47	29.6	35.9	32.4
Sanja	1200	120	6-20	63/57	45	29	74	71.4	50.9	61.7
Total		1414		739/675	153	144	297	20.7	21.3	21.0

Table 6

Prevalence Of Ancylostoma Duodenale (Ad) In Gondar Region

Area Of Study	Elevation (N)	No. Of Individuale Examined	Age Range	Sex (M) (F)	No. of Individuals With Ad			% With Ad.		
					M	F	T	M	F	T
Nefas Mewcha	3150	61	11-26	39/22	-	-	-	-	-	-
Kossoye	3000	61	8-16	47/14	-	-	-	-	-	-
Kimer Dingaye	3000	50	7-18	30/20	-	-	-	-	-	-
Debarq	2950	100	8-20	54/46	-	-	-	-	-	-
Gassay	2850	64	10-18	50/14	-	-	-	-	-	-
Ayekel	2350	64	8-12	33/21	-	-	-	-	-	-
Dib Bahir	2200	70	7-30	40/30	-	3	3	-	10.0	4.3
Tikil Dingaye	2100	101	8-23	49/52	-	-	-	-	-	-
Enfraz	2100	50	7-30	27-23	-	1	1	-	4.3	2.0
Maksegnt	2050	48	10-23	24/24	-	-	-	-	-	-
Chuahit	2050	72	10-30	40/32	-	-	-	-	-	-
Mizaba	2050	101	7-37	26/75	3	6	9	11.5	12.0	8.9
Kola Deba	1950	87	8-23	34/43	-	-	-	-	-	-
Gorgora	1850	100	8-33	47/53	-	-	-	-	-	-
Adi Arkai	1700	120	8-31	55/65	4	1	5	7.3	7.7	4.2
Zarema	1200	145	8-32	81/64	-	-	-	-	-	-
Sanja	1200	120	6-20	63/57	3	3	6	4.8	5.3	5.0
Total		1414		739/675	10	14	24	1.4	2.1	1.7

Table 7

Prevalence Of Necator americanus (Na) In Gojam Region

Area of Study	Elevation (m)	No. of Individuals Examined	Age Range	Sex (M) (F)	No. of Individuals with Na			% With Na.		
					M	F	T	M	F	T
Telema	2550	100	7-25	49/51	-	-	-	-	-	-
Betechena	2550	100	7-18	74/53	-	-	-	-	-	-
Mota	2450	100	7-19	51/49	-	4	4	-	8.2	4.0
Amanuel	2400	70	10-18	52/18	6	4	10	11.5	22.2	5.7
Tilili	2400	150	9-19	84/56	2	-	2	2.1	-	1.3
Dangela	2150	150	10-28	81/69	16	15	31	19.8	21.7	20.7
Dembecha	2100	100	6-12	63/37	12	4	16	19.1	10.8	16.0
Bure	2100	150	8-14	74/76	11	15	26	14.9	19.7	17.3
Merawi	2050	150	10-18	109/41	28	5	33	25.7	12.2	22.0
Dur Bete	200	150	8-16	81/69	25	12	37	30.9	17.4	24.7
Wetet Abay	1900	150	7-19	105/45	37	6	43	35.2	13.3	28.7
Fenote Selam	1900	205	8-24	130/75	40	20	60	30.8	26.7	29.3
Bahirdar	1800	100	8-20	50/50	8	4	12	16.0	8.0	12.0
Chagni	1650	150	7-15	102/48	26	16	42	25.5	33.3	28.0
Total		1825		1088/737	[illegible]	105	316	19.4	14.2	17.3

In Gondar Region elevation and sex ratio were found to be significant. This implies a negative relationship between elevation and N. americanus and that the species seems to be more prevalent among males than females. On the other hand, in Gojam Region elevation was found to be significant while sex ratio was not. In both regions age was not found to be correlated with infection rate.

A simple correlation coefficient between incidence of attack on the one hand and climate/altitude, age and sex on the other, gave the results noted in the scatter-diagrams (Figs. 3 - 8).

4.3. VARIATION OF PARASITES BY ALTITUDE IN EACH OF THE REGIONS

An attempt was made to see the variation of the two species by altitude. This was analysed for each of the species and for both put together. Further classification was also made by region. Our initial hypothesis was that the incidence of the two species does not vary with altitude in each of the regions. Based on this assumption, a standard normal test on the difference between a pair of proportions was applied. The formula used was:

$$Z = \frac{P_1 - P_2}{\left[\dfrac{P_1 q_1}{n_1} + \dfrac{P_2 q_2}{n_2} \right]^{\frac{1}{2}}}$$

