

PREVALENCE AND TYPE OF ANAEMIA  
DUE TO HOOKWORM INFECTION AMONG  
THE POPULATIONS OF WOLISSO

BY

ASTER TSEGAYE

የወላይት ስርዓት  
የትምህርት ማኅበር  
ADDIS ABABA UNIVERSITY  
LIBRARIES

35

PREVALENCE AND TYPE OF ANAEMIA  
DUE TO HOOKWORM INFECTION AMONG  
THE POPULATIONS OF WOLISSO

BY

ASTER TSEGAYE

A THESIS PRESENTED TO THE  
SCHOOL OF GRADUATE STUDIES  
ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE IN BIOLOGY

ADDIS ABABA, ETHIOPIA

JUNE, 1995

## TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS.....	i
LIST OF TABLES.....	iii
LIST OF FIGURES.....	iv
ABSTRACT.....	v
1. INTRODUCTION.....	1
1.1 Anaemia.....	1
1.2 Hookworms.....	8
1.3 Hookworm infection and anaemia.....	10
1.4 Objectives of this study.....	22
2. MATERIALS AND METHODS.....	23
2.1 Study sites and subjects.....	23
2.2 Sample collection.....	24
2.3 Parasitological methods.....	25
2.4 Haematological analysis.....	27
2.5 Biochemical analysis.....	28
2.5.1 Ferritin assay.....	28
2.5.2 Serum iron determination.....	28
2.5.3 Total iron binding capacity (TIBC).....	30
2.5.4 Transferrin saturation.....	31
2.6 Statistics.....	32

	PAGE
3. RESULTS.....	33
3.1 Characteristics of the study population.....	33
3.2 Parasitology results.....	35
3.3 Haematological results and their relationship with hookworm infection.....	35
4. DISCUSSION.....	51
5. CONCLUSION.....	63
6. REFERENCES.....	65

.....

## ACKNOWLEDGEMENTS

I am highly indebted to my advisor Dr. Yalemtehay Mekonnen for her continuous assistance, advice, and encouragement throughout this study. My regards also go to my advisor Dr. Seyoum Tatischeff for his advice at the planning stage, also for facilitating the field work and for all his help throughout this study. My thanks are due to Dr. Hailu Yeneneh, general Manager of the National Research Institute of Health (NRIH), for his fast, positive response whenever I needed the help of the institute.

I am very much grateful to Dr. Hailemichael Gebreselassie for providing me with the ferritin kits, the entire staff of the clinical chemistry laboratory of NRIH, and in particular to Ato Gonfa Ayana, Ato Gebeyehu Damecha, Ato Mengistu Mekuria and Ato Sisay Kinfemichael for their kind help and assistance during the analysis of the biochemical assays.

My heart felt thanks go to Tesfaye Tilahun, Ameha Kebede, Yared Mekonnen, Yonas Tafesse, Ato Hailegnaw Eshete and Ato Tilahun Woldemichael for their invaluable help in the field work. My special thanks are also due to Yared Mekonnen for analysing the data. I gratefully acknowledge the technical assistance of Wondosen Sime of the haematology

laboratory of NRIH, the statistical advice of Ato Amare Dejene and Tekabe Ayalew and the material support of Mesfin Lulu and Mekonnen Kurabachew.

I am grateful to the staff of the drug control and toxicology division of NRIH, the Epharmicor Central Branch and the central medical stores, staff of Industrial chemistry and Immuno-haematology divisions of NRIH and the librarians of both NRIH and the Ethiopian Nutrition Institute who helped me in one way or another.

I am pleased to thank my family and friends who encouraged me during the course of the study and all those who made this study possible. I am also very grateful to W/t Zewdnesch Ayele for typing the manuscript. Lastly, I wish to thank the study subjects and Ato Kuma Gurmessa, health assistant at Obi clinic, and Ato Demsu Dubale, laboratory technician at Wolisso Hospital, for their cooperation.

The laboratory work of this study was supported by the National Research Institute of Health, and the cost of part of the field work and the drugs (anthelmintic and Iron tablets) was covered by the Swedish Agency for Research Cooperation with developing countries (SAREC). Both NRIH and SAREC are gratefully acknowledged.

## LIST OF TABLES

	PAGE
Table 1. Distribution of the study population by age and sex.....	34
Table 2. Mean and standard deviation values for haematological measurements of cases and controls.....	37
Table 3. Mean and standard deviation values for the biochemical assays of cases and controls.....	38
Table 4. Relationship between anaemia parameters (haemoglobin, haematocrit, ferritin, iron, TIBC and percentage transferrin saturation)..	38
Table 5. Association of anaemia parameters with hookworm infection.....	40
Table 6. Anaemia parameters and their relationship to hookworm ova load.....	42
Table 7. Summary of peripheral blood morphology results of the study population.....	46
Table 8. Red cell indices results of the study population.....	48
Table 9. Association of macrocytic anaemia and hookworm infection.....	49

LIST OF FIGURES

	PAGE
Figure 1. Distribution of serum ferritin levels among the study subjects.....	44

## ABSTRACT

A community based case-control study to investigate the relationship between hookworm infection and anaemia was carried out on 227 apparently healthy individuals living around Wolisso area. Of these subjects 155 were cases (individuals infected with hookworm) and 72 were controls (uninfected). It was found that 20.6% of the cases and 5.6% of the controls were anaemic. Chi-squared analysis showed hookworm infection was significantly associated with low haemoglobin (odds ratio = 4.42,  $P < 0.05$ ), low transferrin saturation (odds ratio = 17.18,  $P < 0.001$ ) and low ferritin levels (odds ratio = 14.98,  $P < 0.01$ ). Intensity of infection as expressed in eggs per gram (epg) of faeces showed a highly significant negative relationship with transferrin saturation and serum ferritin levels ( $P < 0.001$ ) but not with haemoglobin levels ( $P > 0.05$ ). When multiple criteria involving elevated mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and macrocytic blood picture were taken as suggestive of a macrocytic type of anaemia, 13.0% of the cases and 5.6% of the controls fell into this category. Chi-squared analysis however, showed no statistically significant association between parameters suggestive of macrocytic anaemia and hookworm infection (odds ratio = 2.54,  $P > 0.05$ ). Further investigation is recommended to clearly indicate the role of hookworms in the genesis of megaloblastic anaemia by employing better biochemical assays.

The intensity of infection was light and the anaemia was generally of mild type.

## 1. INTRODUCTION

### 1.1 Anaemia

Anaemia is of major public health importance throughout the world (DeMaeyer and Adiels-Tegman 1985). According to the World Health Organization (WHO 1972), anaemia is defined as a condition in which the concentration of haemoglobin, haematocrit or the number of red blood cells is lower than that in healthy persons of the same sex and age group in the same environment. Anaemia is an important public health problem in that it challenges the well being of individuals and can affect cognitive function (Pollitt *et al.* 1986), immune function (Dallman 1987) and also work performance (Viteri and Torun 1974).

Anaemia may result from decreased production of red blood cells, or from their increased destruction, or from loss through chronic blood loss or haemorrhage (Seiverd 1983, Rifkind *et al.* 1986). Decreased production of red blood cells can be due to either the failure of the bone marrow to produce the normal number of cells or a deficiency of haemopoietic factors mainly iron, folate and vitamin B<sub>12</sub> (Seiverd 1983, DeMaeyer and Adiels-Tegman 1985, Evatt *et al.* 1992). The common causes of anaemias due to increased red cell destruction called the haemolytic anaemias, include congenital defects of haemoglobin production (Sickle cell

anaemia and thalassaemia) and other extrinsic causes including malaria infection and autoimmunity (Evatt et al. 1992). Chronic blood loss caused by parasites such as hookworms and also schistosomes is also implicated in the aetiology of anaemia in the tropics (Woodruff 1982, DeMaeyer and Adiels-Tegman 1985).

Nutritional anaemia, that is, anaemia due to a deficiency of haemopoietic factors, is by far the most important common type of anaemia world wide (WHO 1968, 1972, 1975). WHO has defined it as follows : "Nutritional anaemia is a condition in which the haemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency (WHO 1992).

Nutritional deficiency of folate and less commonly Vitamin B<sub>12</sub> are the major causes of megaloblastic anaemia (Herbert 1970, Evatt et al. 1992). Foodstuffs with the highest content of Vitamin B<sub>12</sub> are liver and kidney, but other meat, dairy products, poultry, fish and shellfish also contain large amounts. Folic acid is present in most foods; bananas are particularly rich in folic acid (WHO 1970, 1972, Evatt et al. 1992). But folates are heat labile and can be destroyed by prolonged cooking (Herbert 1970). Diagnosis of megaloblastic anaemia is possible from the peripheral blood smear examination which should be confirmed by bone marrow

examination, the red cell indices, serum Vitamin B<sub>12</sub> and folate levels and also by therapeutic trials. Although it is difficult to obtain an accurate one, dietary history is also quite important in the evaluation of megaloblastic anaemia or nutritional anaemia in general. In the absence of extensive laboratory facilities for the biochemical tests and electronic counters which enable the calculation of reliable red cell indices, the findings of oval macrocytes, particularly when accompanied by neutropenia, hypersegmented neutrophils, relative lymphocytosis and sometimes thrombocytopenia, suggests megaloblastic anaemia (Wintrobe *et al.* 1981, Evatt *et al.* 1992).

Iron deficiency anaemia is the most commonly recognized form of nutritional anaemia world wide. It mainly affects women of reproductive age and young children, due to their increased physiological demand for iron (WHO 1968, 1972, 1975, DeMaeyer and Adiels-Tegman 1985, WHO 1989, 1992). The United Nations Administrative Committee on Coordination-Subcommittee on Nutrition (UN ACC/SCN 1991) agreed that iron deficiency affects over one billion people in the world, particularly reproductive women and pre-school children in tropical and sub-tropical zones. It results from consuming diets with insufficient iron, reduced dietary iron availability, increased iron requirements to meet reproductive demands and losses due to parasitic infections; these factors often operate concurrently (UN ACC/SCN 1991).

Iron deficiency anaemia is a slowly evolving condition which passes through three progressive stages, namely the prelatent stage, the latent stage and the manifestation of iron deficiency (Siimes *et al.* 1980, Wintrobe *et al.* 1981, Cook 1982, Finch and Cook 1984). The prelatent stage of iron deficiency is characterized by the depletion of body iron stores. The second stage results from a diminished iron supply to the developing red blood cells, but occurs in the absence of a significant effect on circulating haemoglobin levels. When prolonged, iron deficiency leads to the last stage, that is the development of iron deficiency anaemia. The three stages can be monitored in the laboratory by the measurements of serum ferritin, transferrin saturation or erythrocyte protoporphyrin and haemoglobin respectively (Cook 1982, Finch and Cook 1984, WHO 1989).

