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ASSESSMENT OF MALARIA AND INTESTINAL PARASITES AS PUBLIC HEALTH PROBLEMS BASED ON CLINICAL RECORD, PARASITOLOGICAL SURVEYS AND KAP IN BORENA DISTRICT, SOUTH WOLLO, CENTRAL-NORTH ETHIOPIA

BY

FENTAW BEREDED TIBEBU

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ACRONYMS AND ABBREVIATIONS

AAU	Addis Ababa University
AAUSF	Addis Ababa University Faculty of Science
ACT	Arthemisinin Based Combination Therapy
asl	Above Sea Level
CDC	Center for Disease Control and Prevention
DDT	DichloroDiphenylTrichloroethane
DPCD	Disease Prevention and Control Department
EIR	Entomological Inoculation Rate
GFATM	Global Fund for AIDS, TB and Malaria Control and Prevention
GMCS	Global Malaria Control Strategies
HC	Health Center
HEWs	Health Extension Workers
IRS	Indoor Residual Spray
ITNs	Insecticide Treated bed Nets
KAP	Knowledge, Attitude and Practice
LLINs	Long Lasting Insecticide Treated bed Nets
MDGs	Millennium Development Goals
MDTPs	Malaria Diagnosis and Treatment Posts
MES	Malaria Eradication Service
MIS	Malaria Indicator Survey
MOH	Ministry Of Health
NGOs	Non Governmental Organization
NOCMVD	National Organization for the Control of Malaria and other Vector Born Diseases
PMI	President's Malaria Initiative
PS	Sulfadoxine-Pyrimethamine
Pf	*Plasmodium falciparum*
Pv	*Plasmodium vivax*
RBCs	Red Blood Cells
RBM	Rolls Rack Malaria

RDTs	Rapid Diagnostic Tests
SNNPR	South Nations Nationalities and Peoples Region
SPSS	Statistical Package for Social Science
Km	Kilometer
SSA	Sub Saharan Africa
TB	Tuberculosis
UNICEF	United Nations International Children's Education Fund
WHO	World Health Organization

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ABSTRACT: The analysis of retrospective clinical records from Mekane-Selam Health Center showed high average annual malaria (25.0%) and intestinal parasite prevalence (46.3%). In the cross-sectional survey, both direct wet mount and formal-ether concentration methods were used for microscopic diagnosis of intestinal parasites; malaria diagnosis was based on microscopic diagnosis of thin and thick Giemsa stained blood film. Accordingly, an overall prevalence of 24.5% intestinal parasite infections and 0.96% malaria parasite was determined. The intestinal parasites detected were *E. histolyica/dispar* (10.83%), *A. lumbricoides* (**7**.6%), *G. lamblia* (1.81%), *hookworm* (1.99%), *H. nana* (3.6%) and 0.9% each of E. vermicularis and *S. stercoralis*. Malaria infections were due to *P. falciparum* (0.38%) and *P. vivax* (0.58%). Multiple intestinal parasitic infections were common (12.5%). High prevalence of intestinal parasitic infection was not in general significantly associated with the absence of toilet, source of drinking water, eating raw meat and open field disposal of household waste (P>0.05). Illiteracy and not washing hands before meal and after toilet use were shown to be associated with *G. lamblia* (OR=4.21, P=0.043; OR=9.06, P=0.001) and *E. histolytica/dispar* (OR=17.04, P=0.01; OR=5.104, P=0.001) infection. Relatively high awareness (78.1%) was detected among the heads of household about malaria and its preventive and control measures. Significant discrepancy in prevalence levels between the retrospective clinical record and the cross-sectional survey was observed for malaria. This is to be expected because the prevalence data of clinical records were obtained based on treatment seeking patients reporting to the Health Center. Furthermore, malaria control interventions such as the use of ITNs/LLINs, IRS and prompt treatment with ACT, based on RDT, appeared to have reduced malaria transmission in the study area. On the other hand, the deworming program reportedly under implementation in the study area does not seem to be effective since the prevalence of intestinal helminths remained too high. Therefore, the implementation of control intervention measures for malaria must continue as it is currently practiced and the geo-helminth control measures will require serious improvement in Borena District.

Key words/phrases: Malaria, intestinal parasites, parasitic infection control, health, Borena.

1. Introduction

1.1Global malaria situation

Malaria is still a major public health and medical concern in many parts of the world, especially in countries of tropics and subtropics such as in Africa, South East Asia, Hispaniola (Haiti and Dominican Republic), and the Indian sub continent, the Middle East, Oceania and Latin America. It is also rarely occur in temperate climate. (Dash *et al.,* 2007; Stratton *et al*., 2008; Kaur, 2009). Estimation has shown that 1.2 billion people are at risk of malaria; of this, 300-500 million people are infected and more than 1 million are dying each year globally. The majority of these deaths occur in young children in sub-Saharan Africa where one out of five deaths of children is due to malaria (Bell and Winstanley, 2004; World Malaria Report, 2005). Recent literatures showed that about 40% of the world's population, particularly those in the poorest countries are affected by malaria infection (247million) and about 90% of all malaria deaths (881000) in the world occur in Africa south of the Sahara (World Malaria Report, 2008). But, according to World Malaria Report (2009), 243 million cases of malaria estimated to occur globally, the highest proportion (85%) were in Africa. Similarly, from the estimated global death (863000) due to malaria, 89% were in Africa.

This huge malaria case and death have been due to the fact that, *P. falciparum* causes the majority of infections in Africa. The severity of *P. falciparum* malaria is due to the organism's ability to invade young and old red blood cells, which is not characteristic of the other *Plasmodium* species. In addition, lack and absence of malaria prevention and control services, health services deterioration, parasite resistance to antimalarial drugs, resistance of vector mosquitoes to insecticides, civil unrest coupled with population movement and economic development programs in wetlands, desert fringes and highlands have contributed to the huge malaria case and death in malariuos areas. Moreover, ideal climatic conditions which are exacerbated by some of the world's most efficient malaria vectors, such as *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus* and natural disasters such as El-Nino and tsunamis are among the factors (WHO-UNICEF, 2003; Weinberg, 2006).

The causal microbes for human malaria are four *plasmodium* species: *P. falciparum, P. vivax, P. ovalae and P. malariae.* Of these, *P. falciparum* has caused the most deadly, widespread

and severe malaria. The distribution of *plasmodium* species varies across the world and even among localities in malarious areas. For example, *P. falciparum* predominates in Haiti, Papua New Guinea, and Sub-Saharan Africa, while *P. vivax* is more common in Central America and the Indian subcontinent and causes more than 80 million clinical episodes of illness yearly. The prevalence of these two species is approximately equal in the Indian subcontinent, eastern Asia, Oceania, and South America. *P. malariae* is found in most endemic areas, especially throughout Sub-Saharan Africa, but is much less common than the other species. *P. ovale* is unusual outside Africa, and where it is found accounts for less than one percent of isolates (Breman *et al*., 2006).

The *plasmodium* species life cycle involves both vector mosquitoes and human host. Malaria infected mosquito bites on human beings. It inoculates *sporozoites* into the human blood stream, and then the *sporozoites* travel to the liver. Upon *sporozoite* replication in the liver, *merozoites* release into the blood stream. The *merozoites* bind to the surface then enter the Red Blood Cells (RBCs) via a receptor-ligand interaction (Phillips, 2001). The parasite then undergoes growth through the ring and *trophozoite* stages, finally producing *schizonts* containing multiple *merozoites* (erythrogenic cycle). Matured *schizonts* destruct RBCs and release *merozoites* into the bloodstream, which re-invade new RBCs. Occasionally, parasite maturation will result in the production of gametocytes which may be released into the bloodstream and are subsequently taken up by the mosquito, via a bite. Then gametocytes undergo the sexual stage of development (sporogenic cycle) in the mosquito. When the mosquito takes her next blood meal, 10-14 or more days later, it can again infect a human host (CDC, 2004; Lamb *et al.,* 2006) (Fig. 1).

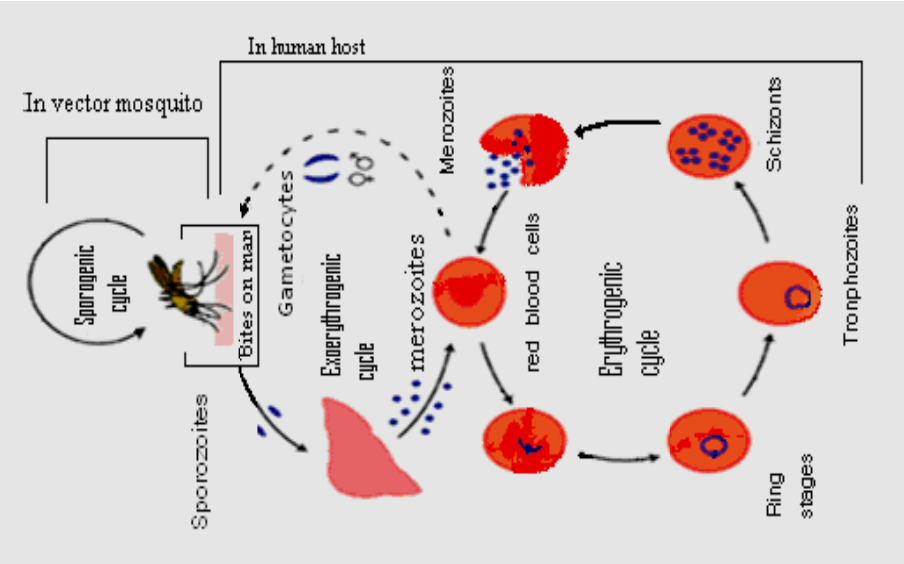

Figure 1: Life cycle of *Plasmodium* species (**Source:** Lamb *et al*., 2006).