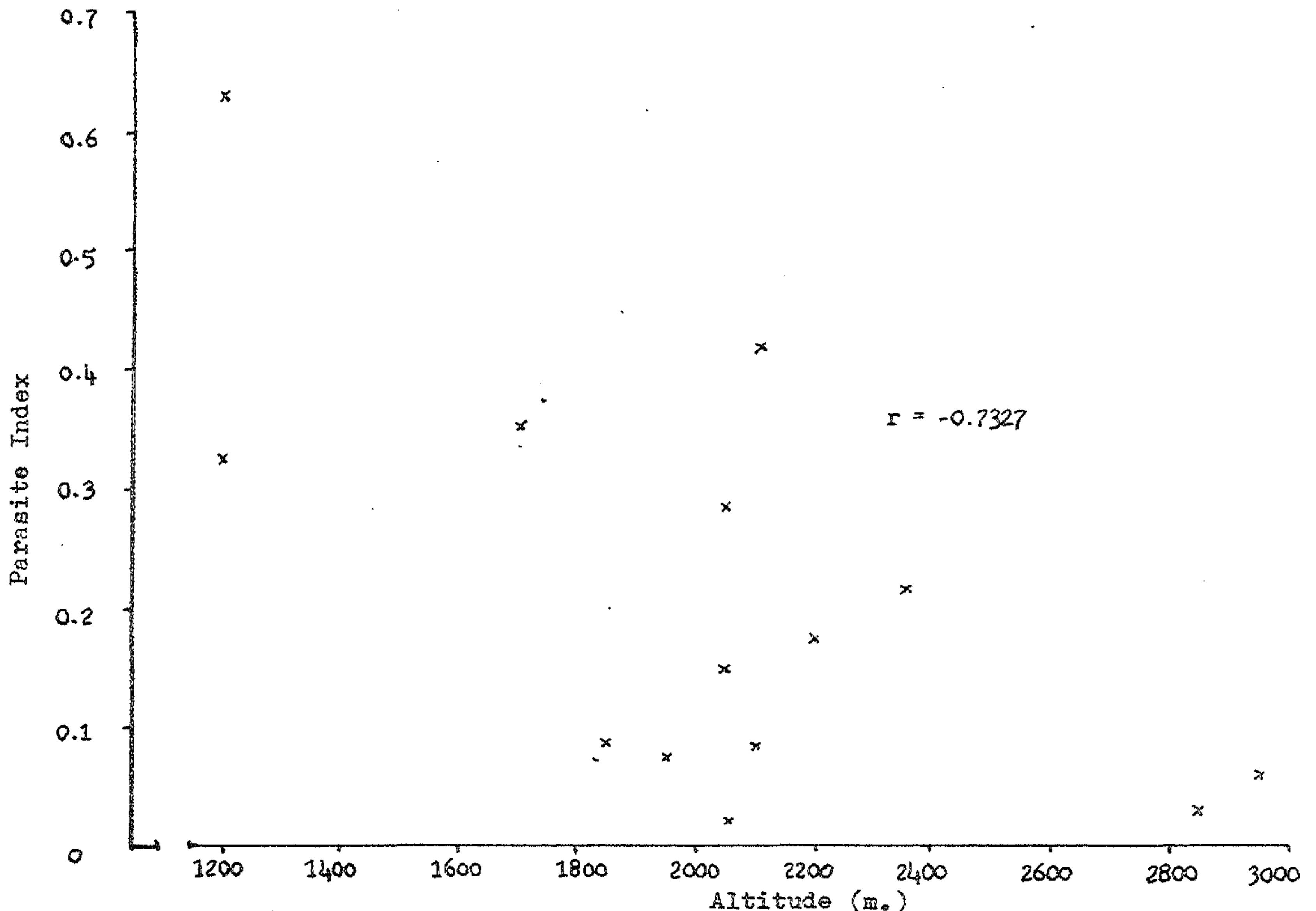


Fig. 3. Scatter diagram showing the relationship of *N. americanus* and altitude in Gondar Region.