Generally, anaemia constitutes a considerable problem of public health throughout the world, especially in developing countries (DeMaeyer and Adiels-Tegman 1985). Data collected indicate that, according to WHO criteria a total of 2,170 million people are anaemic (WHO 1992). According to this report, prevalence rates are higher in developing than in industrialized countries. The regions with the highest overall prevalence of anaemia are South Asia and Africa. In Africa the estimated prevalence of anaemia ranges from 20% in adult males to 63% in pregnant females (DeMaeyer and Adiels-Tegman 1985).

In Ethiopia, anaemia has not been considered as a serious public health problem (Hofvander 1968, Peters 1984, Fleming 1989). This was largely attributed to the high iron content of *teff*, mostly the result of contamination with iron rich clay soil (Besrat et al. 1980, Alemayehu et al. 1993), whose bioavailability is enhanced by the process of fermentation during the preparation of *enjera*, a local home-made bread (Ramachandran and Bolodia 1984).

Even if the prevalence of anaemia in the general population is not known, meager information are obtained from various sources including analyses of medical admissions to various Ethiopian hospitals. Although analysis of hospital admissions is not the best method of studying the disease pattern in a community, an incidence rate of less than 1% has been reported (Tefera and Abdulkadir 1968, Habtegabir et al. 1976, Lester and Tsega 1976). On the other hand, Molineaux et al. (1966) analysed medical admissions to the Gondar hospital and showed an incidence of about 3.7%, the main causes being hookworm infection, nutritional iron deficiency, pernicious anaemia, and anaemias secondary to chronic infections.

Shamebo (1987) reported anaemia, excluding anaemia due to chronic diseases, as one of the common haematological disorders in hospitalized patients accounting for 21.1% of these disorders. Iron deficiency anaemia, megaloblastic

anaemia mostly due to folic acid and also vitamin B<sub>12</sub> deficiencies and haemolytic anaemia, due to *Plasmodium falciparum* (in most of the cases) and autoimmunity, were the common types of anaemia reported (Shamebo 1987). Included in the list were also aplastic anaemia and leukoerythroblastic anaemia, but no cases of haemoglobinopathies (Sickle cell anaemia or thalassemia) as in the previous reports in Ethiopia (Lester and Tsega 1976, Abdulkadir 1977) were observed.

Community based studies, however, indicated a relatively high prevalence of anaemia (40.5%) from northern Ethiopia (Zein and Assefa 1987). Similarly, a prevalence rate of 47.2% in children was reported from this same locality by Zein Ahmed (1991). Moreover, nutritional anaemia was also indicated as a public health problem. Even higher rates of 57% have been reported in high risk groups such as pregnant women in rural Ethiopia (Desalegn 1993, Wakbulcho et al. 1993). Whereas previous reports by Gebermedhin et al. (1976) (Cited in Peters 1984) indicated the rarity of anaemia in pregnancy which was latter challenged by Peters (1984) who reported an anaemia rate of 23% in pregnant women.

In general the conclusions given about the situation of anaemia in Ethiopia seem contradictory. Ethiopia being in the tropics, where the prevalence of anaemia is generally believed to be high (Woodruff 1982), it could be assumed that

anaemia would be fairly common. This would be so when it is considered that *teff*, which is accounted for the rarity of anaemia by most authors, is not a staple diet in many parts of the country. Furthermore, studies have revealed that iron absorption from plant diets is generally less than from animal diets (Layrisse *et al* 1969, WHO 1989) and as in many developing countries (WHO 1989) animal diet intake is lower, even negligible among many rural communities (Bekele *et al.* 1993). In the absence of prevalence data representing the whole country the observed different conclusions are not unexpected. This is because of the differences in social, economical and cultural conditions of the population in addition to the high prevalence of parasitic infestations.

Anaemia is known to be multifactorial. Particularly in tropical countries, blood loss caused by helminthic infections such as hookworms and schistosomes and malaria infection are the common factors (Woodruff 1982). Epidemiological data regarding the common aetiological factors of anaemia in Ethiopia, are scanty. Woldegebriel and colleagues recently concluded that unlike in many developing countries, nutritional anaemia is not a problem of major concern to the country. In their study conducted in central Ethiopia they reported about 18.6% prevalence of clinical anaemia in children which, according to these authors, is due to the effect of infestation with intestinal parasites and malaria (Woldegebriel *et al.* 1993).

Intestinal parasitism and malaria are not uncommon in the country (Nega 1993, Kloos and Tesfayohannes 1993). Among the intestinal parasites, hookworms are implicated as the most important determinant of anaemia in areas where diets are generally inadequate of iron (WHO 1968, Woodruff 1982).

## 1.2 Hookworms

Hookworms, "the important but much neglected, intestinal nematodes of man" (Sturrock 1992), are one of the most prevalent soil-transmitted helminths of man affecting about one billion people worldwide (Gilles 1985). They are highly prevalent in the less developed tropical and subtropical parts of the world, owing to the favourable climate and soil conditions and also poor hygienic conditions (Gilles 1985, Pawlowski et al. 1991).

Hookworm infection is among the ten most common parasitic infections in the world, ranked next to amoebiasis and ascariasis (Bunnag et al. 1987).

Two species of hookworm, *Ancylostoma duodenale* and *Necator americanus*, which flourish where poverty, malnutrition, poor health awareness and inadequate sanitation prevail, infect humans (Pawlowski et al. 1991). Humans transmit hookworms by faecal contamination of the soil. The

eggs which pass in stools, undergo a progressive development to the infective larval stage under favourable conditions, that is, in warm and moist soils. Infection is acquired mainly by skin contact with contaminated soil though other routes such as oral (Pawlowski et al. 1991) and transmammary (Miller 1981) has been suggested for infections with *A. duodenale*. Meat borne ancylostomiasis has been shown to occur in animals and possibly it occurs in humans, although this has yet to be proved (Gilles 1985). The infective larvae after penetrating the skin migrate through the blood stream to the lungs. From the lungs they are passively carried upwards to the trachea, larynx and into the esophagus and then to the small intestine, where they mature. The adult worms live in the small intestine, mainly in the jejunum, attaching themselves to villi. Further details of the life history are described elsewhere (Hogland and Schad 1978, Gilles 1985, Pawlowski et al. 1991).

Even though a dermatitis known as ground itch or coolie itch at the site of larval penetration and mild pulmonary symptoms, due to larval migration through the lungs may occur, the main feature of established infection is the development of anaemia and also hypoalbuminaemia (Gilles 1985, Pawlowski et al. 1991).

Chronic blood loss into the small intestine and the ingestion of blood by the parasites are the major factors in

the aetiology of iron deficiency anaemia world wide (Foy and Kondi 1960, Roche and Layrissé 1966). The loss from the attachment site is now known to be facilitated by the secretion of a proteolytic anticoagulant in the case of *A. duodenale* (Hotez and Cerami 1983). The estimated daily blood loss per worm is 0.14 - 0.26 ml (mean 0.20 ml) for *A. duodenale* and 0.02 - 0.07 ml (mean 0.04 ml) for *N. americanus*. The large amount of blood loss caused by *A. duodenale* as opposed to *N. americanus* is attributed to the large size, toothed mouth part, production of powerful anticoagulant and more migratory habit which leaves more bleeding sites, of the former species (Foy and Kondi 1961).

### 1.3 Hookworm infection and anaemia

The first demonstration, on a very large scale, that hookworms can cause severe and widespread anaemia was in 1880 (quoted in Pawlowski et al. 1991). During this time an epidemic of anaemia occurred amongst labourers digging the Saint Gotthard tunnel in Switzerland. Furthermore, possible explanation for the so called "miners anaemia" which was prevalent in many European countries at that time was given. Mining and tunnel construction are considered as occupational activities which favoured transmission of the parasites due to the regular contact with water and moist soil (WHO 1963, 1987, Pawlowski et al. 1991).

Since its first demonstration, in the early 1900's, a series of studies in different parts of the world have been carried out on the association of hookworm infection and anaemia. It has been universally reported that the type of anaemia associated with hookworm infection is of iron deficiency type with a typical hypochromic and microcytic blood picture (Lehman 1949, Foy and Kondi 1960, Layrisse et al. 1961, Roche and Layrisse 1966, Gilles 1985, Pawlowski et al. 1991).

There are also a few reports, though not well established, suggesting the associations of heavy hookworm infections with deficiencies of vitamin B<sub>12</sub> and folic acid (Borrero et al. 1961, Saraya et al 1972); megaloblastic changes in the bone marrow of hookworm infected individuals have also been observed by Roche and Layrisse (1966). In addition, impairment of folic acid absorption has been detected in heavily infected Venezuelan subjects (Layrisse et al. 1964) suggesting the role that may be played by hookworms in the aetiology of megaloblastic anaemia. In a previous investigation carried out by Lehman (1949) on 44 anaemic patients, it was indicated that hookworm anaemia can exist without iron deficiency and recovery from macrocytic anaemia was possible after removal of a certain load of worms. Other than these few possible suggestions most of the studies are in favour of the iron deficiency type of anaemia due to blood and iron losses.

Roche and Layrisse (1966) based on their own extensive work in Venezuela and on a review of published evidences concluded that, the type of anaemia due to hookworm infection is iron deficiency type. They also concluded that for many parts of the world there is a significant association between haemoglobin level and egg counts. However, the threshold levels of the ova load at which the association between haemoglobin level and egg counts are demonstrated vary. For example 2000 eggs per gram (epg) of faeces for females and children and 5000 for males in Venezuela (Layrisse and Roche 1964) and 5000 epg in rural Nigeria (Udonsi 1984). Even higher threshold levels of above 5000 epg were observed in Fiji, Sierra Leone and in Nigeria in studies quoted by Gilles (1975).

Studies have shown that hookworms were contributory to most of the iron deficiencies seen in anaemic patients from Mulago hospital, Uganda (Lehman 1949), coast province hospital in Mombasa, Kenya (Foy and Kondi 1960), Mauritius (Stott 1961), Dar-Es-Salam (Rowland 1966), Gambia (Topley 1968), Nigeria (Werblinska and Fleming 1979) and Zambia (Fleming 1989).

Lehman (1949) investigated 44 severely anaemic patients in Uganda of which 32 (72.7%) were found to have iron deficiency anaemia due to hookworms. A similar finding was reported by Werblinska and Fleming (1979) from Nigeria where

iron deficiency was primarily the result of hookworm infection in 70.8% of their anaemic patients. Whereas Fleming (1989) found only one-third of the iron deficiency in the iron deficient severely anaemic pregnant Zambians attributed to hookworm infection.