The presence of *sporozoites* in the liver does not result in the symptom of malaria. Symptoms of malaria arise upon the release of *merozoites* from the RBCs. Despite variations within the different *Plasmodium* species, symptoms of malaria are generally non-specific and most commonly include fever, shivering, headache, nausea, vomiting, muscle aches and fatigue. These can rapidly progress to organ failure, delirium, convulsions, coma, and too often death if not treated promptly (Bell and Winstanley, 2004**;** *www.rollbackmalaria.org,* 1998). Even though all the four *Plasmodium* species are causing human malaria, there have been differences in the severity of the disease they cause depending on the species of *Plasmodium* species. For example, infection by any of the four *Plasmodium* species may cause illness, but infection with *P. falciparum,* if not treated promptly may lead to death. Although rarely fatal, malaria due to *P. vivax and P. ovalae* usually develop relapse after symptom free-intervals of up to 2-4 years, respectively (*www.Rollbackmalaria.org*, 1998).

The confirmatory diagnosis of clinical malaria employ both the microscopic demonstration of asexual forms of the parasite in stained peripheral blood smears, *Gold standard method* (Warhurst and Williams, 2004) and the new diagnostic tests using antigen and nucleic acid detection methods, Rapid Diagnostic Tests (RDTs). RDTs tests are promising, but have limitations like species sensitivity (except for *P. falciparum*), parasite quantitation, field feasibility, and costs necessitate further development and evaluation (Murray and Bennet, 2009).

Female anopheles mosquitoes transmit human malaria. Globally, of the 444 formally identified and named anopheline species, more than 60 female *anopheles* species can transmit malaria. The most efficient vectors in the world are those such as *Anopheles* (*An*.) *gambiae, Anopheles arabiensis* and *Anopheles funestus* (Phillip, 2001). They *are also* found in Africa, which are long-lived, occur in high densities in tropical climates, breed readily, and bite humans in preference to other animals. The entomological inoculation rate (EIR)-that is, the number of sporozoite-positive mosquito bites per person per year is the most useful measure of malarial transmission and it varies from less than 1 in some parts of Latin America and Southeast Asia to more than 300 in parts of tropical Africa. That is why 85% of world's malarial death is in Africa. In addition to the vector related factors; factors related to the parasite, the human host, and the environment determine the intensity of malaria transmission (Breman *et al*., 2006; Dash *et al.,* 2007). Malaria could also rarely be transmitted from patients to healthy people through blood transfusion, sharing syringes, organ transplants, and accidental laboratory inoculation (Warhurst and Williams, 1996) .

Studies have shown that rain fall, temperature and humidity have been associated with the dynamics of malaria vector population and hence with the spread of the disease. Ambient temperature plays a major role in the life cycle of the malaria vector. The development of the parasite *(plasmodium*) within the mosquitoes (sporogenic cycle) is dependent on temperature. That is, it takes about 6-10 days at temperature of 28^0c, but stops at temperature below 16^0C. The daily survival of the vector mosquito is dependent on temperature as well. For range of temperature between 16^0C-36 ^{0}C, daily survival is about 90%; while with a temperature above 36^0C survival shown to drop rapidly. Humidity and rainfall have been shown to regulate malaria vector survival. Relative humidity below 60% was related to a decrease in vector life span, hence, which means ultimately low rate of malaria transmission. In contrast, at a humidity level above 60%, the infection rate increases substantially, this can be explained by improved vector survival (Martens *et al*., 1995; Ye *et al*., 2007).

Malaria had once extended widely throughout the old world, reaching as far north as 64ºN latitude and as far south as 32ºS latitude; but today, it is generally a problem of the tropical regions (Hamoud and Sachs, 1999). It is endemic in 109 countries and territories in tropical and sub-tropical zones of the world, except Antarctica and Australia, with intensities of transmission that vary from very low to extremely high (Minakawa *et al.,* 2006). Lack of

control measures and improvements in health and political, economic and moral will in the poorest part of the world and further natural disaster such as tsunamis in south east Asia and El Nino-related climate variability in Botswana, Africa, in addition to man-made climatic variations, had lead to a rapid escalation in the incidence of malaria (Engwerda, 2005; Patz and Olson, 2006). In addition, due to reduction in wide spread use of IRS insecticides following the global consensus to replace malaria eradication with long term malaria control in most countries of Africa, there had been significant resurgence of malaria incidence in a number of countries. For example, recent malaria resurgence in the East African highlands Kenya, Sudan and Ethiopia involves multiple factors: from climate and to drug resistance, variable disease control efforts, and other socio-demographic factors unstable malaria transmission, little or no immunity in the highlanders against malaria antigen, exceeding of *Anopheles* population density above critical levels. The persistent expansion of malaria in the highlands is exacerbated by rapid population growth and massive land use changes (such as deforestation) that can favor mosquito breeding (Malkooti *et al*., 1998; Lindblade *et al.,* 2000; Patz *et al*., 2002; Reiter, 2008).

The greatest altitude at which malaria occurs may differ very much from place to place in tropics with respect to altitudinal range of above 2500m *asl* in Kenya to under 1000m *asl* in Sri-Lanka and in Central Asia such as in Afghanistan at altitude of around 2400m *asl* (Abdur *et al.,* 2003). Throughout most Sub-Saharan Africa (SSA), malaria shows a high endemicity with average altitude of 1400m; but a low epidemic potential except in some areas. Within this endemic altitudinal range, imported malaria from endemic region to areas where the disease has been eradicated was observed for the resurgence of the disease. Such resurgence was seen in America, Azerbaijan, Chechnya, Russia, Tajikistan, Turkey, Madagascar, South Africa and Zanzibar where the disease was previously eradicated using effective control programs (Martens and Hall, 2000).

The rapid increase of the world's urban population has major implications for the transmission and epidemiology of malaria and other vector-borne diseases (Lines *et al*., 1994). Heterogeneity in urban malaria transmission patterns is driven mostly by human activity, urban development and environmental determinants (Wang *et al*., 2006). For instance, the emergence of urban malaria as major public health problem in many small, medium and metropolitan cities of India, which was essentially the result of a man made

problem such as of rapid and casual expansion of the cities, inadequate piped water supply, storage of water in cisterns, disuse or scarce use of wells, developmental activities, aggregation of migrant labor force and overall population movement. Moreover, the dynamics of urbanization in SSA greatly affect eco-systems and, hence, population health; however, this is insufficiently documented (Keiser *et al*., 2004; Donnelly *et al.,* 2005; Hay *et al*., 2005). In most countries of sub-Saharan Africa, malaria transmission is generally intense in rural areas compared to the low-level transmission in the urban areas where population density is high and the number of mosquito breeding sites is reduced. Although malaria widely infects rural communities of Sub Saharan Africa, it has been gaining attention as an urban disease even if suitable vector breeding sites are scarce in highly populated areas. In such urban areas the urban poor are at far higher risk from malaria (Donnelly *et al.,* 2005).

Morbidity and death caused by malaria place a severe economic burden on individuals and their families, communities and on national and global economies. There are multiple ways by which malaria impedes development, including effects on fertility, population growth, saving and investment, worker productivity, school absenteeism, premature mortality and medical costs (Sachs and Malaney, 2002)**.** Association of malaria with socio-economic situation in Africa showed that economic burden of malaria to be about US $12 billion and to slow economic growth by about 1.3%. Besides contributing to loss of life, malaria morbidity may cause anemia and its various complications, miscarriage, brain damage, decreased cognition, and productivity (Rugemalila, *et al*., 2006). A study conducted by Luxemburger *et al.* (2001) in Africa, suggested that women who had contracted malaria during pregnancy developed anemia and contributed to early infant mortality. According to the result of their study, thirty-seven percent (555 of 1,495 the study population) detected anemic during pregnancy due to malaria.

Although no vaccine is available against malaria, effective and low cost strategies are available for the treatment, prevention and control of malaria in Africa and other malaria endemic areas of the world. Using indoor residual spray as a major tool, the WHO led malaria eradication campaign eliminated the risk of malaria infections of several millions of people in countries of Europe, Asia and Latin America within about two decades in the 1950s and 60s. From these times onwards, global malaria prevention and control using ITNs, IRS and anti-malaria drugs and/or injectables have been utilized and had made the save of many

lives particularly in malarious parts of the world (WHO, 1996). Since the launch of the RBM initiatives by WHO in 1998, malaria control has been intensified in endemic countries (WHO, 2008). This resulted in high coverage of malaria control interventions, especially in sub-Saharan Africa and hence the malaria burden has been reduced, however, it varies in all regions of the world (Minakawa *et al*., 2006). In some countries of Africa with high malaria burdens, there is evidence of significantly decreasing malaria incidence and deaths among children and adults (World Malaria Report, 2008a; WHO, 2009). In countries with lower transmission intensities, such as southern Africa and Asia, the malaria burden has been reduced to such an extent that it has ceased to be a major public health problem due to malaria preventive and control measures implemented in the region (Minakawa *et al*., 2006).

1.2 Global Situation of intestinal parasites

Intestinal parasites are those types of entero-parasites, which infect the lumen and lining tissue of the lumen of the small and large intestine. Positive cases of intestinal parasitic infection were defined as the "presence of parasites' egg, larvae, cyst or the parasite in fecal specimen" (Morales-Espinoza *et al.,* 2003). Intestinal parasitic infections are common throughout the world but highly prevalent in the developing countries; particularly in tropical countries (WHO, 1981). These parasites are commonly transmitted through ingestion of contaminated food and/or water because of poor sanitation and hygiene. Sometimes, transmission occurs through close contact between infected and non-infected individuals as in infected food handlers and consumers, respectively (Neghab *et al*., 2006). Recent estimate showed that intestinal parasitic infection affect about 3.5 billion people of which 450 million are ill, the majority being children (Arani *et al*., 2008).