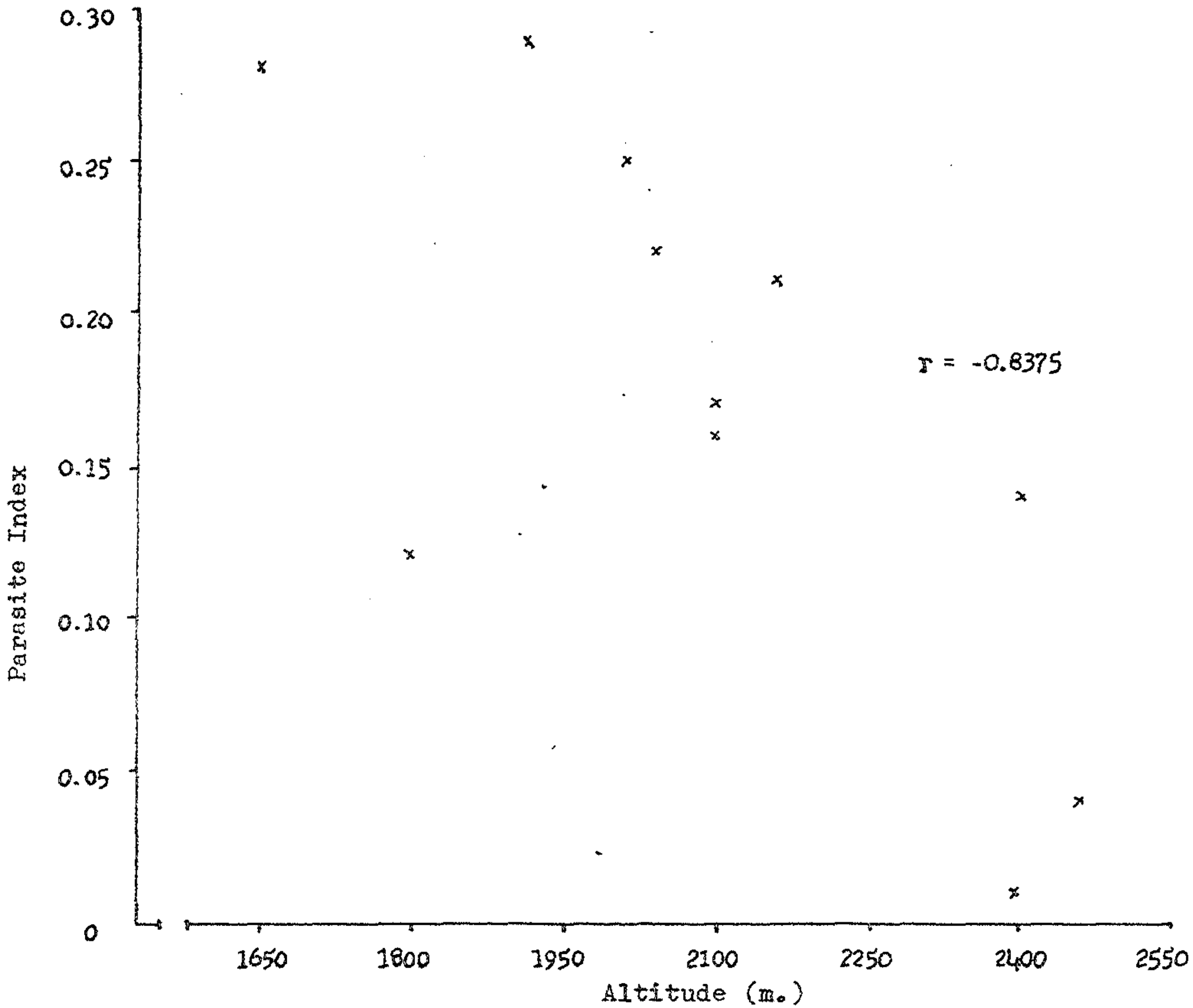


Fig. 4. Scatter diagram showing the relationship of _N. americanus_ and altitude in Gojam Region.