Foy and Kondi (1960), employing radioisotope studies on 15 anaemic adult hospital patients in Kenya, showed the type of anaemia to be iron deficiency. They also demonstrated that species type and worm burden to be of paramount importance in governing the magnitude of blood loss. In fact, others revealed the importance of hookworms even in light infections when the dietary iron content is low (Stott 1961). In his study involving 284 anaemic patients of which 88% had hookworm infection, Stott (1961) found that comparatively light hookworm load to be of importance since the diet was found to be low in iron. Unlike Stott (1961) who observed no significant correlation between intensity and degree of anaemia, Topley (1968) demonstrated significant association ( $r = 0.66$ ) between low haemoglobin and hookworm infection with egg count above 1000 epg in rural Gambia. On the other hand, evidence for such associations came from studies involving patients with heavy hookworm infection with an egg count above 5000 epg in Venezuela (Layrisse et al. 1961, 1964) and above 11,000 in Colombia (Borrero et al. 1961).

In addition to studies on patients, mentioned so far,

community based studies revealed a significant relationship between hookworm load and anaemia (Layrisse and Roche 1964, Latham *et al.* 1983, Udonsi 1984, Stephenson *et al.* 1985, Srinivasan *et al.* 1987, Foo 1990).

Layrisse and Roche (1964) studied 1142 rural Venezuelans and found significant relationship between circulating haemoglobin levels and hookworm load represented by above 2000 epg for women and children and above 5000 epg for adult men. Similar findings were reported by Udonsi (1984) from Nigeria, Foo (1990) from Malaysia, the former involving large number of individuals from all age groups and the latter primarily school children. The findings of Foo (1990) also suggests that hookworm may be an important determinant of chronic protein-energy malnutrition as well. This is due to the fact that, in this study in addition to having a significantly lower haemoglobin, children with hookworm infection were significantly shorter in height and lighter in weight than children without the infection.

Not only does heavy hookworm infection is related to anaemia, but a significant association between light hookworm infections and anaemia has been reported from one of the districts of Kenya (Latham *et al.* 1983) and India (Srinivasan *et al.* 1987). The respective egg intensities harboured by the majority of the study subjects of Latham *et al.* (1983) and Srinivasan *et al.* (1987) were less than 500 and 1000 epg.

In contrast to the above reports, for various reasons a number of authors have reported absence of a statistically significant association between hookworm infection and anaemia from several countries : from South Africa (Mayet and Powell 1966, Mayet et al 1985), Georgia (Martin 1972), Kenya (Latham et al. 1983), South Benin (Herceberg et al. 1986), Guinea Bissaw (Caristensen et al. 1987), North Peru (Johns and Lewis 1989), Thailand (Egger et al. 1990, Sanchaisuriya et al. 1993), Papua New Guinea (Pritchard et al. 1991) and Panama (Robertson et al. 1992).

The justification given by most of these workers for the lack of correlation was the low intensity of infection (Latham et al. 1983, Herceberg et al. 1986, Pritchard et al. 1991, Robertson et al. 1992, Sanchaisuriya et al. 1993). Nonetheless, even at lower intensities the effect of hookworm was observed to be aggravated and to cause anaemia by other factors such as low iron status of the study population (Stott 1961) and the co-existence of other parasites such as *Trichuris trichiura* (Robertson et al. 1992) which could interfere with the reabsorption of the iron in the lower intestine. Robertson et al. (1992) reported that children with concomitant *T. trichiura* and hookworm infections were significantly more likely to have blood hemoglobin levels indicative of anaemia than children who are uninfected or had single infections with either of these helminthes except for heavier *T. trichiura* infections (>5000 epg).

In the case of the study in Georgia (Martin 1972) adequate diet was the contributory factor for the lack of statistically significant association between hookworm infection and anaemia. Whereas in the South African studies nutritional factors are found to be of importance than hookworm infection (Mayet and Powell 1966, Mayet et al 1985). The other authors (Caristensen et al. 1987, Johns and Lewis 1989, Egger et al. 1990) did not have information with regard to intensity of infection, i.e., no egg count was provided, which they indicated as a limitation of their study.

Pritchard et al. (1991), employing both egg counts and counts of expelled worms as a measure of intensity of infection, found no consistent correlation between haemoglobin level or haematocrit and measures of hookworm intensity (i.e, neither egg count nor worm burden). They also found no significant relationship between plasma ferritin and egg count. However, they demonstrated a significant negative correlation between plasma ferritin and hookworm burden. This result suggests that estimation of hookworm burden in association of ferritin levels reflects more accurately the degree of progressive iron deficiency in the infected populations . It also suggests the importance of an accurate measurement of hookworm burden, instead of egg counts, in assessing the role of hookworms in the aetiology of anaemia.

Regarding the effect of hookworms on the other major haemopoietic factors other than iron , there are a few documentations. For example, Herceberg *et al.* (1986) reported no significant relationship between egg count and folacin status, while Mayet and colleagues (1985) reported the absence of any relationship with vitamin B<sub>12</sub> levels though the effect of heavy hookworm infection on these two nutrients have been indicated earlier (Borrero *et al.* 1961, Layrisse *et al.* 1964). Here also the same reason which was given for the absence of association between iron status parameters and egg count held true. That is, Herceberg *et al.* (1986) attributed the absence of association to the low intensity of infection. Whereas other confounding factors for anaemia were of importance than hookworm infection to affect the vitamin B<sub>12</sub> levels in the case of Mayet and colleagues' (1985) study.

Generally, not all infected individuals suffer from the life-threatening anaemia that reflects heavy burdens of hookworms. Development of hookworm anaemia depends on the iron content of the human diet, the state of the iron reserves, other iron losses, the type of species and the intensity and duration of infection (Gilles 1985, Pawlowski *et al.* 1991). In other words it depends on a balance between iron utilized by the body for haemoglobin production and that which is lost via the hookworm, other channels of loss probably being negligible (Roche and Layrisse 1966).

With regards to the situation in Ethiopia, both *A. duodenale* and *N. americanus* are known to exist; but the latter is significantly more common (Armstrong and Chane 1975, Jemaneh and Tedla 1984, Tedla and Jemaneh 1985) though in some localities *A. duodenale* is reported as the dominant species (Wondimagegnehu et al. 1992). Hookworm infections are widespread particularly in the humid western and southwestern low lands, where coffee and tea plantations are largely practiced. And higher infection rates between 60% and 80% were reported from Kefa, Welega and Illubabor (Kloos and Tesfa-Yohannes 1993) and even over 80% in Western Shoa (Wolisso) (Integrated family planning, health education and parasite control project (IP) base-line report 1990, Taticheff et al. 1992).

Hookworm infection, which is regraded as an occupational disease of the farming community in many tropical countries (Gilles 1985), is also reported as a serious occupational health hazard in the Awash Valley agricultural development schemes (Kloos et al. 1980, Wondimagegnehu et al. 1992).

The main pathologic feature of hookworm infection is known to be anaemia (Gilles 1985, Pawlowski et al. 1991). And anaemia reduces the ability to perform energy demanding tasks because of the reduced amount of oxygen delivered to tissues (Viteri and Torun 1974). Despite the high prevalence of hookworm in the irrigation development schemes (eg. the

cotton Scheme, Wonji and Metehara sugar plantations, the Awara Melka cotton / fruit farm and Melka Sedi banana plantations) or the farming community in general, and the debilitating effect of its important sequel anaemia, the association between the two is not well assessed and documented.

The available information in Ethiopia is obtained either from hospital patients (Molineaux *et al.* 1966, Seboxa *et al.* 1986, Kahssai and Woldesemayat 1991), which are generally regarded as unsatisfactory representatives of a population (WHO 1972, Hennekens and Buring 1987), or from a community-based study with its own limitations indicated by the authors (Bulto *et al.* 1992).

Molineaux and colleagues (1966) by analysing medical admissions to the Gondar hospital between the years 1963-1965 (3508 patients) reported hookworm anaemia to be the major cause of the anaemias encountered. Another study conducted on one-hundred consecutive out patients in the same administrative region revealed a significant negative correlation between haemoglobin level and egg load ( $r = -0.47$ ) (Seboxa *et al.* 1986). In this study, all the patients were found to have hookworm infection with mean egg loads of 11,392 (range 150 - 91,100). These workers concluded the type of anaemia to be iron deficiency from the clinical pictures, the peripheral blood morphologies and bone marrow

examinations. Similar conclusion was given by others from southwestern Ethiopia where patients with severe hookworm were found to respond rapidly to iron therapy suggesting the type of anaemia to be of iron deficiency type (Kahssai and Woldesemayat 1991). In view of the scarcity of other community based studies regarding the association between hookworm infection and anaemia, these hospital based studies can shade some light on the existence of the problem in the country.

A better picture regarding the relation of hookworm and anaemia in a community was provided by Bulto and Co-workers (1992) in a study undertaken in Gambella. Even though these workers didn't take care of the effect of other confounding factors which can result in anaemia, and also didn't provide data on egg intensity, they showed a significant association (odds ratio 2.65) between low haematocrit and hookworm infection in the resettled population.

In addition to the short comings stated above, the study of Bulto and colleagues (1992) lack information on the nature of the anaemia since the only parameter used was the haematocrit. This parameter indicates only the presence or absence of anaemia (Evatt et al. 1992) but not other conditions such as the type of anaemia and the progressive stages of anaemia. Utilization of only simple measurements such as haemoglobin and haematocrit could oversimplify the

of  
hy  
so  
a.  
a,  
  
ds  
)  
th  
5  
of  
ls  
ls  
nt  
ho  
st  
al  
nd  
re  
ng  
a  
re

omitted on account of taking anthelmintic treatment which was distributed by IP.

The subjects were interviewed using a simple questionnaire. Hookworm infected individuals were treated with Mebendazole (Vermox<sup>®</sup>) with the standard dose of 100 mg twice a day for 3 days as recommended (Gilles 1985). Moreover, those with a haemoglobin value below the cut-off levels (WHO 1972) were also given ferrous gluconate tablets through the health center.

The project protocol was evaluated and accepted by the Ethical committee of the National Research Institute of Health (NRIH).

## 2.2 Sample collection

Faecal samples were collected in a pre-coded screw capped plastic containers and processed at the field laboratory on the same day of collection for examination by the Kato thick smear method (Martin and Beaver 1968).