The global prevalence and intensity of intestinal parasitic infections in man have shown considerable variation in distribution and in seasonal occurrence due to geographical and climatic factors and to human activities changing the environment (Pawlowski, 1980). For example, a range of 30-60% estimated intestinal parasitic infection prevalence rates in developing countries of Central America, especially Guatemala, Honduras, and Mexico was very high compared to that ≤ 2% in developed countries (Saab *et al*., 2004).

Both micro-parasites (helminths) and macro-parasites (protozoans) are important causes of intestinal parasitic infections. These include nematodes, cestodes, trematodes and protozoan parasites. Helminths are worms with multi-cells. Usually, they cannot multiply in the human body (Haque, 2007). Helminthic infection is among the most prevalent intestinal parasitic infections of humans from developing parts of the world where there is low coverage of hygiene and sanitation, such as in Latin America, China and East Asia, and Sub-Saharan Africa. This infection is distributed virtually throughout the world and has been causing morbidity most commonly associated with infections of heavy intensity (Haque, 2007; Hotez *et al.,* 2008*;* WHO, 2008).

The transmission of most of the intestinal parasites reflects the level of sanitation and the availability and quality of water. For instance, in human communities in which poverty is deep-rooted and clean drinking water, sanitation, health care, and health awareness are inadequate, there has been wide distribution of roundworms, hookworms, and whipworms (Crompton and Nesheim, 2002; Ostan *et al.,* 2007; Yilmaz *et al*., 2007). In addition, the high prevalence of *ascariasis* is good indicator of improper fecal disposal and high prevalence of *giardiasis* frequently reflects the lack of water or its quality. Others such as Hookworm interfere with nutrition while others such as *taeniasis*, spread in contaminated food (WHO, 1987). Moreover, diseases such as HIV/AIDs, which reduce the immunocompetence of patients, may contribute for the frequent incidence of intestinal parasites and to their high morbidity and mortality burden even in the well-developed countries where intestinal parasites had been considered controllable (Gomez-Morales *et al*., 1995).

Intestinal parasites have been thought to cause considerable worldwide morbidity and mortality, especially in developing countries and people with co-morbidity (Awolaju and Morenikeji, 2009). According to WHO (2000), an estimate of 3.5 million people have been infected and around 450 million children are ill due to intestinal parasitic infection globally. Helminths alone had high global prevalence: estimated 1.5 million cases of *Ascaris lumbricoides,* 1.2 million cases of hookworm, 1.05 million cases of *T. trichiura,* and 200–300 million cases of schistosomiasis had been reported (Ezeamama *et al*., 2005). It was estimated that greater than one-fourth of the world's people are infected with Hookworm, *A. lumbricoides*, *T. trichiura*, and schistosomes only, which are particularly prevalent among school-age children in developing countries, making *amoebiasis, ascariasis*, hookworm and

trichuriasis among the ten most common infections (WHO, 1987; Kremer and Miguel, 2001). An estimation of 850 million people (WHO, 1996) and 1.5 billion people (Crompton, 1999) (quarter of the world's population) were infected with *A. lumbricoides*. This high infection of *ascariasis* is concentrated in developing world with poor sanitation and personal hygiene (de` Silva *et al.,* 1997). Several research work reports indicated that intestinal parasitic infection have main impact on malnutrition, vitamin A deficiency, and iron deficiency which could reduce working capacity and productivity in adults and impaired growth, learning and school performance in children. Moreover, they cause economic burden of medical expense and compounding the personal burden of disease and disability (WHO, 2006; Al-Mekhlafi *et al*., 2007; Mengistu *et al.,* 2007; Ngrenngarmlert *et al.,* 2007).

In addition to helminths, there is a wide range of worldwide intestinal protozoa infection on humans. But the range of species and their prevalence has been high in developing countries with low standards of environmental sanitation and hygiene, whether in temperate or the tropical locations (Sackey, 2001). The protozoan species that infect the human intestinal tract include *Giardia lamblia, Cyclospora caytanensis, Entameoba histolytica/dispar*, *Cryptosporidium* and *Microsporidia* species and *Isospora belli*. Most of these parasites are either strict or opportunistic intestinal parasites and their presence in stool specimen indicates that they are obtained through contamination of food or water (Mills and Goldsmid, 1995).

According to WHO's recommendation, prevention and control of intestinal parasitic infection constitutes several activities: universal or selective deworming of population groups that are at risk of developing morbidity and chronic diseases using anthelminthic drugs. In combination to deworming, improving environmental sanitation, personal hygiene, food and water hygiene and appropriate health education are useful measures for more permanent control of transmission and preventing re-infection (WHO, 2002).

1.3 Malaria and intestinal parasites in Ethiopia

1.3.1 Malaria in Ethiopia

Malaria is a leading public health problem in Ethiopia where an estimated 75% of the total area of the country and 48 million people (65-68% of the population) live in areas at risk of malaria and the problem is compounded by increasing frequency and magnitude of malaria

epidemics (WHO, 2002; Deressa *et al*., 2006; MOH, 2008). In this country, endemicity of malaria was reported for the first time by scientists from Britain and Italy starting from the mid 1930`s. Since then, many malaria epidemics were recorded in the country. For example, in 1958 severe outbreak of malaria epidemic that devastated the lives of many people occurred in the Dembia plain near Lake Tana (Covell, 1957). In addition, there was epidemic malaria in 1991 and more than 100,000 malaria cases were recorded in the epidemic year of 1998. Moreover, malaria epidemics had occurred in the country in 2003 and 2004 that affected 40 million people in several regions of Ethiopia (World Malaria Report, 2005). The disease is among the most important public health problems surpassing other communicable diseases such as *helminthiasis, amoebiasis*, and bacterial *pathogenesis* (MOH, 2005). By 2002/03 malaria was reported as the first cause of morbidity and mortality, where 15.5% outpatient consultation, 20.4% admissions and 27% inpatients death were notified (MOH, 2004). Malaria, followed by *helminthiasis* and TB, was reported to be the leading infectious disease in the country by 2004/05 (MOH, 2005).

Human malaria in Ethiopia has been caused by four *Plasmodium* species: *P. falciparum, P. vivax, P. ovalae* and *P. malarae. P. falciparum and P. vivax* are the most commonly encountered human malaria parasites in Ethiopia. *P. malariae* is found sporadically in some areas (Deressa *et al*., 2006). The most deadly malaria is caused by *P. falciparum,* but two or more species can overlap in the same area and in a person at the same time (WHO, 2002).

All human malaria is transmitted by female *anopheles* mosquitoes only (Harbach, 2004; WHO, 2008). In Ethiopia, about forty-three anopheles mosquito species have been recorded in the country. Some of these are *Anopheles (An.) arabiensis, An. funestus, An. nili, An. pharoensis, etc,* (MOH, 2002). Malaria transmission in Ethiopia depends substantially on A*nopheles arabiensis* Patton [principal malaria vector in the country], a member of the *An. gambaie* Giles complex, in the intermediate highlands of Ethiopia. *An. funestus* Giles is the second most important malaria vector in Ethiopia. *An. nili* Theobald is an important local malaria vector in the low land region of south-west Ethiopia (Woyessa *et al.*, 2002; MOH, 2007).

The epidemiology of malaria may vary considerably within relatively small geographic areas (Breman *et al.,* 2006). The transmission of malaria in Ethiopia is generally unstable and

seasonal. Frequent malaria epidemics in highland areas of Ethiopia where previously had not known have been currently occurring (Woyessa1 *et al*., 2004). The transmission patterns and intensity vary greatly due to the large variations in altitude, rainfall, humidity and population movement (Negash *et al.,* 2005). Unstable malaria occurs in moist parts of the country especially in the lowland-highland fringes where climatic conditions are suitable for malaria transmission (WHO, 2009). Transmission usually occurs at altitudes below 2000m *asl*. Areas below 2,000m *asl* are malarious (potentially malarious) (Paulander *et al.*, 2009). There are two malaria transmission seasons in the country: one is the major malaria transmission season that occurs between September and December following the rain from June to September and the second, relatively low, occurs between April and May due to the February and March rains (MOH, 2000; MOH, 2003). Some localities may also experience perennial malaria as the environmental and climatic situations permit the continual breeding of vectors in permanent breeding sites (Gebre-Mariam *et al*, 1998; Mouchet *et al*., 1998).

Ethiopia comprises regions of largely differing malaria endemicity and transmission (Schunk *et al.*, 2006): "Kolla" or hot zone below 1500m, "Woina-dega" (and temperate zone) between 1500-2500m and the "Dega"or the cold region above 2500m *asl* (WHO, 1991). Areas above 1500m are among mostly affected parts by seasonal malaria because of the varying physiographic and climatic factors in which all age groups are affected due to the absence of protective immunity (Tulu, 1993).

1.3.2 History of malaria prevention and control in Ethiopia

The control of malaria in Ethiopia has a history of more than 40 years (WHO, 2009*)*. Initially, malaria control had began as pilot control project in the 1950's and then it was transformed to a national malaria eradication campaign in the 1960's then to a control strategy in the 1970's (Deressa *et al*., 2003). In 1976, the National Organization for the Control of Malaria and Other Vector-borne Diseases (NOCMVD), emerged from the Malaria Eradication Service (MES). Until the early 1990s, malaria control was organized by sectors, (PMI, 2008). Sector Malaria Control Offices were responsible for Malaria Detection and Treatment Posts (MDTPs) which collected data on microscopically confirmed cases in each sector. Despite reduced prevalence and level of transmission in many areas, the eradication of malaria by that time was not successful partly because of some technical and financial

constraints in both the country and the institutional organization supporting the project (MOH, 2000; Deressa *et al*., 2006).

Following the global malaria control strategy by WHO in 1992, Ethiopia had made a major reorganization and decentralization in health administration starting from 1993. Regions took over responsibility for many aspects of the program and malaria control was integrated with other parts of the health system. In a subsequent reorganization of the Federal Ministry of Health (MOH), malaria control became a 'team' under the Disease Prevention and Control Department (DPCD) rather than a separate department. At the central level, cores of professionals are responsible for formulation of policies, provision of technical guidelines to regions, assistance in training, conducting operational research and support in anti-malarial drugs, insecticides and equipment (Roberts *et al.*, 1997; MOH, 2002).