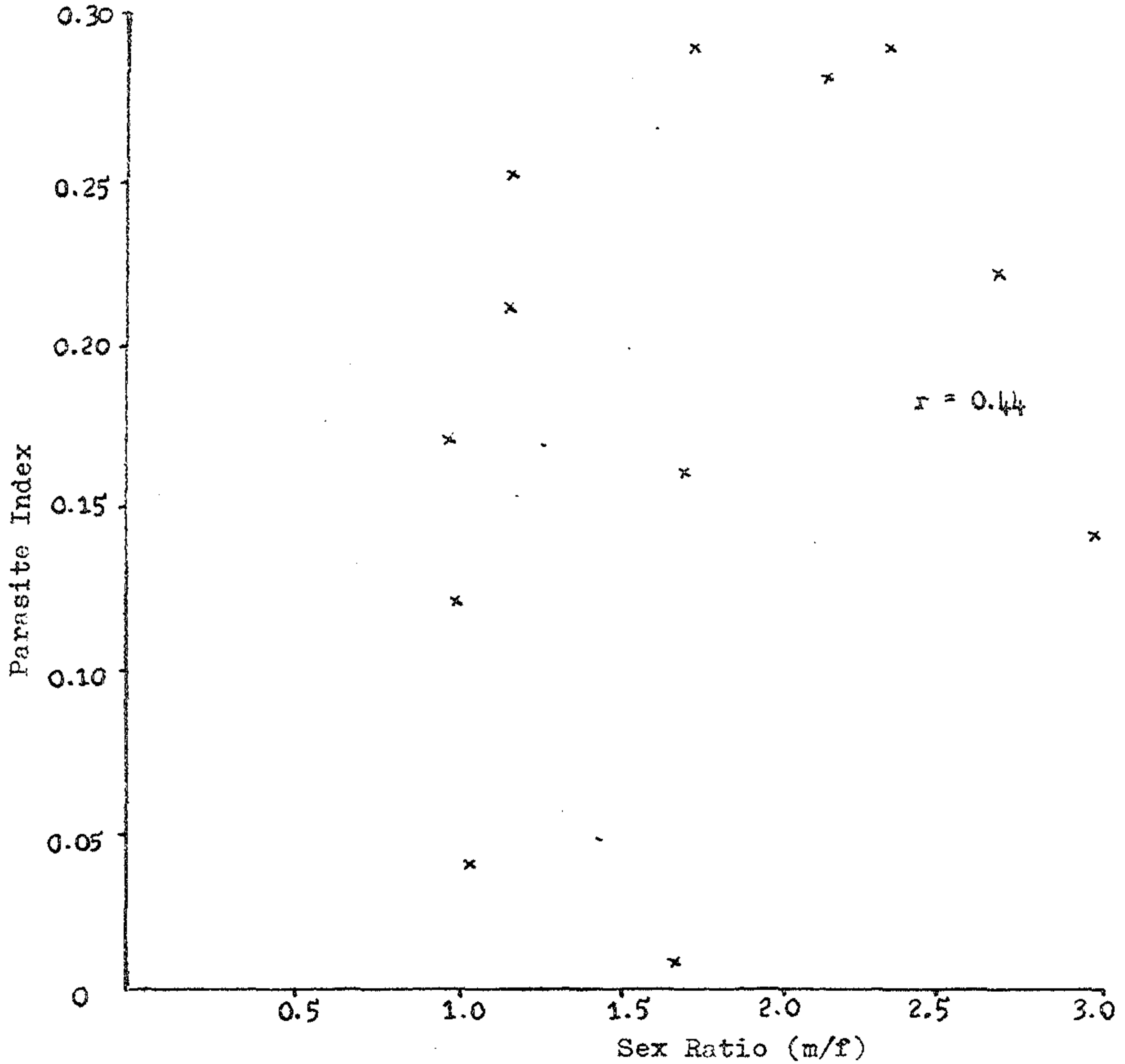


Fig. 6. Scatter diagram showing the relationship of N. americanus and sex ratio of schoolchildren in Gojam Region.