Approximately a total of 15 cc of venous blood was collected from each subject into two tubes using the vacutainer system (Venoject, Terumo, Belgium). About 5 cc of the blood was collected into one of the tubes containing

limitation is that, the delicate, thin-shelled hookworm eggs usually disappear soon after the thick smear has become clear enough for examination. Eggs may have become invisible after 60 minutes (Pawlowski et al 1991). However, in the present study all possible measures were taken to read the preparations in time. Each stool from each cup was transferred to a piece of disposable paper and filtered through a disposable sieve using applicators. Each sample was then applied on a card plate with a hole, placed on a slide, that can hold about 43 mg stool. After removing the card plate, the faecal sample was covered with a cellophane strip which has been soaked in glycerine-malachite green solution at least 24 hrs. The preparation was then pressed, to spread the smear, using another slide until evenly distributed and allowed to clear for about 30 min before reading. A single slide was prepared for each subject. The number of hookworm eggs per slide were counted and multiplied by 23 to obtain the number of eggs per gram (epg) of faeces.

Malarial infection was determined from thick and thin smears stained with Giemsa stain following standard procedures (WHO 1980). Based on this, one individual who was found to be positive for *Plasmodium falciparum* species was excluded during the selection of cases and controls.

## 2.4 Haematological analysis

Haematological assays were performed using Coulter counter model T-540, which automatically dilute, lyse and count the white blood cells (WBC), the red blood cells (RBC), the platelets (Plt), and measures the haemoglobin (Hb) content and the haematocrit (Hct) (Instruction manual for Coulter counter 1989). The red cell indices, i.e, the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of RBC, Hb and Hct using the following standard formulae (Seiverd 1983).

$$\text{MCV} = \frac{\text{Hct reading}}{\text{RBC in million / cumm}} \times 10$$

$$\text{MCH} = \frac{\text{Hb in gram / 100 cc}}{\text{RBC in million / cumm}} \times 10$$

$$\text{MCHC} = \frac{\text{Hb in gram / 100 cc}}{\text{Hct reading}} \times 100$$

The slides were stained by the May-Grunwald Giemsa stain and inspected for any morphological abnormality; abnormalities were recorded as slight, moderate or marked

according to Seivered (1983). The percentage of the different white cells were determined by counting 200 cells per slide.

## 2.5 Biochemical analysis

### 2.5.1 Ferritin assay

The biochemical analysis was carried out within two months of sample collection. Ferritin concentration was determined by an enzyme linked immuno-sorbent assay, ELISA/2-step sandwich assay, using kits supplied by Boehringer Mannheim GmbH, Germany on ES 300, a fully automated multibatch immunoanalyser. Parallel with the test sera, control plasmas with both high and low ferritin values were run for quality check.

### 2.5.2 Serum iron determination

For serum iron determination various methods were sought and the one which is discussed in Evatt et al. (1992) was adopted for it was possible to get a suitable standard control plasma to check accuracy and reproducibility of the procedure. This procedure employs ferrozine as a coloring reagent and is based on comparing the color that develops

when the iron (ferrous) in serum is treated with a chromogen reagent, with that which develops from a standard iron solution.

Determination of serum iron is based on the principle that at an acid pH and in the presence of a suitable reducing agent, transferrin bound iron ( $\text{Fe}^{3+}$ ) dissociates to form ferrous ions which when reacted with ferrozine form a color complex with a peak absorption near 560 nm. Based on this principle the procedure used was as follows.

The test sera which were kept at  $-20^{\circ}\text{C}$  was first allowed to thaw and mixed and then 0.5 ml was dispensed to each of labelled iron-free tubes [that is, tubes which had been cleaned following standard procedures (Evatt et al 1992)]. To each of these tubes and to two other separate tubes each containing 0.5 ml iron standard solution (product of bioMerieux, France containing 1 mg/l iron) and 0.5 ml distilled water (blank), 0.5 ml of protein precipitant was added. Each tube was mixed vigorously, using a vortex mixer, for at least 1 min and allowed to stand for 5 min. Tubes containing test samples were centrifuged for 10 min at 3000 rpm to obtain a clear supernatant. Then 0.5 ml of the serum supernatant and 0.5 ml of the contents from each of the tubes containing the standard and the blank was dispensed into separate tubes and to each of these 0.5 ml of the chromogen reagent was added. Each tube was then mixed well and stood

for 10 min. Finally, the absorbance was read at 550 nm against the blank, using Coulter Kem-O-mat, after transferring the contents of each test tube into new cuvettes, and the total serum iron was calculated using a standard formula (see below). With each batch of test a control plasma was run to check the quality.

$$\text{Serum iron (mg/l)} = \frac{\text{A Sample}}{\text{A Standard}} \times n$$

A = Absorbance

n = Concentration of iron standard

### 2.5.3 Total iron binding capacity (TIBC)

The principle behind the measurement of the TIBC is first saturating the transferrin by an iron solution and then adsorbing the excess iron and finally determining the total iron in the supernatant using one of the serum iron determination procedures (Piccardi et al 1972).

The first step, that is saturation of transferrin was done using a kit (TIBC additif product of bioMerieux, France), which contains saturating iron solution and an adsorbent, magnesium hydroxycarbonate. Then for the determination of the TIBC, the method for the determination

of iron discussed by Evatt et al. (1992) was followed.

To each of an iron-free labelled tubes, 0.5 ml of test serum was dispensed. To all tubes 1 ml of saturating iron solution, containing 5 mg/l iron, was added, mixed and stood for 5 min. Then 1 level spoonful magnesium hydroxycarbonate was added to each tube to adsorb unbound iron, allowing to stand for 20 min, shaking intermittently and then centrifuged at about 3000 rpm for 10 min. The supernatant obtained was treated as serum by following the method discussed above for the determination of serum iron and finally the TIBC was calculated using the following standard formula.

$$\text{TIBC (mg/l)} = \frac{\text{A TIBC Sample}}{\text{A Standard}} \times n$$

A = Absorbance

n = concentration of iron standard used in iron determination x serum dilution factor

#### 2.5.4 Transferrin saturation (TS)

The transferrin saturation was calculated for each study subject from the values of the total serum iron and the TIBC. This value was obtained by dividing the serum iron

concentration by the TIBC, and multiplying by 100 to express the result as a percentage (WHO 1989).

## 2.6 Statistics

Data entry and analysis were done using a Dbase III<sup>+</sup> and SPSS Pc<sup>+</sup> programs, respectively. Statistical methods employed include: Chi-squared ( $X^2$ ) test for investigating associations, one way analysis of variance (ANOVA) for testing differences between means; t-test to compare differences between two means and F-test for comparing group means, and correlation analysis for testing relationship between variables. A P-value less than 0.05 was taken as statistically significant. As the distribution of ova count was very skewed, the mean egg count was calculated after logarithmic transformation, however, the results are presented in the original units.

### 3. RESULTS

#### 3.1 Characteristics of the study population

The study population, aged 15 to 75 years, was composed of three ethnic groups. 94.4% were Oromo, 4.8% Amhara, 0.4% Gurage and 0.4% mixed (Amhara and Gurage); and all were christians. The age and sex distribution of the study population is depicted in Table 1. The ratio of male to female was 1.18 : 1. Farmers constituted 52.4% of the subjects, housewives 21.1%, students 17.7% and miscellaneous occupations 8.8%.

From the dietary information, the major staple diet was Kocho (60.0%) followed by maize (29.1%), teff (8.4%) and a mixture of Kocho, maize, teff and sorghum (2.5%). Meat was almost absent in the diets of 86.8% of the subjects. Whereas milk and milk products consumption was found to be better in that about 85% of the subjects got these products. Legumes particularly vetch were the major constituents of the diets though cabbage and tomato were reported to be eaten seasonally. Most of the subjects (87.2%) confirmed the lack of fruits in their diets.

Table 1. Distribution of the study population by age and sex.

Age (Years)	Cases <sup>a</sup>			Controls		
	Male	Female	Total	Male	Female	Total
15-29	42	26	68	19	23	42
30-44	14	19	33	8	6	14
45-59	17	15	32	8	6	14
60 <sup>+</sup>	14	8	22	1	1	2
All subjects	87	68	155	36	36	72

<sup>a</sup> cases are individuals who are positive for hookworm ova.

negatively with the total iron binding capacity. Of all the parameters, a very strong correlation was found between haemoglobin and haematocrit ( $r= 0.977$ ,  $P<0.001$ ); hence haemoglobin values were taken to classify individuals as anaemic and non-anaemic. Correlation between the percentage saturation of transferrin and the serum iron levels was also strong ( $r= 0.840$ ,  $P<0.001$ ).

Table 2. Mean and standard deviation values for haematological measurements of cases and controls.

Parameters <sup>a</sup>	cases		Controls		p <sup>b</sup>
	(n=155)		(n=72)		
RBC	4.62	(0.09)	4.85	(0.42)	< 0.01
Hb	14.33	(1.41)	14.87	(1.09)	< 0.01
Hct	42.99	(4.27)	44.60	(3.37)	< 0.01
MCV	93.08	(4.47)	103.75	(99.17)	NS
MCH	31.06	(1.77)	30.28	(3.81)	< 0.05
MCHC	33.36	(0.87)	33.35	(0.67)	NS
Plt	313.42	(95.81)	297.15	(85.38)	NS
WBC	7.83	(2.91)	7.65	(2.45)	NS

<sup>a</sup> The units for haematological parameters are as follows: RBC =  $\times 10^{12}/L$ , Hb in g/dl, Hct in %, MCV = femto litre (fl), MCH = pico gram (pg), MCHC = g/dl, WBC =  $\times 10^9/L$ , Plt =  $\times 10^9/L$ . Figures in parentheses are standard deviations.

<sup>b</sup> t-test for significant difference between means. NS for non-significant difference.

Table 3. Mean and standard deviation values for the biochemical assays of cases and controls.

Parameters	Cases (n = 155)	Controls (n = 72)	P <sup>a</sup>
Serum iron (mg/L)	0.84 (0.32)	1.05 (0.34)	< 0.001
TIBC (mg/L)	3.58 (0.79)	3.21 (0.56)	< 0.001
TS (%)	24.45 (10.25)	33.39 (10.81)	< 0.001
Ferritin (ng/ml)	42.38 (24.98)	66.13 (45.78)	< 0.001

<sup>a</sup> t-test for significant difference between means.

Table 4. Relationship between anaemia parameters (haemoglobin, haematocrit, ferritin, iron, TIBC and percentage transferrin saturation).