The recent malaria control and prevention in Ethiopia has been governed by a five-year strategic plan for 2006–2010 based on ITNs, IRS (IRS in selected villages) distribution and prompt treatment of malaria patients using ACT based on diagnosis of patients (WHO-African Region, 2007). The main objectives of the malaria strategic plan is to reduce the overall burden of malaria by 50% for 2010 as compared to the baseline level (determined in 2006, 80%) and maintain malaria free areas through strong surveillance and preventative measures. The operation is targeted on only for altitudes below 2000m *a.s.l* and based on the criteria such as altitude, morbidity data, and history of epidemic (MOH, 2008).

Indoor Residual Spray (IRS) is the application of long-lasting insecticides (up to six months) on the walls of dwellings. Insecticides repel mosquitoes from entering houses and/or kill it, thereby preventing subsequent transmission (Jima, 2007). In Ethiopia, IRS operations are currently implemented in Kebeles selected within each District based on local knowledge using information. The number of annual spray rounds required is determined by: the history of malaria cases, altitude and the presence of nearby anopheline breeding sites with rainfall patterns. It is believed that *An. pharoensis* and *An. arabiensis* breeding in lakes may be focal points for initiating epidemics when the rainy season begins, thereby allowing anopheline populations to spread (PMI, 2008).

As with IRS, the vector control effects of ITNs become more apparent when household coverage increases (Binka *et al*. 1998; WHO, 2006). The distribution of ITNs in Ethiopia was started since 1998 using ordinary nets, with low coverage and re-impregnation (Jima, 2007). Later on, the Long Lasting Insecticide Treated Nets (LLINs) was introduced in 2005, followed by rapid scale-up in the LLINs distribution to provide 2 nets per household in 2007. Therefore, national ITNs coverage rates have been increased from 3.4% in 2005 to 53.3% in 2007 (MOH, 2007). The national malaria indicator survey (MIS), 2007 showed that an average of 60% of children under age five years and 65.7% of pregnant women had slept under an ITN in households that owned at least one ITN, in malarious areas and also nationwide (MOH, 2008).

In addition to the vector control measures described above, distribution of anti-malarial drugs; environmental management; larvicides; *health education and counseling* are also essential to combat the disease (MOH, 2003; Tren *et al.,* 2008). Therefore, now in Ethiopia, Sulfa-doxine-pyriethamine (Fansider) at 36% clinical failure rate was successful replaced by new, highly effective Artemisinin-Combination Drug treatment (ACT) as the first line treatment drug. This has been done with the help of Rapid Diagnostic Tests (RDTs) kit that can detect malaria cases quickly in their home (MOH, 2004).

In addition, environmental management as vector control intervention in Oromia regional health bureau, Ethiopia, showed implementation of vector mosquitoes identification and filling-in breeding sites with low community participation (PMI, 2008) In northern Ethiopia, Tigray; filling, draining and shading of potential mosquito-breeding habitats was carried out by the community of Deba (study village) as a pilot study and relatively few malaria cases were recorded after control interventions (Yohannes *et al*., 2005). Along with environmental management, the application of chemical insecticides, called larvicides, may be applied to all mosquito-breeding sites or targeted to the breeding sites of specific vectors in conjunction with environmental control measures. The most common water-soluble chemical used in Ethiopia is temephos (Abate). It is safe for human and therefore it can be applied to drinking water (MOH, 2003).

Moreover, the health extension workers (HEWs) have been giving health services to the community such as case management of childhood illnesses (e.g., pneumonia, malaria, etc)

and delivery of preventive interventions such as LLINs, immunization, promotion of healthy behavior and mobilization of communities (Haines *et al*., 2007; Negusse *et al*., 2007).

According to MOH (2008), Ethiopia has seen a significant reduction in malaria morbidity, mortality and in the number of life threatening epidemics, which is the result of integrated malaria prevention and control intervention measures.

1.3.3 Intestinal parasites in Ethiopia

Intestinal parasitic infection has cosmopolitan in distribution. There has been wide distribution of intestinal parasites in Ethiopia (Mengistu *et al*., 2007). Similar to other developing countries, wide distribution of intestinal parasites in Ethiopia could be due to low level of environmental sanitation, personal hygiene, food and water contamination with human excreta and unaware of simple health promotion practices such as personal hygiene, food hygiene, the effect of altitude, urbanization, irrigation, and resettlement within the country (Haile *et al*., 1994; Jemaneh 1998; Endeshaw, 2005).

The most important intestinal parasites predominantly distributed in the county include *A. lumbricoides, G. lamblia,* hookworm*, H. nana, T. trichiura, E. vermicularis, and E. histolytica/dispar*; with varying prevalence in different areas. Heliminthic infection (both geohelminthiasis and schistosomiasis) are common in the country and are the second most predominant cause of outpatient morbidity in the country (Erko and Medhin, 2003; Mengistu *et al.* 2007). It was shown that the prevalence of intestinal parasitic infection in Ethiopia is different in different parts of the country. For example, Jemaneh (1998) reported that the distribution of the three common helminths; *A. lumbricoides*, *T. trichiura* and the hookworm in schoolchildren in several communities of three altitudinal regions in Ethiopia have been shown to be different. That is, the prevalence of *A. lumbricoides* infection was 29% in the highlands, 35% in the temperate areas and 38% in the lowlands. The prevalence of hookworm infection was highest in the lowlands (24%) followed by the temperate (15%) and highland (7%) areas, while *T. trichiura* infection exhibited similar prevalence in all altitudinal regions (13% on the average). In addition, Legesse and erko (2004) reported that 83.8%, of 259 surveyed students, had one or more intestinal parasites which include hookworm (60.2%), *S. mansoni* (21.2%), *T. trichuria* (14.7%), *Taenia* species (13.9%), *E.*

histolytica/dispar (12.7%), *A. lumbricoides* (6.2%), *G. duodenalis* (6.2%) and *S. stercoralis* (5.8%) from rural area close to the southeast of Lake Langano, Ethiopia. Moreover, Mengistu *et al.* (2007) showed that *T. trichiura, A. lumbricoides, E. histolytica/dispar, G. lamblia, S. stercoralis, H. nana,* intestinal *schistosome, T. saginata*, *E. vermicularis* and hookworm with prevalence of 60.9%, 40.9, 17.1% 13.9%, 17.5%, 2.1% 5.0%, 2.3%, 14.8% and 1.1% respectively were diagnosed from study groups in Jimma, southwestern Ethiopia.

Stool samples examination based on morphology are commonly employed for the detection of the parasite ova, larvae, cyst or the parasite (Ngrenngarmlert *et al.,* 2007). Antigen-detection tests are now commercially available for the diagnosis of all three major intestinal protozoan parasites to resolve the problem of morphological similarity such as the pathogenic *E. histolytica* and the non-pathogenic *E. dispar*. However, the diagnosis and treatment of intestinal helminthic infections have not been changed much and the traditional microscopic method can be used for their diagnosis (Haque, 2007)

Despite substantial investment and research, no vaccine is yet available for prevention of human intestinal parasitic infections. Prevention is based on avoidance strategies, which are based on sanitary disposal of feces, keeping personal hygiene and provision of purified water together with deworming of helminthic infection (WHO, 1987). In connection with the hygiene and sanitary based control of intestinal parasites, deworming of helminthiasis on targeted groups of the community (children of age below 5 years old) and in/outpatient treatment of protozoan infection have been implemented in Ethiopia.

Although nationwide information on malaria and intestinal parasite has gradually been increasing in Ethiopia, there is no documented information on malaria and intestinal parasites in Borena District. According to informants from the District and records from health facilities, there are year round intestinal parasitic infections and seasonal malaria in the District. Therefore, the present study was designed to see the prevalence of malaria and intestinal parasitic infections in the Borena District.

Hypothesis*:* The prevalence of malaria and intestinal parasites in Borena District may be relatively high and hence they constitute an important public health concern.

2. Objectives

2.1 General objective

- To assess the prevalence of malaria and intestinal parasitic infections and the effectiveness of the preventive and control measures implemented in Borena District.

2.2 Specific objectives

- To assess the prevalence of malaria and intestinal parasites in the District
- To assess the effective utilization of malaria and intestinal parasites control measures
- To predict the risk of malaria and intestinal parasite infection for the communities under consideration.

3. Materials and Methods

3.1 The study area

The study was conducted in Borena District, South Wollo, Amhara Region (Fig.2), between November and December 2008. The District covers an area of about 11,000 Km^2 and has 36 Kebeles. Among these, almost all 19 Kebeles are partially malaria endemic. Borena District is 580 kms North of Addis Ababa and 180 Km Northwest of Dessie on an all weather gravel road.

The District is found within the altitudinal range of below 1500 to 3660m above sea level (*asl*). The District covers three agro-ecological zones: Kola (below 1500m *asl*), Woina-Dega (between 1500 and 2500m *asl*) and Dega (above 2500m *asl*) (WHO, 1991). There are a number of tributary rivers that discharge their volume downstream to Blue Nile. The District is characterized by two rainy seasons: June to September-the main rainy season and March to May-the small rainy season.

According to the 2007 national census, the population of the District is 158,920 of which 78,988 are males and 79,932 are females. The rural population is 149,440 and the urban is 9,480. Every Kebele has at least one health extension worker who is assigned to provide home-to-home health service to the community.