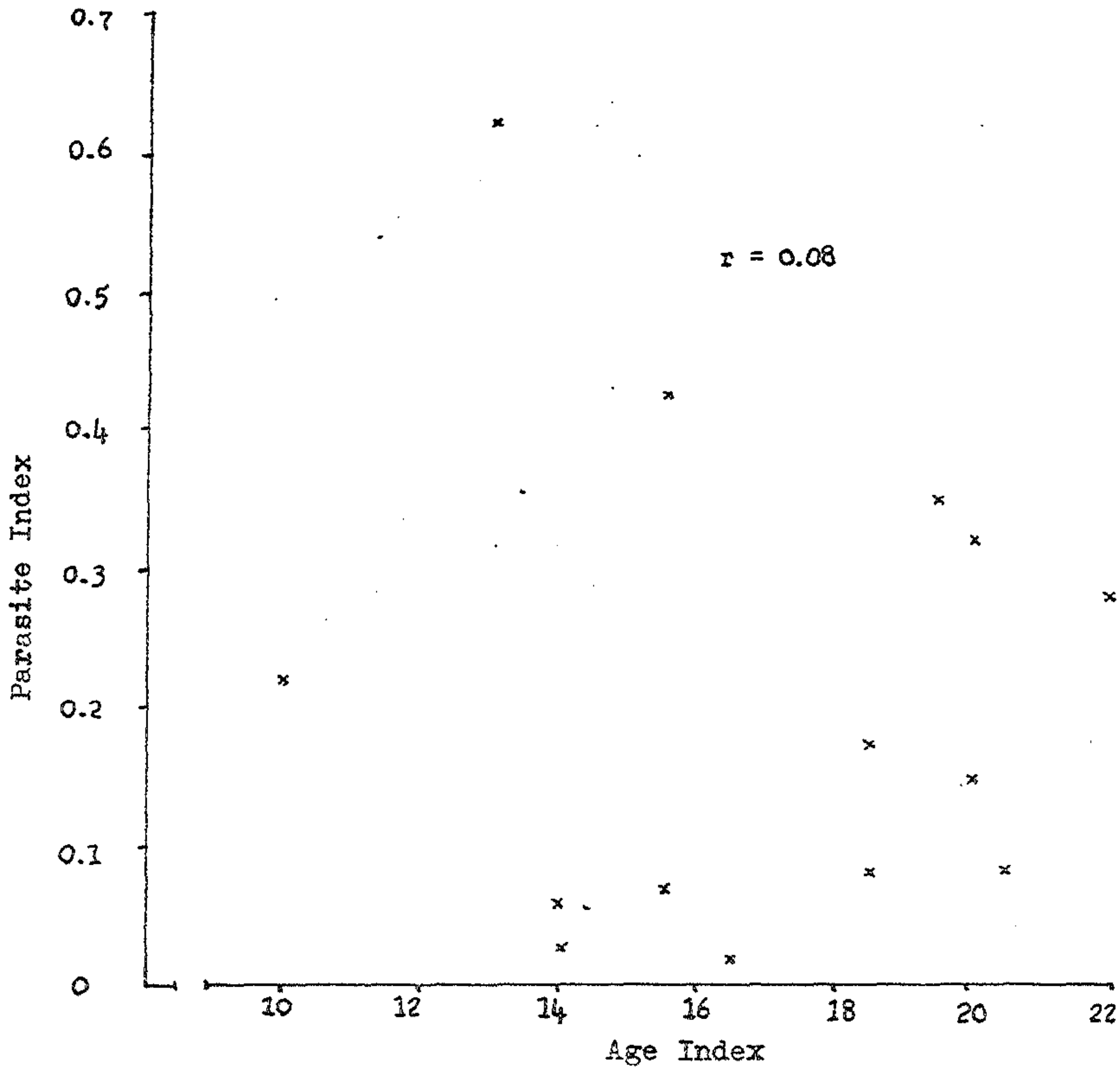


Fig. 7. Scatter diagram showing the relationship of N. americanus and age of schoolchildren in Gondar Region.

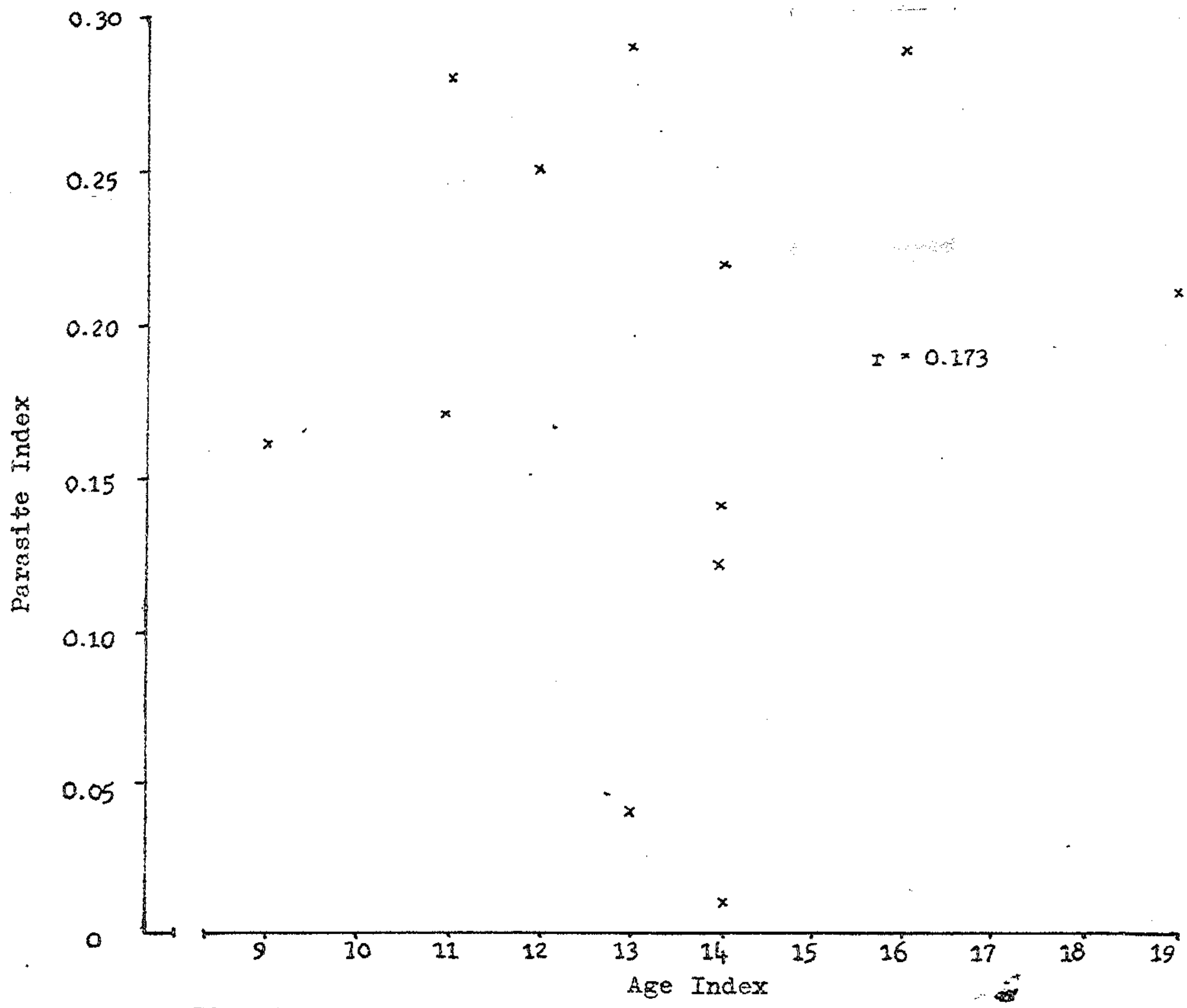


Fig. 8. Scatter diagram showing the relationship of N. americanus and age of schoolchildren in Gojam Region.