	Hb(g/dl) <sup>a</sup>	Hct(%) <sup>a</sup>	Ferritin (ng/ml) <sup>a</sup>	Iron (mg/L) <sup>a</sup>	TIBC (mg/L) <sup>a</sup>
Hb (g/dl)	-				
Hct (%)	0.977***	-			
Ferritin (ng/ml)	0.259***	0.251***	-		
Iron (mg/L)	0.388***	0.394***	0.335***	-	
TIBC (mg/L)	0.030	0.021	-0.168*	0.002	-
TS (%)	0.408***	0.420***	0.355***	0.840***	-0.421***

<sup>a</sup> Correlation coefficients, n = 227. Statistical significance indicated thus: \* = P < 0.05, \*\*\* = P < 0.001.

The total prevalence of anaemia, as judged by the altitude adjusted WHO (1972) cut-off levels, regardless of hookworm positivity was 15.9%. Individuals at this altitude (about 2100 m) would be judged to be anaemic if their haemoglobin concentrations were less than 12.8 for females and less than 13.9 for males. These values are regarded as the altitude-adjusted "equivalent" cut-offs for haemoglobin (WHO recommendation + 7%) to characterize anaemia prevalence in the Ethiopian highlands between 2000-2500m (Peters 1984). The other measurements were considered to be abnormal at the following levels: Transferrin saturation below 16% (WHO 1989) and serum ferritin below 20 ng/ml (Rifkind et al. 1986). For the red cell indices the normal ranges given by Harrison's (1991) was taken. Thus the normal values for  $MCV = 90 \pm 7$  fl,  $MCH = 29 \pm 2$  pg and  $MCHC = 34 \pm 2$  g/dl.

When judged by haemoglobin values 20.6% of hookworm positive individuals and 5.6% of the controls were found to be anaemic (Table 5). The results presented in Table 5 also show that there was a significant association between hookworm infection and the three parameters of progressive stages of anaemia.

Table 5. Association of anaemia parameters with hookworm infection

Parameters	Cases		Controls		odds ratio	X <sup>2</sup>	P <sup>a</sup> <
	n	%	n	%			
Ferritin							
normal (NR)	128	82.6	71	98.6	14.98	10.25	0.01
below NR	27	17.4	1	1.4			
TS							
normal	125	80.5	71	98.6	17.18	12.08	0.001
below NR	30	19.5	1	1.4			
Hb							
normal	123	79.4	68	94.4	4.42	7.30	0.05
below NR	32	20.6	4	5.6			

<sup>a</sup> Chi-squared (X<sup>2</sup>) analysis for testing significant associations between anaemia parameters and hookworm infection.

Intensity of infection as expressed in epg showed a highly significant relationship with transferrin saturation and serum ferritin values ( $P < 0.001$ ). There was no significant association between intensity of infection and haemoglobin levels at the 5% level (Table 6).

Table 6. Anaemia parameters and their relationship to hookworm ova load.

egg count (epg)	n <sup>o</sup>	Haemoglobin (g/dl) <sup>a</sup>	TS (%) <sup>a</sup>	Ferritin (ng/ml) <sup>a</sup>
0	72	14.66 ± 2.05	32.93 ± 11.43	65.22 ± 46.12
1-100	67	14.44 ± 1.18	26.13 ± 9.32	51.62 ± 27.52
101-500	59	14.41 ± 1.69	24.59 ± 11.13	39.17 ± 20.86
501-1000	14	13.91 ± 1.24	19.50 ± 10.62	28.02 ± 21.38
> 1000	15	13.93 ± 1.28	20.93 ± 8.78	27.13 ± 12.62
		P <sup>b</sup> > 0.05	P <sup>b</sup> < 0.001	P <sup>b</sup> < 0.001

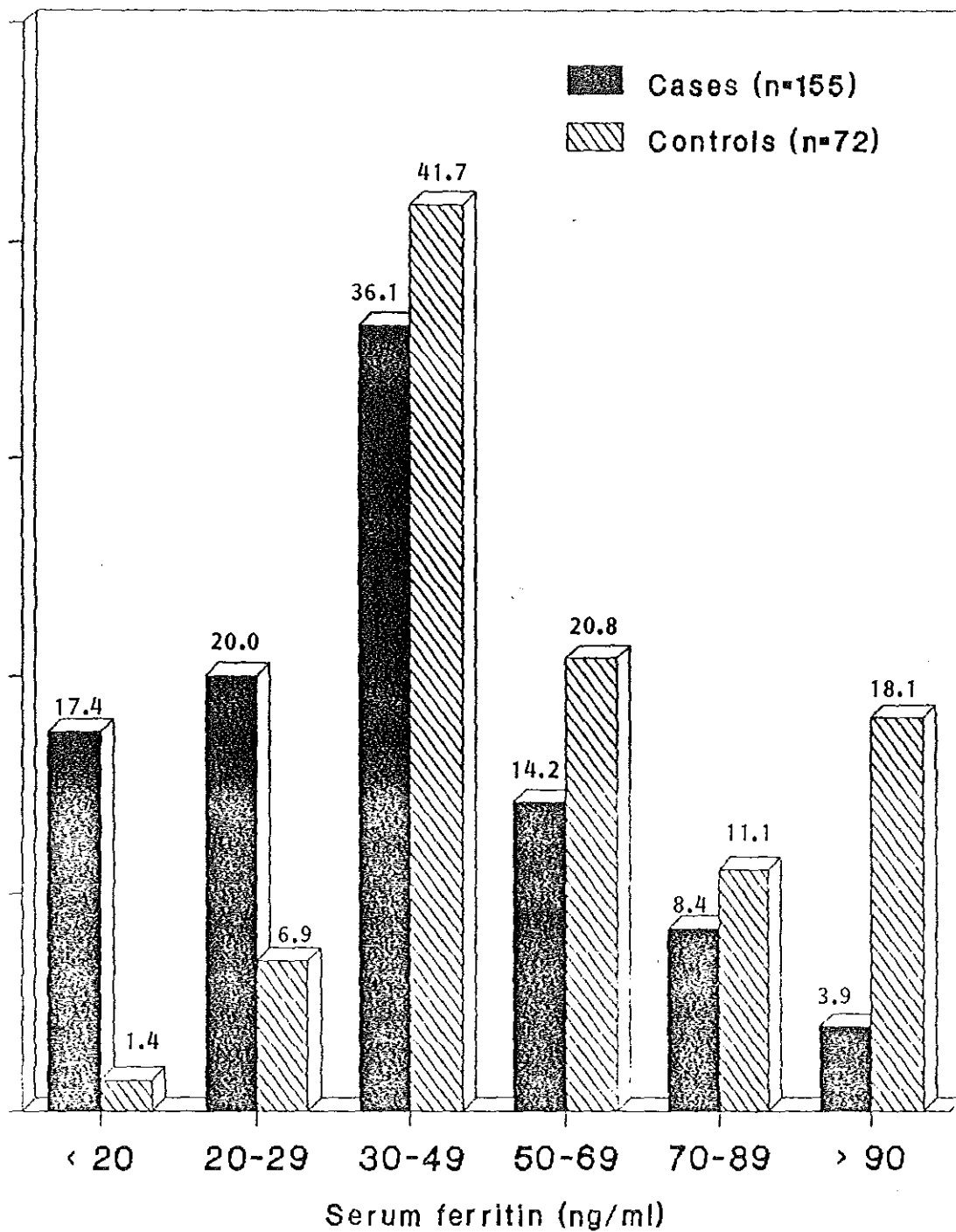
<sup>a</sup> Mean ± standard deviation.

<sup>b</sup> One way analysis of variance testing a null hypothesis of no significance difference between group means.

<sup>o</sup> Number of subjects.

Assessment of iron status as determined by the serum ferritin levels showed relatively higher proportion of hookworm infected individuals had precarious iron stores than the non infected ones. On the other hand, only a few hookworm positive individuals had higher ferritin values. Figure 1 shows the distribution of serum ferritin levels in the study population. As it is shown in the figure, as the levels of serum ferritin increases the proportion of cases falling to each category decreases while that of the controls increases.

## Percentage of subjects



distribution of serum ferritin levels among the study subjects. The numbers on each of the histogram show percentage of subjects for the given range of serum ferritin.

Examination of the peripheral blood morphology showed a normochromic normocytic (NCNC) blood picture in approximately 65% of the cases and 90% of the controls. The respective percentage of abnormalities encountered in the cases and the controls include 12.2% and 1.4% anisocytosis, 5.2% and 1.4% hypochromia with microcytosis, and 17.4% and 6.9% macrocytosis. Poikilocytosis in 0.6%, polychromatic cells in 1.9%, hypersegmented neutrophils in 0.6%, and 2% nucleated red blood cells (NRBC) in 0.6% of hookworm positive individuals were observed. But none of these abnormalities were found in the controls. Summary of blood morphology results is given in Table 7. When morphological abnormalities were graded as slight, moderate and marked, all were found to be slight to moderate except very few marked abnormalities. Eosinophilia was observed in 80.6% of the cases with a mean count of  $12.32 \pm 9.08\%$ , which differ significantly ( $P < 0.001$ ) from that of the controls. The mean eosinophil count of the controls was  $7.67 \pm 7.21\%$ .

Table 7. Summary of peripheral blood morphology results of the study population.

	Cases (n=155)		Controls (n=72)	
	n	%	n	%
NCNC <sup>a</sup>	101	65.2	65	90.3
Anisocytosis	19	12.2	1	1.4
Hypochromic- microcytosis	8	5.2	1	1.4
Macrocytosis	27	17.4	5	6.9
Poikilocytosis	1	0.6	0	0
Polychromatophilia	3	1.9	0	0
NRBC <sup>b</sup> (2%)	1	0.6	0	0
Hypersegmented neutrophils (few)	1	0.6	0	0

<sup>a</sup> NCNC = normochromic normocytic.

<sup>b</sup> NRBC = nucleated red blood cell.

Results of the red cell indices based on Harrison's (1991) normal ranges, indicated a high MCV in 15.5% of the cases and 5.6% of the controls, high MCH in 17.4% of the cases and 8.3% of the controls. Low MCV and MCH values were observed in 1.9% and 2.6% of hookworm infected individuals respectively. But none of the controls had low MCV and MCH values. 1.9% of cases and 1.4% of the controls were found to have low MCHC (Table 8).

Multiple criteria involving elevated MCV, MCH and macrocytic blood picture were taken as suggestive of a macrocytic type of anaemia. When these criteria for classifying the anaemia as macrocytic were taken, 13.0% of the cases and 5.6% of the controls fell into this category. Further chi-squared analysis showed no statistically significant association between parameters suggestive of macrocytic anaemia and hookworm infection (odds ratio= 2.54,  $P > 0.05$ ) (Table 9).

Table 8. Red cell indices results of the study population.