According to the clinical records from the Health Center, malaria and intestinal parasitic infections are common in the District. Hence, malaria prevention and control interventions are being implemented. These include annual indoor residual spray (IRS), distribution of ITNs/LLINs in selected areas of malarious Kebeles and prompt treatment of malaria cases using ACT-Coartem as a first line treatment based on rapid diagnosistic Tests (RDTs). Moreover, there has been deworming of intestinal helminth infections based on targeted segment of the community (children less than age five) from selected Kebeles based on clinical report of the diseases. This has been carried out with the support of the Enhanced Outreach services (EOS) for child intervention funded by CIDA, Canada (Personal communication, Yoseph Mulugeta, Malaria and Other Infectious Diseases control unit, February 2009, Mekane-Selam).

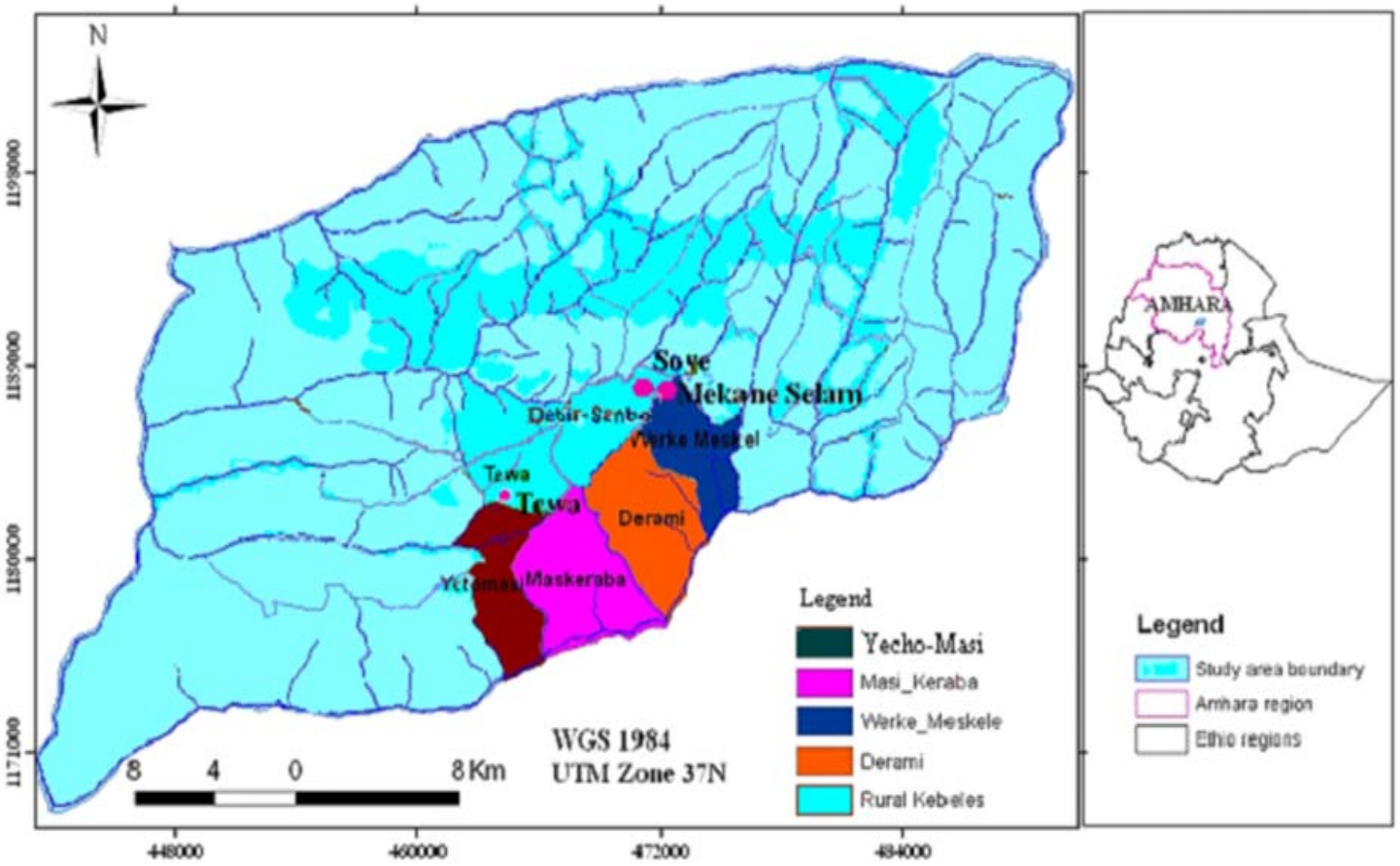

Figure 2. Map showing the study area (Adapted from Borena District office of agriculture).

3.2 The study population and the sampling technique

A cross-sectional survey was conducted during the main malaria transmission period, after the main rainy season, from Nov.28-Dece.27/2008. Random sample of households from the sample Kebeles were participated for providing blood film and stool sample by the law of voluntarism. Along with sample collection, face-to-face interview of selected heads of household was conducted using a pre-formatted Amharic language questionnaire, which constituted sociodemographic information. During sample collection, participants who had been absent at the time of sampling and those relatives who had come during the sampling time were excluded without any replacement.

Sample size was determined using the formula for estimating single proportion at the level of 95% CI [$Z\alpha/_2 = 1.96$]. Using the clinical record prevalence of intestinal parasitic infection from Mekane-Selam Health Center, we took average prevalence (P)=48.7 (Table 3). Using the formula, $n=Z^2xPx(1-P)/d^2$ a minimum of 600 samples (n) was estimated taking the marginal error (d) 4% as shown below:

$n=Z^2 P (1-P)/d^2$	Where, n-sample size
$n=(1.96)^2x\ 0.487x(1-0.48.7) / (0.04)^2$	P- average prevalence
n= 0.96/ 0.0016	Z^2 - value at 95% CI, from table
n= 600	d-worst accepted value (marginal error).

Hence, once the minimum sample was obtained, a contingency of 5% was added and then the sample size reached 630.

The survey was conducted in four sample Kebeles which were selected for convenience of sampling and their accessibility (Awasthi *et al*., 2008). Using the assumption of six average families (WHO, 2005), 105 heads of household were selected. By using proportional stratified random sampling method, the households were proportionally distributed among the sample Kebeles (sample sites). Hence, sample households from each Kebele were selected using systematic random sampling technique. The stratification was based on the proportion of samples for each sample Kebele (study site) since the sample Kebeles have different population sizes. Five hundred fifty four households consented for stool sample collection and five hundred twenty for blood film.

3.3 Parasitological study

Before collecting blood sample for malaria diagnosis, the finger was cleaned with alcohol-moistened cotton, dried with dry cotton, and then punctured by using sterile disposable blood lancet. Using the drop of blood, thin and thick blood smears were made on a single glass per individual. The smears were air-dried and the thin smear was fixed with 100% methanol for 30 seconds in Mekane-Selam health center laboratory. Following this, the smears were stained with 3% Giemsa for 30 minutes. Staining and blood film examination was performed by following the standard protocol of WHO (Payne, 1988; Garcia, 2001). Experienced laboratory technician and technologist examined the slides. The presence of malaria parasites on thick blood smear was examined by using high power magnification objective (40x) and the identification of *Plasmodium* species from the thin blood smear was through oil immersed objective (100x). The xylene cleaned and appropriately, dried slides were kept in the slide box and transported and re-examined in the biomedical science laboratory, Department of Biology, Addis Ababa University (AAU).

During stool sample collection, plastic sheets were given to each participant and told to bring about 4gm of stool. The samples brought by the participants were collected after registering the names and giving identification number to each participant. Direct wet mounts were prepared with normal saline (0.85% NaCl solution). The smear was diagnosed for parasites under light microscope of 10x and then with 40x objectives in Mekane-Selam Health Center laboratory. The rest of the sample was preserved in 10% formalin solution for future examination.

Each stool sample was processed and examined by using the Formal Ether Concentration technique according to the guidelines set by WHO bench aid for the diagnosis of intestinal parasites (WHO, 1981). This was done in the biomedical laboratory, Department of Biology, Addis Ababa University. About 1gm of stool from the 10% formalin preserved stool sample was taken using the applicator stick and placed in conical 15ml centrifuge tube containing 10 ml formalin. The stool was suspended after thorough mix using applicator stick. Then the suspension formed was filtered using cotton gauze into clean and labeled centrifuge tube, discarding the debris along with the cotton gauze. Then it was centrifuged at 2000 rpm for one minute. On removal from the centrifuge the supernatant fluid was decanted, 10ml of

10%, formalin added to the sediment then thoroughly mixed, and after 10 minutes of fixation, 3ml of Diethyl-ether was added and shaken for thorough mix. The mixture was centrifuged at 2000 rpm for 2 minutes. The sediment and supernatant liquid were separated by decantation. Finally, stool smear with iodine stain were prepared and observed for intestinal parasites under 10x and 40x objectives.

During blood film and stool sample collection, two laboratory experts, one supervising nurse, and one guide participated.

3.4 Questionnaire

Using a questionnaire, all the heads of household were interviewed during sample collection. The questionnaire included socio-demographic information, experience with malaria and intestinal parasitic infection and use of anti-malarial and anti-intestinal parasite drugs, about use of malaria and intestinal parasitic infection preventive, control measures, and other information relevant to malaria and intestinal parasites.

3.5 Ethical consideration

The Addis Ababa University, Faculty of Science, Department of Biology ethical committee had issued ethical clearance before the study was conducted through an official letter to the Borena District health office. Thus, in agreement with the letter received, the District health office in collaboration with the District capacity building office agreed and collaborated in many aspects of the study.

Before starting sample collection, written consent (appendix 1**)** was obtained from the participants and parents or guardians for children. Privacy was assured during interview of the study participants (heads of household). Those participants who were positive either for malaria and intestinal parasites were treated with anti-malarial drugs, chloroquine (*P. vivax*) and Coartem (*P. falciparum*), and anti-helminthic drugs (Albendazol as a broad wormcide) free of charge by Mekane-Selam Health Center. The cost of anti-helminthic drugs was covered by the study. In addition, the Health Center professional staffs gave health education relevant to malaria and intestinal parasitic infection prevention and control.