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where;

P_1, P_2 is the proportion of cases positive in any two altitudinal regions in Gondar and Gojam, respectively

q_1, q_2 is the complement of P_1 and P_2, respectively

n_1, n_2 is the sample size for Gondar and Gojam used for estimating proportions.

Table 8 shows the proportions of positive cases in each area and altitudinal region. Table 9 shows the value of the Z statistic on the difference between two proportions. The results of Table 9 suggest that there is significant difference in the incidence of the parasites when classified by altitude. The only exception was the incidence of A. duodenale between temperate and lowland areas for Gondar Region. The latter did not show significant difference. The conclusion is thus the incidence varies inversely with altitude, i.e., as we go higher we get fewer propobtion of cases.

Table 8

Proportion of Cases Positive for the Two Species of Hookworms Arranged by Altitude and Region

(Value in parenthesis is the sample size; Na = *Necator americanus*, Ad = *Ancylostoma duodenale*)

Altitudinal Region	Region					
	Gondar			Gojam		
	Na	Ad	Na+Ad	Na	Ad	Na+Ad
Highland	.02 (336)	0 (336)	.02 (336)	0 (200)	--	0 (200
Temperate	.22 (506)	.03 (506)	.25 (506)	.16 (370)	--	.1 (87
Lowland	.31 (572)	.02 (572)	.33 (572)	.27 (755)	--	. (75

Table 9

Z statistic on the Variability of the Incidence by Altitude and Region for the Two Species of Hookworm

(Na = *N. americanus*; Ad = *A. duodenale*)

Comparison of altitudinal region	Region					
	Gondar			Gojam		
	Na	Ad	Na+Ad	Na	Ad	Na+Ad
Highland vs Temperate	20.00	3.66	11.45	14.14	-	14.14
Highland vs Lowland	13.57	3.34	14.32	15.06	-	15.06
Temperate vs Lowland	3.29	0.71*	2.98	5.09	-	5.09

* Not significant result.

4.4. VARIATION OF PARASITES BETWEEN REGIONS

Table 10 shows the proportion of positive cases in each region along with the Z statistic between two proportions.

Table 10

Proportion of Cases with One or Both Species in Each Region, along with the Z statistic

(Na = *N. americanus*; Ad = *A. duodenale*)

Region	Species		
	Na	Ad	Na + Ad
Gondar	0.21	0.02	0.23
Gojam	0.17	-	0.18
Z value	2.88	-	3.48

Table 10 suggests that the parasites are more prevalent in Gondar than in Gojam Region. One may have to do further research on the higher prevalence of the parasites in Gondar than in Gojam.

4.5. PROPORTION OF CHILDREN WITH HOOKWORM WITH/ WITHOUT REGARD TO SPECIES IN THREE DIFFERENT ALTITUDINAL REGIONS

Table 11 shows the proportion of schoolchildren with hookworm (without regard to species) in three different altitudinal regions and the proportion of children with pure and mixed infections of *N. americanus* and *A. duodenale* in the three different altitudinal regions is indicated in Table 12.

Table 11

Proportion of Children with Hookworm (without regard to species) in Three Different Altitudinal Regions

Region	No. of children examined	Proportion positive for hookworm (%)
Highland	536	1.49
Temperate	1376	18.46
Lowland	1327	29.39

Table 12

Proportion of Children with Single and Mixed Infections of *N. americanus* and *A. duodenale* in Three Different Altitudinal Regions

(Na = *N. americanus*; Ad = *A. duodenale*)

Infections	Highland (536)* proportions positive (%)	Temperate(1376) proportions positive (%)	Lowland(1327 proportions positive(%)
Na only	1.49	17.00	27.95
Ad only	-	0.94	0.83
Ad when with Na	-	17.95	28.71

* The numbers of children examined in each altitudinal region are shown in parenthesis.

As shown in Table 11 and 12, the proportion of children infected by hookworms is highest in the lowlands (29.39%) followed by the temperate regions (18.46%). The highest infection by *N. americanus* is found in the lowlands (27.95%) followed by the temperate areas (17.00%). Infection due to this parasite is also found in the highlands, though uncommonly (1.49%), contrary to what has been indicated in the literature.