Parameters <sup>a</sup>	Cases (n=155)		Controls (n=72)	
	n	%	n	%
MCV (fl)				
normal (NR)	128	82.6	68	94.4
above NR	24	15.5	4	5.6
below NR	3	1.9	0	0
MCH (pg)				
normal	124	80.0	66	91.7
above NR	27	17.4	6	8.3
below NR	4	2.6	0	0
MCHC (g/dl)				
normal	152	98.1	71	98.6
above NR	0	0	0	0
below NR	3	1.9	1	1.4

<sup>a</sup> Red cell indices parameters are considered as normal, above normal and below normal based on the normal range given by Harrison's (1991). Thus, the normal values for MCV =  $90 \pm 7$  fl, MCH =  $29 \pm 2$  pg and MCHC =  $34 \pm 2$  g/dl.

Table 9. Association of macrocytic anaemia<sup>a</sup> and hookworm infection.

Macrocytic anaemia <sup>a</sup>	Cases (n=155)		Controls (n=72)		odds ratio	X <sup>2</sup>	P <sup>b</sup>
	n	%	n	%			
Yes	20	13.0	4	5.6	2.54	2.13	>0.05
No	135	87.0	68	94.4			

<sup>a</sup> Multiple criteria involving elevated MCV, MCH and macrocytic blood picture are taken as suggestive of macrocytic anaemia.

<sup>b</sup> Chi-squared analysis for testing significant association between macrocytic anaemia and hookworm infection.

With regards to diet, no significant difference at the 5% level was observed in the dietary patterns of anaemic and non-anemic individuals. Ferritin levels were rather associated with animal diet intake ( $P < 0.05$ ); none of the subjects taking animal food had ferritin levels below normal.

#### 4. DISCUSSION

The present work has attempted to examine the relationship between hookworm infection and anaemia among the populations living in rural Wolisso. The results indicate the importance of the role of hookworms in the aetiology of anaemia in this community.

Even if about 90.3% of the study population had egg counts less than 1000 epg, a significant association was found between hookworm infection and anaemia (odds ratio = 4.42,  $P < 0.05$ ). The result indicates that hookworm infected individuals are about four times at risk of being anaemic than uninfected subjects. This result is more or less in agreement with the findings of Bulto and colleagues (1992) who reported about three times risk of development of anaemia in their hookworm positive adult subjects.

As it is shown in Table 5, 20.6% of the cases and 5.6% of the controls were anaemic. The high proportions in the cases emphasizes that hookworm infection possibly accounts for most of the anaemias in this community. Layrisse and Roche (1964) by taking the difference between the prevalence of anaemia in infected and non infected Venezuelans, 46% and 30% respectively, reported the percentage of individuals with anaemia due to hookworm to be 16%. In a similar manner, in the present study the prevalence of anaemia due to hookworm

as a possible factor could be about 15%.

When the degree of anaemia was graded into mild, moderate and severe as described in the document of WHO (1989), all the anaemic individuals had what would be described as mild anaemia (haemoglobin value between 10 and the cut-off value, i.e., 12.8 g/dl for females and 13.9 g/dl for males), whereas neither moderate nor severe anaemia was detected. This could be attributed to the low intensity of infection observed in these populations.

The demonstration of a significant association between hookworm infection and anaemia in these lightly infected subjects agrees with other studies conducted in Mauritius (Stott 1961), in Kenya, on road workers, among whom about 80% had egg counts of less than 500 epg (Latham et al. 1983) and in India (Srinivasan et al. 1987). However, in contrast to Latham and colleagues (1983) and Srinivasan and colleagues (1987), no significant association was observed between hookworm ova load and haemoglobin levels in the present study.

In fact, the proportion of individuals who had egg counts above 2000 epg, which is generally regarded as a threshold load above which anaemia is likely to occur (Roche and Layrisse 1966), was rather small in both Srinivasan et al. (1987) and the present study. That is, 3.2% of the

subjects in the present study and 4% in the Srinivasan *et al.* (1987) study had egg counts above 2000 epg. The possible explanation for this difference in the results of the Indian group (Srinivasan *et al.* 1987) and the present study could be, the present study is a controlled one. In addition, according to the information obtained from the subjects, no remarkable dietary difference was observed between anaemic and non anaemic individuals. Whereas Srinivasan *et al.* (1987) did not rule out the effect of other factors. And they suggested that factors such as nutritional status, pregnancy history and basic physiological causes may have had a greater bearing on the extent of haemoglobin variation accounted for by hookworm ova load.

Nevertheless, the present result is in agreement with those of Stott (1961) who demonstrated a significant association between hookworm infection and anaemia but no association with intensity of infection.

On the other hand, this result is in sharp contrast to other studies, who failed to demonstrate association between hookworm infection and anaemia at lower intensities, including studies in Kenya (Latham *et al.* 1983), in Panama (Robertson *et al.* 1992) and in Thailand (Sanchaisuriya *et al.* 1993). One of the possible reasons for the observed disagreement between these reports and the present result, could be variations in the duration of infection, which could

not be definitely known. Roche and Layrisse (1966) have pointed out that infections which are light at the time of examination may have been much heavier previously. Perhaps, the subjects of the present study could have been heavily infected previously. So, the temporal divorce between the cause of blood loss and the resulting lowering of haemoglobin may have accounted for the observed differences between the present study and those who reported no association between light hookworm infection and anaemia.

On the contrary, others have demonstrated a significant association between intensity of infection and haemoglobin levels when infections were severe. For instance, in the study of Stephenson *et al.* (1985) which was conducted on Kenyan School children, a significant negative correlation between haemoglobin level and hookworm ova load was observed where about 36% of the subjects had what is generally regarded as heavy infection, that is, above 2000 epg. In Udonsi's report from Nigeria such relation was observed where nearly 60% of the study subjects had counts of 10000 epg or more including 29% with counts of at least 15000 (Udonsi 1984). Similarly, Seboxa and colleagues (1986) also reported a significant negative correlation between haemoglobin level and ova load in their heavily infected subjects, with mean ova loads of 11392 epg, from Gondar, north-western Ethiopia.

association between serum ferritin levels and hookworm egg count in their lightly infected subjects, with 1% of the subjects having egg counts above 2000 epg. Eager *et al.* (1990) from Thailand also reported absence of a statistically significant association between ferritin levels and hookworm infection in the Thai children, though no information was provided with regard to the worm load.

The observed significant association between intensity of infection and iron status parameters, that is, transferrin saturation and ferritin levels, ( $P < 0.001$ ) and absence of significant association with haemoglobin levels ( $P > 0.05$ ) (Table 6) emphasize the effect of such low variation in the intensity of infection on the iron status of the subjects. But, the variation in intensity was not as such remarkable to affect the haemoglobin values at the various intensity categories. The present finding is generally in line with the concept that iron deficiency first affects iron stores, then transferrin saturation and lastly haemoglobin production (Verloop 1970, Siimes *et al.* 1980).

In the present study, the iron status of hookworm infected subjects, as determined by the serum ferritin levels, was very low as compared to the control groups, though the anaemia was generally mild. The fact that about 20% of infected subjects had sub normal levels of serum ferritin (20-29 ng/ml) as opposed to the 6.9% control groups

(see Figure 1) suggests that these people are at risk of being iron deficient if exposure to hookworms is prolonged. On the other hand, as it is presented in Figure 1, only 3.9% of the cases and 18.1% of the controls had higher ferritin values (above 90 ng/ml). The finding of apparently large proportion of the control groups with higher ferritin levels as opposed to the cases indicates the impact of hookworms on the iron status of the infected subjects. The effect was found to be less serious in infected individuals who reported meat as part of their diet. There was a significant association between ferritin levels and meat consumption ( $P < 0.05$ ). None of the infected subjects who had meat in their diet were found to have ferritin values below the lower normal limit (20 ng/ml). It is known that animal foods not only contain the more absorbable form of iron, but also enhance iron absorption from vegetable foods (Layrisse *et al.* 1968, Gillooly *et al.* 1983). Therefore, the consumption of meat may have helped these subjects to have better iron stores so as to resist the effect of such light hookworm infections.

In general, the biochemical analysis results show that iron deficiency to be the major cause of anaemia in the infected subjects. As it is clear from the chi-squared analysis result of serum ferritin levels (Table 5), hookworm infected individuals are at great risk of being iron deficient than the negative counterparts (odds ratio = 14.98,

$P < 0.01$ ). The role of hookworms in the aetiology of iron deficiency anaemia with a typical hypochromic microcytic blood picture has been universally reported (Foy and Kondi 1960, Roche and Layrisse 1966, Gilles 1985, Pawlowski et al. 1991). In the present study however, even if 17.4% of the cases and 1.4% of the controls (Table 5) had lower ferritin levels, a slight hypochromic microcytic blood picture was observed in about 5% of the cases and 1.4% of the controls (Table 7). On the other hand, about 2% of the cases and none of the controls had microcytic hypochromic red cells on the basis of the red cell indices values. This observation might not be surprising since the anaemia in these subjects is generally mild. Moreover, even in iron deficiency anaemia the erythrocytes do not become hypochromic or microcytic until several months after iron stores have been depleted (Wintrobe et al. 1981).

The possible explanation for the slight discrepancies observed between the red cell indices and the blood morphology results would be, the red cell indices are single, mean values which cannot express the variation that may occur within a population of cells.

The other finding of the present study is the occurrence of macrocytosis in 17.4% of the cases and 6.9% of the controls (Table 7). Based on the red cell indices values, 15.5% of the cases and 5.6% of the controls had high MCV; and

17.4% of the cases and 8.3 of the controls had high MCH values (Table 8). As it is already stated, 13.0% of the cases and 5.6% of the controls had a macrocytic type of anaemia when judged by multiple criteria (Table 9).

Even though in Ethiopia, no report is available for the existence of the problem in the community at large, Shamebo (1987) reported that about 9.3% of the haematological abnormalities in hospitalized patients are due to megaloblastic anaemia. In this report (Shamebo 1987) 6.7% of the megaloblastic anaemias were due to folic acid deficiency and 2.7% due to vitamin B<sub>12</sub> deficiency.

Previously, Abdulkadir (1977) also suggested that folic acid deficiency, the major cause of megaloblastic anaemia, would be more common than appears to be the case otherwise in Ethiopia. This is so because the consumption of fresh vegetables is very low in many communities; and folates are known to be destroyed by prolonged cooking (Herbert 1970). The subjects of the present study were not an exception to this. The majority of them have reported the absence of fruits in their diet.

The finding for the association between parameters suggestive of macrocytic anaemia and hookworm infection, though not statistically significant (odds ratio 2.54,  $P > 0.05$ ) (Table 9), indicates that hookworm positive individuals are about 2.5 times at risk of developing

macrocytic anaemia than non-infected individuals. As far as the controls are concerned, the observed abnormality can be attributed to nutritional factors. In the case of the infected subjects, hookworm infection might have aggravated the situation.