3.6 Data analysis

SPSS version 15.0 was used for statistical data analysis. Descriptive statistics was used to give a clear picture of population characteristics such as age, sex, and distribution of *Plasmodium* species and intestinal parasites. Univariate analysis based on Odds Ratio was conducted to show the association of risk factors with disease. Association of sex with proportion of malaria and intestinal parasites was made using chi-square test. Statistical significance was defined at P-values less than 0.05 ($P<0.05$).

3.7 Significance of the study

This study will help to provide significant information on the level of current situation of malaria and intestinal parasite prevalence transmission in the Borena District. In addition, it will help to evaluate the effectiveness of malaria and intestinal parasite control interventions measures. Moreover, future research in the District may use the result as base line information.

4. Results

4.1 Malaria from outpatient clinical record of Mekane-Selam Health Center in Borena District

According to the retrospective clinical record from Mekane-Selam Health Center (2003-2008), the *Plasmodium* species detected in Borena District were *P. falciparum* and *P. vivax*. The cumulative prevalence of malaria out of the whole clinical record of the past six years showed a decreasing trend (Tables 1 and 2). For example, the highest annual cumulative prevalence (33.1%) of malaria was detected in the 2003 malaria year. This was much higher than that in 2008 (7.0%). Moreover, patients who were visiting the Health Center and the number of positives for malaria infection had generally been decreased from 2003 onwards (Table 1).

In the past six year clinical record, malaria prevalence peaks were detected mostly from September to December. For example, in 2003, there were high malaria infection peaks in the months of January (45.1%), June (39.3%), September (38.7%), October (42.6%), November (41.4%) and December (24.2%). But, in 2004 the malaria prevalence peaks were in February (29.8%), March (35%) and December (31%) and in 2005, high malaria prevalence was detected almost throughout the year (Table 1).

Table 1: Malaria prevalence by month and year from the outpatient clinical records oMekane-Selam Health Center in Borena, South Wollo, Ethiopia, 2003-2008

Year	2003		2004		2005		2006		2007		2008	
Month	Total examined	No. (%)	Total examined	No. (%)	Total examined	No. (%)	Total examined	No. (%)	Tot. examined	No. (%)	Total examined	No. (%)
January	144	65(45.1)	204	40(19.6)	79	32(40.5)	77	14(18.2)	97	16(16.5)	20	0
February	211	62(29.4)	121	36(29.8)	107	51(47.7)	135	36(26.7)	141	35(24.8)	10	0
March	96	23(24.0)	134	48(35.8)	144	43(29.9)	149	23(15.4)	79	3(3.8)	8	0
April	83	15(18.1)	75	21(28.0)	24	8(33.3)	89	12(13.5)	87	1(1.1)	5	0
May	76	8(10.5)	77	10(13.0)	110	42(38.2)	*NR*	*NR*	84	14(16.7)	5	2(40.0)
June	89	35(39.3)	56	14(25.0)	102	37(36.3)	*NR*	*NR*	24	2(8.3)	9	0
July	114	33(28.9)	74	20(27.0)	121	27(22.3)	*NR*	*NR*	55	5(9.1)	4	3(75.0)
August	100	29(29.0)	71	19(26.8)	152	45(29.6)	*NR*	*NR*	77	5(6.5)	15	1(6.7)
September	191	74(38.7)	119	24(20.2)	176	72(40.9)	110	8(7.3)	64	4(6.3)	64	6(9.4)
October	251	107(42.6)	142	39(27.5)	175	72(41.1)	170	33(19.4)	112	5(4.5)	58	5(8.6)
November	249	103(41.4)	180	44(24.4)	208	46(22.1)	127	38(29.9)	82	3(3.7)	79	5(6.3)
December	265	64(24.2)	88	28(31.8)	122	26(21.3)	47	14(29.8)	113	0	78	5(6.4)
Total	1869	618(33.1)	1341	343(25.6)	1520	501(33.0)	904	178(19.7)	1015	86(8.5)	355	25(7.0)

Key: *NR*-no clinical records in the indicated months.

In 2003, the annual cumulative prevalence of malaria was 33.1%, which is above the overall average malaria prevalence (25.0%). In 2004, it was reduced to 25.6%, which again went up to 33.0% in 2005. Then, in the years beyond 2005 annual cumulative malaria prevalence followed a decreasing trend of 19.7%, in 2006; 8.5% in 2007 and 7.0% in 2008 (Table 2). In almost all the malaria years, it seemed that, more males were infected than females. For instance, in the year 2003 from the total of 1869 patients examined for malaria in the Health Center, only 601 (33.2%) were females while the rest, 1268 (67.8%) were males of which 71 female and 274 male were detected malaria infected, respectively (Table 2).

Analysis of the clinical record of malaria showed that the relative prevalence of *P. falciparum* to that of *P.vivax* was detected with very small difference within a year. Nevertheless, a relatively more dominant *P. falciparum* infection was detected. For example, relative prevalence of *P. falciparum* to that of *P.vivax* was 18.5%:14.6%, in 2003; 11.4%:14.2%, in 2004; 17.0%:15.9% in 2005; 11.5%:8.2%, respectively.

Table 2: Malaria prevalence by parasite species from outpatient clinical records of Mekane-Selam Health Center in Borena, South Wollo, Ethiopia, 2003-2008

Year	Total examined	*Pf* (no. %)			*Pv* (no. %)			Total
		M	F	total	M	F	total	
2003	1869	274 (14.7)	71 (3.8)	345 (18.5)	194 (10.4)	79 (4.2)	273 (14.6)	618 (33.1)
2004	1341	118 (8.8)	35 (2.6)	153 (11.4)	139 (10.4)	51 (3.8)	190 (14.2)	343 (25.6)
2005	1520	174 (11.4)	85 (5.6)	259 (17.0)	187 (12.3)	55 (3.6)	242 (15.9)	501 (33.0)
2006	904	74 (8.2)	30 (3.3)	104 (11.5)	62 (6.9)	12 (1.3)	74 (4.9)	178 (19.7)
2007	1015	45 (4.4)	16 (1.6)	61 (6.0)	15 (1.5)	10 (1.0)	25 (2.5)	86 (8.5)
2008	355	8 (2.3)	4 (1.1)	12 (3.4)	10 (2.8)	3 (0.8)	13 (3.7)	25 (7.0)
Total	7004	693 (9.9)	241 (3.4)	734 (10.5)	607 (8.7)	210 (2.9)	817 (11.7)	1751 (25.0)

Key: *Pf-Plasmodium falciparum* and *Pv-Plasmodium vivax.*

4.2 Intestinal parasites from outpatient clinical record of Mekane-Selam Health Center in Borena District

Analysis of clinical record from Mekane-Selam Health Center revealed high cumulative annual prevalence of intestinal parasitic infection (46.5%) over the six years (2003-2008). Eight types of intestinal parasites were identified in the Health Center: *E. histolytica/dispar, A. lumbricoides, H. nana,* hookworm, *G. lamblia*, *S. stercoralis, E. vermicularis* and *Taenia* species. Of these intestinal parasitic infections, *E. histolytica/dispar* (27.7%) was the most frequent infection followed by *G. lamblia* (9.4%), *A. lumbricoides* (3.7%) and *H. nana* (2.1%) infections (Table 3). However, the rest of intestinal parasitic infections were relatively low (Table 3). The highest degree of reduction in infection prevalence was observed for *G. lamblia* from 2003 (30%) to 2008 (3.1%). Such high reduction in annual prevalence was not observed for the geo-helminths, *E. histolytica/dispar,* and *Taenia* species (Table). However, the annual cumulative prevalence of intestinal parasitic infection between year 2003 (71.1%) and 2008 (36.5%) remained very high.

Table 3: The prevalence of intestinal parasites by species and year from outpatient clinical records of Mekane-Selam Health Center in Borena, South Wollo, Ethiopia, 2003-2008

Year	total examined	*Gl*	*Al*	hw	*H.nana*	*Eh/dis*	*Ev*	*Ss*	*T*.spp	Total
2003	651	195 (30)	16 (2.5)	8 (1.2)	15 (2.3)	208 (32)	11 (1.7)	6 (0.9)	4 (0.6)	463 (71.1)
2004	1009	167 (16.6)	36 (3.6)	10 (0.9)	26 (2.6)	223 (22.1)	15 (1.5)	11 (1.1)	0 (0)	488 (48.4)
2005	1049	92 (8.8)	59 (5.6)	14 (1.3)	43 (4.1)	253 (24.1)	20 (1.9)	6 (0.6)	8 (0.8)	495 (47.2)
2006	880	69 (7.8)	40 (5.1)	7 (0.9)	10 (1.3)	271 (30.8)	3 (0.4)	9 (1.2)	1 (0.12)	410 (46.6)
2007	1073	9 (0.8)	27 (2.5)	8 (0.7)	22 (2.1)	379 (35.4)	3 (0.3)	6 (0.6)	6 (0.6)	460 (42.9)
2008	1467	46 (3.1)	46 (3.1)	27 (1.8)	11 (0.7)	366 (24.9)	13 (0.9)	14 (1)	12 (0.8)	535 (36.5)
Total	6129	578 (9.4)	224 (3.7)	74 (1.2)	127 (2.1)	1700 (27.7)	65 (1.1)	52 (0.85	31 (0.51)	2851 (46.5)

Key: *Gl-G. lamblia, Al-A. lumbricoides*, hw-hookworm, *Eh/dis-E. histolytica/dispar*, *Ev-E. vermicularis, Ss-S. stercolaris* and T.spp-Taenia species.

Malaria was the most prevalent disease among the four diseases during the years 2003, 2004 and 2005 (Fig.3). After 2005, amoebiasis due to *E. histolytica/dispar* became the most prevalent infection among the four parasites compared. The other parasitic diseases on record such as giardiasis showed nearly the same infection rate in the six years clinical records; while helminthiasis had shown a general fluctuation in prevalence (Fig. 3).