Surprisingly enough, infection by *A. duodenale* is also creeping into the lowlands. In the lowlands and in the two other altitudinal regions, the prevalence of hookworm infection among schoolchildren is mainly accounted by *N. americanus*.

5. DISCUSSION AND CONCLUSION

Among the specific objectives of this study were to make a survey for an accurate and reliable estimate of the infection in defined altitudinal areas of the two regions, to help the physicians of the regions know the identity of the most important cause of hookworm disease and thereby help them control it and to help lay the foundation for a thorough investigation in other regions of the country.

It is true that school age groups do not represent all the age groups in a community. In spite of this restriction, many studies use this parameter to infer community prevalence of intestinal parasites (Lemma et al. 1968; Desowitz and Wiebenga, 1975). This study used schoolchildren as its sample population because the schoolchildren share the same environmental conditions common to all age groups in the community. In addition, the sample population has also included the age 6 -20 yrs. group which shows the highest prevalence of intestinal parasites in community studies (De Bei, 1955; Ricci, 1956).

Many investigators have analysed the effect of altitude in the distribution of hookworm infection. Watson et al. (1956) indicated that increasing altitude caused a diminishing incidence of hookworm. Wang (1965) found a significant difference between children living in highlands and those in lowlands (68.8% and 95% infections, respectively).

Diesfeld (1969) noted that the incidence of hookworm d creased with rising altitude. Diesfeld (1970) has also reported that spread of hookworm occurs most readily in low-lying moist areas. Kelly and Garavusi (1974) indicated a high incidence of hookworm infection in mountainous regions. Ebrahimzadeh (1974) found hookworm to be predominant in areas with warm, humid and oceanic climate.

Similar results have been obtained in this study. In examining 1317 schoolchildren from 10 localities in lowland areas, over-all infection rates of 29.39% hookworm were revealed. In 1376 schoolchildren from 14 localities studied in temperate areas over-all infection rates of 18.46 hookworm were demonstrated, and in 536 pupils from 7 localities of highland areas 1.4% of the children were shown to be hookworm-infected. Statistical analysis has indicated an inverse relationship between incidence of infection and altitudinal region. This may be related to the absence of warm and moist environmental conditions necessary for the development of the larvae. Svensson (1956) has indicated that high altitudes and dryness prevent development of immature helminths to the infectious stage outside the host elsewhere, and the same opinion is expressed by Chandler and Read (1961).

The prevalence rate of hookworm was found to be higher in Gondar Region than in Gojam Region. However, both regions enjoy similar climatic conditons and other human factors such as occupation, agricultural practices, defecation behaviour and food habits, which make them equally vulunerable for hookworm infection. This difference in prevalence rates needs further investigation.

Variation in local and micro-environment may influence hookworm intensity and infection rate. Thus an infection rate of 41.6% prevailed at Tikil Dingaye at an altitude of 2100m. in Gondar Region and 19.3% infection rate at Bure at the same altitude in Gojam Region.

Earlier reports (Schaller and Kuls, 1972) indicated that ancylostomiasis is a serious health problem in almost all Ethiopian provinces in places up to 1800m. Later McConnell and Armstrong (1976) have indicated that hookworm infections were numerous between Debre Markos and Axum at altitudes between 1300-2100m., and low infection rates were encountered at well below 2200m. along the eastern edge of the plateau. In our study, however, contrary to what has been indicated hookworm infection has been obtained at well over 2200m. streching up to 2500m.

According to Sadun (1955), prevalence of intestinal parasitism showed an increase up to the ages of 10 - 14 and a remarkable decrease after age 30. Hinz (1967) concluded that ages 6 - 12 show the highest prevalence rate. Beaver _et al._ (1952) state that in human hookworm infections the maximum incidence occurs somewhere between the ages 15 - 25.

Duncan (1978) found hookworm to be evenly distributed among the different age groups. This study, however, revealed that there was no significant age difference in the prevalence of hookworms.

There is no consistent evidence to indicate that there is a difference in susceptibility to helminthic infections between the sexes in man. Sadun (1955) indicated that the infection rate in males was significantly higher than in females from the ages of 10 yrs. onwards for hookworm. The infection rate for Ascaris lumbricoides and Trichuris trichiura was higher in females than in males. The results of this study was supported by Hinz (1967) and Molineaux (1967). These studies actually indicate not a sex difference per se but the outcome of sex-related occupational differences. In our study, however, significant sex-related differences was obtained in Gondar Region only. Infection with hookworm was more prevalent in males than in females.

N. americanus was first reported (Ferro-Luzzi, 1949; Sofia, 1949) in the natives of the Eritrean highlands and D'Ignazio and Mira (1949) revealed the presence of A. duodenale. Later Armstrong and Chane (1975) indicated the presence of both N. americanus and A. duodenale.