A much better information about the role of hookworm infection in the genesis of megaloblastic anaemia could have been obtained if analysis of serum folic acid and vitamin B<sub>12</sub> levels had been made. This is a limitation of the present study. Nevertheless, the finding of macrocytic cells in the peripheral blood combined with the red cell indices values could be considered as a possible morphological evidence of either folic acid or vitamin B<sub>12</sub> deficiency, or deficiencies of both (Wintrobe *et al.* 1981, Evatt *et al.* 1992).

From the world wide perspective, there is still some doubt about the association of hookworm infection with folic acid and vitamin B<sub>12</sub> deficiencies. Some investigators have observed megaloblastic changes in the bone marrow of heavily infected subjects in Colombia (Borrero *et al.* 1961), in Venezuela (Roche and Layrisse 1966) and in India (Saraya *et al.* 1972). Layrisse and associates (1964) also observed impairment of folic acid absorption in their heavily infected subjects. On the other hand, Herceberg and Colleagues (1986) failed to demonstrate any relationship between light hookworm infection and serum folic acid levels. In fact these workers

(Herceberg *et al.* 1986), contrary to the finding of the present study, did not find significant relationship between hookworm infection and any of the anaemia parameters including the iron status parameters.

In general, the type of anaemia resulting from hookworm infection has been properly indicated by Gilles in his serial publications, about the features of hookworm infection and anaemia (Gilles 1975, 1985). Gilles stated the occurrence of a super added folic acid megaloblastic anaemia, in some parts of the tropics, which is often masked by the severe iron deficiency anaemia and which only becomes overt after a partial haematologic response to iron therapy. He further pointed out that the classic anaemia of uncomplicated hookworm disease is, however, hypochromic microcytic anaemia.

In the case of the present study, though light infection was observed, the possibility that heavy infection could have been present previously cannot be ruled out. Thus, the mild infection was found to be enough to have a highly significant association with iron status parameters (transferrin saturation and ferritin) but not with parameters suggestive of macrocytic anaemia. However, even if no statistically significant association was demonstrated with the latter at the 5% level, the result gives some clue as to the possibility that hookworm infection might be an aetiologic

agent of megaloblastic anaemia in this particular community  
in question.

## 5. CONCLUSION

The result reported herein shows that hookworm infection in this community leads to the development of anaemia. In spite of the fact that infections are generally light, the anaemia was of mild type and not associated with intensity of infection. However, the application of biochemical analysis for the determination of iron status has enabled to show that hookworm infected individuals had depleted iron stores, and the depletion relates to the intensity of infection. This would possibly lead to iron deficiency anaemia unless and otherwise measures for both deworming and correction of the iron level are taken. Though, control priorities naturally lie with acute and possibly fatal infections, any hookworm control program should target both at the parasite and its important sequel, anaemia.

Information obtained from this study suggests that macrocytic anaemia may also be a problem in the area. With this regard further investigation needs to be carried out to clearly indicate the role of hookworms in the genesis of megaloblastic anaemia by employing better biochemical measurements of folic acid and vitamin B<sub>12</sub> levels. In addition, bone marrow analysis is also helpful to confirm the results obtained from peripheral blood morphology examinations.

Considering the most important consequences of hookworm infection on the well being of individuals and also productivity, by way of causing anaemia, no doubt that the control of hookworms should not be ignored. Information about the prevalence and the extent of hookworm anaemia in such communities is rather of great help for decision making on preventive and control measures. Hence, the present study could serve as a basis in designing preventive and controlling methods.

## 6. REFERENCES

- Abdulkadir J. (1977). Diagnosis and management of anaemia. *Ethiop. Med. J.* 15, 107-116.
- Alemayehu A., Seifu K., Ingram J. and Davis RHD. (1993). The iron content of *teff* [*Eragrostis tef* (Zucc.) Trotter] and its controversy. *Sinet (Ethiop. J. Sci.)*. 16, 5-13.
- Armstrong JC. and Chane T. (1975). Identification of hookworm species in Ethiopia. *Ethiop. Med. J.* 13, 13-18.
- Bekele A., Woldegebriel A. and Kloos H. (1993). Food, Diet and Nutrition. In: *The Ecology of Health and Disease in Ethiopia*. H. Kloos and Zein Ahmed Zein (editors). Westview Press Colorado. pp. 85-102.
- Besrat A., Admasu A. and Ogbai M. (1980). Critical study of the iron content of *teff* (*Eragrostis tef*). *Ethiop. Med. J.* 18, 45-52.
- Borrero J., Restrepo A., Botero D. and Guillermo L. (1961). Clinical and laboratory studies on hookworm disease in Colombia. *Am. J. Trop. Med. Hyg.* 10, 735-741.

- Bulto T., Hailemeskel F., Endeshaw T. and Dejene A. (1992). Prevalence of hookworm infection and its association with low haematocrit among resettlers in Gambela, Ethiopia. *Trans. R. Soc. Trop. Med. Hyg.* 86, 184-186.
- Bunnag D., Cabrera BD., Crompton DWT., Gilles HM., Kale OO., Kan-Chua SP., Kilama WL., Marsden PD., Martinez-Palomo A., Ozeretskovskaya N. and Schultz MG. (1987). Public health significance of parasitic infections. *Bull. WHO.* 65, 575-588.
- Carstensen H., Hansen HL., Kristiansen HO. and Gomme G. (1987). The epidemiology of cryptosporidiosis and other intestinal parasitoses in children in southern Guinea-Bissau. *Trans. R.Soc. Trop. Med. Hyg.* 81, 860-864.
- Cook JD. (1982). Clinical evaluation of iron deficiency. *Semin. in Haematol.* 19, 7-18.
- Dallman PR., Marti MD., Siimes A. and Stekel A. (1980). Iron deficiency and childhood. *Am. J. clin. Nutr.* 33, 86-118.
- DeMaeyer E. and Adiels-Tegman M. (1985). The prevalence of anaemia in the world. *Wld. Hlth. Stat. Quart.* 38, 302-316.

- Desalegn S. (1993). Prevalence of anaemia in pregnancy in Jima town, Southwestern Ethiopia. *Ethiop. Med. J.* 31, 251-258.
- Egger RJ., Hofhuis EH., Bloem MW., Chusilp K., Wedel M., Intarakhao C., Saowaknotha S. and Schreurs WHP. (1990). Association between intestinal parasitoses and nutritional status in 3-8 year old children in North east Thailand. *Trop. Geogr. Med.* 42, 312-323.
- Evatt BL., Gibbs WN., Lewis SM. and McArthur JR. (1992). *Anaemia: Fundamentals of diagnostic haematology*. 2<sup>nd</sup> ed. published jointly by Centers for Disease Control (CDC), Atlanta and WHO, Geneva. pp. 1-112.
- Finch CA. and Cook JD. (1984). Iron deficiency. *Am. J. Clin. Nutr.* 39, 471-477.
- Fleming AF. (1989). Anaemia in pregnancy in tropical Africa. *Trans. R. Soc. Trop. Med. Hyg.* 83, 441-448.
- Fleming AF. (1989). The aetiology of severe anaemia in pregnancy in Ndola, Zambia. *Ann. Trop. Med. Parasitol.* 83, 37-49.

Hogland KE. and Schad GA. (1978). *Necator americanus* and *Ancylostoma duodenale* : life history parameters and epidemiological implications of two sympatric species. *Exp. Parasitol.* 44, 36-49.

Hotez PJ. and Cerami A. (1983). Secretion of a proteolytic anticoagulant by *Ancylostoma duodenale*. *J. Exp. Med.* 157, 1594-1603.

*Instruction manual for Coulter Counter <sup>2</sup> Model T-540,*  
Coulter Electronics Limited, England 1989.

*Ip Base-line survey report, NRIH, 1990. pp 38-48.*

Jemaneh L. and Tedla S. (1984). The distribution of *Necator americanus* and *Ancylostoma duodenale* in School populations, Gojam and Gondar administrative regions. *Ethiop. Med. J.* 22, 87-91.

Johns WL. and Lewis SM. (1989). Primary health screening by haemoglobinometry in a tropical community. *Bull. Wld. Hlth. Org.* 67, 627-633.

Kahssai H. and Woldesemayat G. (1991). Diagnosis and management of severe hookworm anaemia in Gambela, South-western Ethiopia. *Abstracts of the 27<sup>th</sup> annual medical conference. Abstract 49. Addis Ababa: Ethiopian Medical Doctors' Association.*

Kloos H., Lema A., Kirub B., Gebre A., Mazengia B., Feleke G. and Desole G. (1980). Intestinal parasitism in migrant farm labour populations in irrigation schemes in the Awash Valley, Ethiopia, and in the major labour source areas. *Ethiop. Med. J.* 18, 53-62.

Kloos H. and Tesfayohannes T. (1993). Intestinal parasitism. In: *The Ecology of Health and Disease in Ethiopia.* H. Kloos and Zein Ahmed Zein (editors). Westview Press, Oxford. pp. 223-235.

Latham MC., Stephenson LS., Hall A., Wolgemuth JC., Elliot TC. and Crompton DWT. (1983). Parasitic infections, anaemia and nutritional status : a study of their interrelationships and the effect of prophylaxis and treatment on workers in Kwale District, Kenya. *Trans. R. Soc. Trop. Med. Hyg.* 77, 41-48.

Latham MC., Wolgemuth JC. and Hall A. (1983). Nutritional status, parasitic infections and health of road workers in 4 areas of Kenya : part II Krinyaga and Murang'a Districts, the highlands. *East. Af. Med. J.* 60, 75-80.

Layrisse M., Blumenfeld N., Carbonell L., Desenne J. and Roche M. (1964). Intestinal absorption tests and biopsy of the jejunum in subjects with heavy hookworm infections. *Am. J. Trop. Med. Hyg.* 13, 297-305.

Layrisse M., Cook JD., Martinez C., Roche M., Kuhn IN., Walker RB. and Finch CA. (1969). Food iron absorption : a comparison of vegetable and animal foods. *Blood.* 33, 430-443.

Layrisse M. Martinez-Torres C. and Roche M. (1968). Effect of interaction of various foods on Iron absorption. *Am. J. Clin. Nutr.* 21, 1175-1183.

Layrisse M., Paz A., Blumenfeld N. and Roche M. (1961). Hookworm anaemia: Iron metabolism and Erythrokinetics. *Blood* 18, 61-72.

Layrisse M. and Roche M. (1964). The relationship between anaemia and hookworm infection. *Am. J. Hyg.* 79, 279-301.