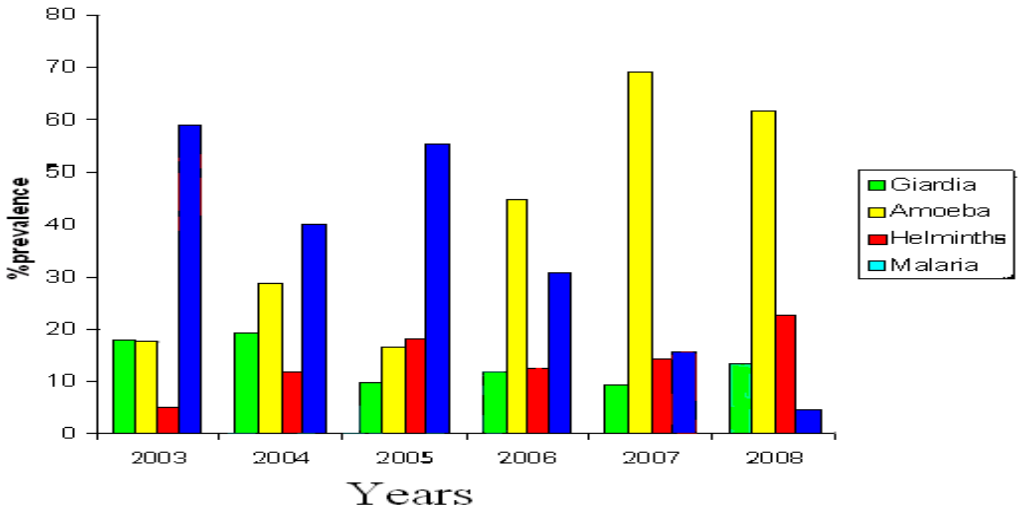

Figure 3: The prevalence of malaria and other parasitic diseases from outpatient records of Mekane-Selam Health Center, in Borena, South Wollo, Ethiopia, 2003-2008.

4.3 Parasitological survey

From the sample Kebeles, 554 (88%) individuals were participated for stool sample provision and 520 (83%) provided blood film. Although very low malaria prevalence was detected in the present study, it was detected from Derami, Worke-Meskele, Masi-Keraba and none from Yecho-Massi kebele. The same prevalence (0.4%) of malaria was detected in Derami and Masi-Keraba (Table 4). Intestinal parasites are detected most frequently (12.6%) in Worke-Meskele followed by Derami (6.7%). There was no co-infection of malaria with intestinal parasites in the present study; whereas multiple parasitic infections within intestinal parasites were common (12.5%).

Table 4. Malaria and intestinal parasites prevalence of the survey population from Borena, South Wollo, Ethiopia, 2008

Sample Kebeles	Parasitic infection								
	Malaria (N=520)		Intestinal parasites (N=554)						
	Pf	*Pv*	*Gl*	*Eh/d*	*Al*	hw	*Ev*	Ss	*H.nana*
Derami	1(0.2)	1(0.2)	2(0.4)	13(2.3)	13(2.3)	3(0.5)	0(0.0)	1(0.2)	5(0.9)
Worke-Meskele	0(0.0)	1(0.2)	5(0.9)	29(5.2)	17(3.1)	4(0.7)	2(0.4)	2(0.4)	11(2.0)
Yecho-Masi	0(0.0)	0(0.0)	1(0.2)	7(1.3)	4(0.7)	2(0.4)	2(0.4)	2(0.4)	2(0.4)
Masi-Kera	1(0.2)	1(0.2)	2(0.4)	11(2.0)	8(1.4)	2(0.4)	1(0.2)	0(0.0)	3(0.5)
Total	2(0.38)	3(0.58)	10(1.81)	60(10.83)	42(7.6)	11(2.0)	5(0.9)	5(0.9)	20(3.6)

Key: *Pf-P. falciparum* and *Pv-P.vivax, Gl-G. lamblia, Al-A. lumbricoides*, hw- hookworm, *Eh/dis-E. histolytica/dispar*, *H.nan-H.nana, Ev-E. vermicularis* and *Ss-S. stercolaris.*

4.3.1 Malaria prevalence determination from the survey population in Borena District

Among the 520 individuals that participated for providing blood film, only 5(0.96%) were found infected with malaria (Table 4). The *Plasmodium* species identified were *P. falciparum* and *P. vivax. P. falciparum* was found infecting two of the participants while three individuals were infected with *P. vivax*. Regarding age, 20% of malaria positive individuals were below the age of five years and 80% were above 5 years old. Furthermore, the few malaria cases detected were in the age groups below 5 and 15 and above years (Table 5).

Table 5: Age specific malaria infection and *Plasmodium* species prevalence among the survey population in Borena, South Wollo, Ethiopia, Nov.-Dec., 2008 (N=520)

Age group	Number examined	*Pf*	*Pv*	Total
< 5	5	1(20)	0(0.0)	1(20)
5-14	67	0(0.0)	0(0.0)	0(0.0)
≥ 15	448	1(0.22)	3(0.67)	4(0.89)
Total	520	2(0.38)	3(0.58)	5(0.96)

Key: *Pf-Plasmodium falciparum* and *Pv-Plasmodium vivax.*

Comparison of malaria cases detected in the survey sample population and that on clinical record for the same period of time of the year showed the prevalence from clinical records (0.98%) to be similar to the prevalence (0.96%) detected in the survey (Table 6).

Table 6: Comprison of malaria prevalence of the survey population and clinical records from outpatients of Mekane-Selam Health Center in Borena, South Wollo, Ethiopia, 2008/09

Age group (n=102)	Survey participants Nov.-Dec., 2008 (n=520)			Clinical records Nov.-Dec., 2008		
	Pf	*Pv*	Total	*Pf*	*Pv*	Total
< 5	1(0.19)	0(0.0)	1(0.19)	0(0.0)	0(0.0)	0(0.0)
≥ 5	1(0.19)	3 (0.58)	4(0.77)	0(0.0)	1(0.98)	1(0.98)
Total	2(0.38)	3 (0.58)	5(0.96)	0(0.0)	1(0.98)	1(0.98)

Key: *Pf-Plasmodium falciparum* and *Pv-Plasmodium vivax.*

4.3.2 Intestinal parasite prevalence determination from the survey population in Borena District

Microscopic examination of the stool sample detected seven types of intestinal parasite species, namely: *E. histolytica/dispar, A. lumbricoides, H. nana,* hookworm, *G. lamblia*, *S. stercoralis and E. vermicularis* in order of decreasing frequency (Table 7). The overall prevalence among the survey population in terms of the number of individuals diagnosed was 136 (24.6%). Of this, single parasitic infection was detected in 119 (87.5%) among intestinal parasite positives. Multiple parasitic infection was identified in 17 (12.5%) among intestinal parasite positives and 3.1% among the survey population. The mean age of the participants who had intestinal parasite was 34.05 [SD=18.53]. Of these, 20 are below the age of 18years while the rest were above and including 18years.

Two sample Kebeles have hosted all the seven intestinal parasites detected in this study. Highest infection of *A. lumbricoides* and *E. histolytica/dispar,* 13.8% of each, was detected in Derami. The highest intestinal parasitic infection was detected from Worke-Meskele followed by Derami. The difference in terms of intestinal parasite prevalence between Worke-Meskele and that of Derami was significant (P=0.017). There was also significant difference in the prevalence of intestinal parasites between Worke-Meskele and Masi-Kera (P=0.043) (Table 7).

Table 7: Intestinal parasite prevalence distribution over sample *Kebeles* in Borena, South Wollo, Ethiopia, Nov.-Dec. 2008

Sample sites	Protozoa		Nematodes				Cestodes
	Gl	*Eh/d*	*Al*	hw	*Ev*	*Ss*	*Hnana*
Derami (n=94)	2(2.1)	13(13.8)	13(13.8)	3(3.2)	0(0.0)	1(1.1)	5(5.3)
Worke-Meskele (n=205)	5(2.4)	29(14.1)	17(8.3)	4(2.0)	2(1.0)	2(1)	11(5.4)
Yecho-Masi (n=143)	1(0.7)	7(4.9)	4(2.8)	2(1.4)	2(1.4)	2(1.4)	2(1.4)
Masi-Keraba (n=112)	2(1.8)	11(9.8)	8(7.1)	2(1.8)	1(0.9)	0(0)	3(2.7)
Total (N=554)	10 (1.81)	60 (10.83)	42 (7.6)	11 (1.99)	5 (0.9)	5 (0.9)	20 (3.6)

Key: *Gl-G. lamblia, Al-A. lumbricoides*, hw-hookworm, *Eh/dis-E. histolytica/dispar*, *Ev-E. vermicularis* and *Ss-S. stercolaris.*

The prevalence of *E. histolytica/dispar* was the highest (10.83%) in the present study. The second highest parasitic infection was by *A. lumbricoides* (7.6%). In addition, *H. nana* with prevalence of 3.6% was the third highest infection. Least prevalence was detected for the rest of intestinal parasites (Table 8). In the present study, more males were infected with intestinal parasites than that of females. The difference was significant (P=0.001). There were more females who are infected with *E. histolytica/dispar* than that of males. But, the difference was not significant (P=0.178). More prevalence of *A. lumbricoides*, *H. nana* and hookworm infections were detected in male than that in female. In all of these, the differences were not significant (P>0.05).