The cultivation technique used in this study proved the existence of both A. duodenale and N. americanus infection in Gondar Region, with the latter species occuring about 13 times more frequently than the former. Only N. americanus infection was found in Gojam Region. Similar patterns of differential existence of infection by the two species have been encountered elsewhere.

De Carneri (1974) indicated that N. americanus prevails over A. duodenale in the territory stretching from northern portugal to northern Italy, through northern Turkey and Soviet Transcaucasia, right into northern Iran. According to Canese and Canese (1976), in Paraguay, in the east of the country the A. duodenale/N. americanus ratio was 1 : 44, in the west it was 1 : 16.5 and over the whole country it was 1 : 25.6. Furthermore, N. americanus has been shown to be the prominent species in El Salvador (Bloch and Ruiz, 1966), Singapore (Zaman and Loh, 1974), Mexico (Chacon et al. 1975), and the Phillipines (Carney et al. 1980). Franz et al. (1964). Sturrock (1966), Bakker (1969), Miller (1970), Ricciardi (1974) and Oyerinde (1978) have also indicated N. americanus to outnumber A. duodenale in various parts of Africa.

The preponderance of A. duodenale over N. americanus has also been reported. In Taiwan, A. duodenale was more prevalent than N. americanus (Hsieh et al. 1965). In Korea, Choi et al. (1973) reported that culture of 182 stool specimens yielded only A. duodenale. In many parts of India the preponderance of A. duodenale over N. americanus has been indicated by Bahandari and Shrimali (1969). In Africa, though not predominating, the occurrence of *A. duodenale* has been reported by Hsieh *et al.* (1972) in Liberia, Rep and Shaba (1976) in Tanzania, Huys *et al.* (1976) in Rwanda and Colaert *et al.* (1978) in Kinshasa.

The findings of investigations on the differential distribution of the hookworm species of man reviewed so far try to relate the difference to climatic, altitudinal and other related environmental factors in general. But in this study the regional differences in climatic factors are favorable for wide dispersal of *A. duodenale*, but this has not occurred. Temperature, rainfall and humidity vary somewhat in these regions, but the range of variations in these factors is still within the limits demonstrably favorable in other parts of the world for endemicity of *A. duodenale* (Diesfeld, 1970; Daniel, 1977).

A fundamental fact in hookworm endemicity is that soil pollution maintains the life-cycle of the parasites. It needs to be considered then whether, with a generally favorable climate, the differences in the capacity of *N. americanus* and *A. duodenale* to maintain themselves and spread in different parts of the regions may be associated with the character of the soils of the regions.

Elsewhere, Keller *et al.* (1940) undertook a re-survey in 8 southern American states of the hookworm situation in the period 1930-38, examining over 400,000 persons. They concluded that " while there is a wide spread distribution of hookworm, the areas of highest incidence are confined to the coastal plain and sandy-soil areas of each site." Their observation relate to where the infective agent is _N. americanus_ and they emphasize a favorable relationship between _N. americanus_ parasitism and sandy soil. Vieira and Fraga De Azvedo (1966) also report that hookworms (_N. americanus_) were most common in soils which contained more organic matter.

No comparable data are available for _A. duodenale_. Kim (1965) and Vinayak _et al_. (1979) found higher hookworm prevalence where both species are present and where soils had more sand, but did not report any generic predilection.

According to Hsieh _et al_. (1971), _N. americanus_ showed high incidence and intensity in all parts of Liberia, regardless of demonstrably different textural characterstics of the soil in different regions. Instead, _A. duodenale_ was found to be highest in the villages where the percentage of sand in the upper surface soil was 90% or higher, or the percentage of clay was below 10%. Whether the accompanying change in amount of organic matter played a role was not clear.

It is obvious that differences in the clay, silt, sand and organic matter of the soil may greatly affect such physical properties of the soil as porosity, aeration, moisture condition, density, etc. These differences doubtless influence the diffusion rate of oxygen and carbon dioxide into and from the soil. When studying helminthiasis one has to take note of these factors.

In the regions of this study there are no reports on studies of soil textures. This awaits the future.

Perhaps, of greater consequence is the bearing this study makes available in the study of problems of clinical and non-clinical hookworm in pre- and post- treatment phases, on the efficacy of drugs and other related problems.

As N. americanus was identified in 92.52% of the hookworm infection in Gondar Region and in all cases in Gojam Region, routine single-dose therapy against hookworm when faecal specimen are not cultured for larval identification should be with tetrachlorethylene or other drugs active against N. americanus selectively. WHO (1963) reported tetrachlor-ethylene has been found more selective against Necator species than bephenium hydroxynaphthoate.

It was found by this study that there are significant differences in infection with hookworm between high and low altitude areas. Infection was found to be lower in highland than in lowland areas, thus suggesting that climate/altitude factors play an important role in the transmission. The possibility of exploiting the climate/altitude factors in the designs for human settlements deserves consideration if this does not conflict with developmental endeavours.

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