Lehman H. (1949). Macrocytic anaemia in Central Africans in relation to Ancylostomiasis and other diseases. *Lancet* 1, 90-95.

Lester FT. and Tsega E. (1976). The pattern of adult medical admissions in Addis Ababa, Ethiopia. *East. Af. Med. J.* 53, 620-634.

Martin LK. (1972a). Hookworm in Georgia. I. Survey of intestinal helminth infections and anaemia in rural school children. *Am. J. Trop. Med. Hyg.* 21, 919-29.

Martin LK. and Beaver PC. (1968). Evaluation of Kato thick smear technique for quantitative diagnosis of helminth infections. *Am. J. Trop. Med. Hyg.* 17, 282-291.

Mayet FGH. and Powell SJ. (1966). Hookworm infection and iron deficiency anaemia in Durban. *S. Af. Med. J.* 40, 244-246.

Mayet FGH., Schutte CHJ. and Reinach SG. (1985). Anaemia among the inhabitants of a rural area in Northern Natal. *S. Af. Med. J.* 67, 458-62.

Miller GC. (1981). Helminths and the transmammary route of infection. *Parasitol.* 82, 335-342.

Molineaux L., Plorde J. and Dasnoy J. (1966). Analysis of medical admissions to Gondar hospital, 1963-1965. *Ethiop. Med. J.* 5, 47-65.

Nega A. (1993). Malaria. In: *The Ecology of Health and Disease in Ethiopia*. H. Kloos and Zein Ahmed Zein (editors). Westview Press Oxford. pp. 341-352.

Pawlowski ZS., Schad GA. and Stott GJ. (1991). *Hookworm infection and anaemia: approach to prevention and control*. WHO, Geneva. pp. 3-25.

Peters WH. (1984). Haematocrit and haemoglobin levels in adult males and in pregnant and non-pregnant females in northern Ethiopia. *Ethiop. Med. J.* 22, 17-27.

Pollitt E., Saco-Pollitt C., Liebel RL. and Viteri FE. (1986). Iron deficiency and behavioral development in infants and preschool children. *Am. J. Clin. Nutr.* 43, 555-565.

Pritchard DI., Quinnell RJ., Moustafa M., McKean PG., Slater AF., Raiko A., Dale DD. and Keymel AE. (1991). Hookworm (*Necator americanus*) infection and storage iron depletion. *Trans. R. Soc. Trop. Med. Hyg* 85, 235-238.

Piccardi G., Nyssen M., Dorche J. (1972). Determination of serum iron binding and concentration. *Clin. Chem. Acta.* 40, 219-228.

Ramachandran K. and Bolodia G. (1984). The effect of fermentation on the iron, phosphorus and zinc content of teff (*Eragrostis tef*). *Ethiop. Med. J.* 22, 45-48.

Rifkind RA., Bank A., Marks PA., Kaplan KL., Ellison RR. and Lindenbaum J. (1986). *Fundamentals of haematology*. 3<sup>rd</sup> ed. year book medical publishers, INC. Chicago. pp. 7-40, 209.

Robertson LJ., Crompton DWT., Sanjur D. and Nesheim MC. (1992). Haemoglobin concentrations and concomitant infections of hookworm and *Trichuris trichiura* in Panamanian primary schoolchildren. *Trans. R. Soc. Trop. Med. Hyg.* 86, 654-656.

Roche M. and Layrisse M. (1966). Nature and causes of hookworm anaemia. *Am. J. Trop. Med. Hyg.* 15, 1031-1101.

Rowland HAK. (1966). Anaemia in Dar-ES Salam and methods for its detection. *Trans. R. Soc. Trop. Med. Hyg.* 60, 143-169.

- Sanchaisuriya P., Pongpaew P., Saowakontha S., Supawan V., Migasena P. and Schelp FP. (1993). Nutritional health and parasitic infection of rural Thai women of the child bearing age. *J. Med. Assoc. Thai.* 76, 138-145.
- Saraya AK., Tandon BN. and Ramachandran K. (1972). Study of Vitamin B<sub>12</sub> and folic acid deficiency in hookworm disease. *Am. J. Clin. Nutr.* 24, 3-6.
- Seboxa T., Jemaneh L. and Tedla S. (1986). The clinical picture of hookworm anaemia in northwestern Ethiopia. *Ethiop. Med. J.* 24, 206.
- Seiverd CE. (1983). *Haematology for medical technologists* 5<sup>th</sup> ed. Lea and Febiger, Philadelphia. pp. 231, 285-298, 368-375, 648-698.
- Shamebo M. (1987). Pattern of haematological diseases among adult hospitalized Ethiopians. *Ethiop. Med. J.* 25, 113-118.
- Siimes MA., Refino C., and Dallman PR. (1980). Manifestation of iron deficiency at various levels of dietary iron intake. *Am. J. Clin. Nutr.* 33, 570-574.

Srinivasan V., Radhakrishna S., Ramanathan AM. and Jabbar S. (1987). Hookworm infection in a rural community in South India and its association with haemoglobin levels. *Trans. R. Soc. Trop. Med. Hyg.* 81, 973-977.

Stephenson LS., Latham MC., Kurz KM., Kinoti SN., Oduori ML. and Crompton DWT. (1985). Relationship of *Schistosoma haematobium*, hookworm and malaria infections and metrifonate treatment to haemoglobin level in Kenyan school children. *Am. J. Trop. Med. Hyg.* 34, 519-528.

Stott G. (1961). Hookworm infection and anaemia in Mauritius. *Trans. R. Soc. Trop. Med. Hyg.* 55, 20-25.

Sturrock RF. (1992). Hookworm infections. Human parasitic diseases. Vol. 4. Gilles HM. and Ball PAJ. (editors). Book review. *Trop. Dis. Bull.* 89, 403-404.

Taticheff S., Woldemichael T., Eshete H., Dejene A., Wakbulcho M., Gudunfa A. and Haile D. (1992). Intestinal helminthiasis : An. IP-Base-line survey in Addis Ababa and Wolisso, a paper presented at the 3<sup>rd</sup> annual scientific conference of the Ethiopian Public Health Association.

Tedla S. and Jemaneh L. (1985). Distribution of *Ancylostoma duodenale* and *Necator americanus* in Ethiopia. *Ethiop. Med. J.* 23, 149-158.

Tefera A. and Abdulakadir J. (1968). Analysis of medical admissions to Princess Tsehai Memorial Hospital from April 1966 to March 1967. *Ethiop. Med. J.* 6, 95-102.

Topley E. (1968). Common anaemia in rural Gambia I. Hookworm anaemia among men. *Trans. R. Soc. Trop. Med. Hyg.* 62, 579- 594.

Udonsi JK. (1984). *Necator americanus* infection : a cross-sectional study of a rural community in relation to some clinical symptoms. *Ann. Trop. Med. Parasitol.* 78, 443-444.

United Nations Administrative Committee on Coordination/ Sub Committee on Nutrition (UN ACC/SCN). (1991). *Controlling iron deficiency*. A report based on an ACC/SCN workshop. S. gillespie, J. Kevany and J. Mason (editors). pp. 1-5.

Verloop MC. (1970). Iron depletion without anaemia: A controversial subject. *Blood.* 36, 657-671.

Viteri FE. and Torun B. (1974). Anaemia and physical work capacity. *Clin. in Haematol.* 3, 609-626.

Wakbulcho M. Gudunffa A. and Moller B. (1993). Prevalence of anaemia in pregnancy in an urban and a rural community of Ethiopia : a preliminary study. A paper presented at the 4<sup>th</sup> annual conference of the Ethiopian Public Health Association. Addis Ababa, Ethiopia.

Wintrobe MM., Lee GR., Boggs DR., Bithell TC., Foerster J., Athens JW. and Lukens JN. (1981). Clinical Haematology. 5<sup>th</sup> ed. Lea and Febiger, Philadelphia, USA. pp. 536-635.

Werblinska B. and Fleming AF. (1979). Treatment of iron deficiency in Nigerians with daily intramuscular Ferastral. *Ann. Trop. Med. Parasitol.* 73, 149-159.

Woldegebriel Z., West C., Gebru H., Tadesse A., Fisseha T., Gabre P., Aboye C., Ayana G. and Hautvast JGAJ. (1993). Interrelationship between vitamin A, iodine and iron status in Schoolchildren in Shoa Region, Central Ethiopia. *Br. J. Nutr.* 70, 593-607.

Wondimagegnehu T., Woldemichael T. and Assefa T. (1992). Hookworm infection among the Melka Sedi banana plantation residents, Middle Awash Valley, Ethiopia. *Ethiop. Med. J.* 30, 129-134.

Woodruff AW. (1982). Recent work concerning anaemia in the tropics. *Semin. in Haematol.* 19, 141-147.

- WHO (1963). African conference on Ancylostomiasis. *WHO Tech. Rep. Ser. No. 255*, 5-12.
- WHO (1968). Nutritional anaemias. *WHO Tech. Rep. Ser. No. 405*, 5-28.
- WHO (1970). Requirements of Ascorbic acid, vitamin D, vitamin B<sub>12</sub>, Folate and Iron. *WHO Tech. Rep. Ser. No. 452*, 5-56.
- WHO (1972). Nutritional anaemias. *WHO Tech. Rep. Ser. No. 503*, 5-29.
- WHO (1975). Control of nutritional anaemia with special reference to iron deficiency. *WHO Tech. Rep. Ser. No. 580*, 5-49.
- WHO (1980). *Manual of basic techniques for a health laboratory*. WHO, Geneva. pp. 193-196.
- WHO (1987). Prevention and control of intestinal parasitic infections : report of WHO expert committee. *WHO Tech. Rep. Ser. No. 749*, 5-22.

WHO (1989). *Preventing and controlling Iron deficiency anaemia through primary health care : A guide for health administrators and programme managers.* (author DeMaeyer EM. with collaboration of Dallman P., Gurney PM., Hallberg L., Sood SK. and Srikanta SG.). WHO, Geneva. pp. 1-28.

WHO (1992). *The prevalence of anaemia in women : Tabulation of available information.* 2<sup>nd</sup> ed. WHO/MCH/MSM/92.2. Geneva. pp. 2-12.

Zein Ahmed Z. (1991). Haematocrit levels and anaemia in children, Gondar. *Abstracts of the 27<sup>th</sup> annual medical conference. Abstract 58. Addis Ababa: Ethiopian Medical Doctors' Association.*

Zein Ahmed Z. and Assefa M. (1987). The prevalence of anaemia among populations living at different altitudes in North-western Ethiopia. *Ethiop. Med. J.* 25, 105-111.