Table 8: Gender related distribution of intestinal parasites among the survey population in Borena, South Wollo, Ethiopia, 2008/09

Type of parasite	No. (%)		
	Male(n=72)	Female(n=64)	Total (n=136)
G .lamblia	6(8.3)	4(6.25)	10(7.4)
E. histolytica/dispar	27(37.5)	33(51.6)	60(44.1)
A. lumbricoides	27(37.5)	15(23.4)	42(30.9)
hookworm	6(8.3)	5(7.8)	11(8.1)
E. vermicularis	1(1.4)	4(6.25)	5(3.7)
S. stercoralis	2(2.8)	3(4.7)	5(3.7)
H. nana	10(13.9)	10(15.6)	20(14.7)
Total	72(52.9)	64 (47.1)	136 (100)

High prevalence of intestinal parasite infection were seen in the age groups 0-9, 10-14 and above 14 years with prevalence of 81%, 64.1%, and 20.3%, of which the most prevalent infection was seen in the age group 0-9years (81%) (Table 9). Age group 0-9 years has no infection with *E. vermicularis*. *E. histolytica/dispar, A. lumbricoides* and *G. lamblia* infections were shown to be the most frequent infections in the age group of 0-9 years. The most frequent *E. histolytica/dispar* infection followed by *A. lumbricoides* and *H. nana* infections were seen in the age group 10-14 years. Hookworm infection was the most frequent in the age group 0-9, while it showed a decreasing trend in the age groups above 9 years. *E. histolytica/dispar* was the most frequent infection among the age group ≥15 years. Analysis showed that there was no significant difference in the prevalence of intestinal parasitic infections between age groups (P>0.05).

Table 9: Age related distribution of intestinal parasites among the survey population in Borena, South Wollo, Ethiopia 2008/09

Age groups	Number examined	*Gl*	*Eh/disp*	*Al*	*H. nana*	hw	*Ss*	*Ev*	Total
0-9	21	3(14.3)	5(23.8)	4(19.0)	2(9.5)	2(9.5)	1(4.8)	0(0.0)	17(81)
10-14	64	4(6.3)	16(25.0)	9(14.1)	7(10.9)	3(4.7)	1(1.6)	1(1.6	41(64.1)
≥ 15	469	3(0.6)	39(8.3)	29(6.2)	11(2.3)	6(1.3)	3(0.6)	4(0.9)	95(20.3)
Total	554	10(1.81)	60(10.83)	42(7.6)	20(3.6)	11(1.99)	5(0.9)	5(0.9)	136(24.5)

Key: *Gl-G. lamblia, Al-A. lumbricoides*, hw-hookworm, *Eh/dis-E. histolytica/dispar*, *H.nan-H.nana, Ev- E. vermicularis* and *Ss-S. stercoralis.*

*17 (153-136) multiple parasitic infection.

The overall prevalence of protozoan infection (48.5%) identified in the present study was lower than that of helminthic infection (51.5%). Similar trend was seen in the age group of 0-9 years. The prevalence of protozoan infection had generally higher infection in the age groups 0-9 and 10-14 year). Helminthic infection prevalence had also shown similar trend (Table 9).

Multiple intestinal parasitic infection prevalence (3.1%) was detected among the survey population and 12.5% among the intestinal parasite positive individuals. Most combination of infections was bi-parasitic where as only a single triple infection was detected (Fig.9). Relatively high prevalence of multiple parasitic infections (5.1% and 4.4%) was observed in the age groups of 5-14 followed by ≥ 15, years respectively.

The co-infection of hookworm with *A. lumbricoides*, *A. lubricoides* with *E. histolytica/dispar* and hookworm with *E. histolytica/dispar* in double infection is 17.6% each. In addition *A. lubricoides* with *E. vermicularis* (11.8%), S. stercorlis with *A. lubricoides*, *H. nana* with *A. lubricoides*, *E. histolytica/dispar* with S. stercoralis and *G. lamblia* with *E. vermicularis*, 5.9% each, were contributors to the double infection in the present study. Single infection was due to *E.histolytica/dispar* (39%), *G.lamblia* (23.2%), *A. lubricoides* (22.8%), *E. vermicularis* (6.5%), hookwarm (3.0%) *and S. Stercorlis* (3.0%). Triple infection was due to the concurrent infection of *G.lamblia* with *A. lubricoides* and hookworm. *A. lumbricoides* was the most frequent contributor to the double infection followed by *E. histolytica* and hookworm in this study. There was relatively less multiple parasitic infection prevalence (29.4%) in males than that (70.6%) in females among the survey population. However, the difference was not statistically significant (P > 0.05).

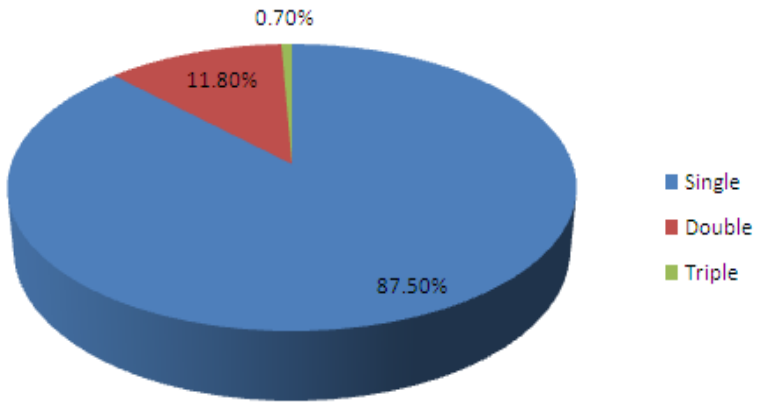

Figure 4: Pie chart showing multiple parasitic infections among the survey population from Borena, South Wollo, Ethiopia 2008/09.

4.4 Assessment of Knowledge, Attitude and Practices (KAPs) towards malaria and intestinal parasite control and prevention in Borena District

All the 105 heads of household properly responded to the questionnaire. Out of these, 65 (61.9%) were males while 40 (38.1%) females. The mean age of the heads of household was 46.40 [STD=11.72356]. All of the interviewees were farmers of differing educational levels: 16.2% who cannot read and write, 25.7% those that can read and write, 33.3% who dropout from elementary school, 14.3% dropout from high school and 10.5% those who completed

high school education. There was no association between educational status and malaria infection prevalence in this study (P > 0.05).

Among the interviewees, 76 (72.4%) reported of malaria infection, of which 71 (93.4%) attended health services for treatment (Table 10) a month before the time of interview. Insignificant number of malaria infected heads of household, 5 (6.6%), were not treated for the reported malaria infection. Most of the respondents, 60 (57.1%), reported that they did not know the name of the anti-malaria drug even though they knew its importance. Only 45 (42.7%) of household heads reported that the anti-malaria drugs used were Chloroquine and Coatrum and have been supplied freely and they know well its importance.

Table 10: The proportion of heads of household who reported experience of malaria infection, ITN and anti-malarial drug use in Borena, South Wollo, Ethiopia, 2008

Sample kebeles	N0.(%)				
	Interviewed hh heads	Malaria infected	Treated (n=76)	ITN used hh heads (N=105)	Sick after ITN use (n=103)
Yecho-Masi	17	8(47.1)	8(100)	17	1(5.9)
Masi-Keraba	40	31(32.5)	28(90.3)	40	0
Worke-Mesk	27	20(74.1)	19(95)	27	0
Derami	21	17(81.0)	16(94)	19	0
Total	105	76(72.4)	71(93.4)	103(98.1)	1(.97)

Key: hh=household

Most of the interviewees responded that anti-mosquito insecticide spray was conducted once every year, particularly, in September after the cease of the main rainy season. From the 105 interviewees, 103 (98.1%) have been using insecticide treated bed nets (ITNs/LLINs), supplied by the government of Ethiopia , every day and all the family members until the survey time; while 2 (1.99%) have not used the bed net due to large family size, leaving it for children. Regarding malaria infection after ITNs/LLINs use, 102 (99.03%) heads of household reported that they were not infected with malaria after starting ITNs/ LLINs use ; but 1 (0.97%) reported malaria infection after the use of ITNs. From the above information, it could be generalized that the respondents have good awareness towards the use and

knowledge of anti-malarial drugs and malaria prevention and control measures such as ITN/LLIN for the preventing malaria.

No respondent was moved to other mlarious areas within a month before the survey time. Heads of household of 15 (14.3%) responded that they often work at night in their garden; but working in the garden at night was not significantly associated with malaria infection (P= 0.0645). Twenty-five respondents reported that they have used to keeping their cattle out door at night. It was analyzed that no significant association between malaria infection and outdoor cattle keeping at night (P=0.783) in the present study.

Analysis of the records of information from the interview for intestinal parasitic infection showed that all the 105 heads of household responded to the interview. Out of these, intestinal parasitic infections were detected in 15 (14.3%) males and 10 (9.5%) females, and with an overall prevalence of 23.8%. Of these intestinal parasite infected individuals, 10 (40%) had received anti-intestinal parasite treatment from health services. Among the heads of household (70.5%) had always washed their hands before eating food items and after toilet use, 64 (61%) never eat raw meat, 48 (45.7%) did not eat uncooked vegetable foods, 34 (32.4%) only drank boiled water. In addition, 52 (49.5%) of the heads of household had disposed household wastes on the open field and 34 (31.4 %) had prepared toilet. Generally, low awareness on average (47.2%) was shown by the heads of household towards the knowledge of risk factors to intestinal parasite infection.

Analysis of the risk factors for intestinal parasitic infection showed that there was no significant association between sex (being female or male) and the risk of intestinal parasitic infection (P=0.175). However, some of the heads of household were complaining of having signs and symptoms related to intestinal parasites such as abdominal pain and abdominal cramp 25 (23.8%), bloating 12 (11.4%) and nausea 14 (13.3%). Abdominal pain and cramp (OR= 36.00, P=0.000), bloating (OR= 27.76, P=0.001) and nausea (OR= 24.7, P=0.000) were significantly associated with intestinal parasitic infections. Diarrhea in general, was reported by 55 (52.3%) and bloody diarrhea by 10 (3.8) of the heads of household with bivariate analysis showing its significant associated with the risk of intestinal parasitic infection (OR=9.00; P=0.005).

The risk factors considered in the present study were sex, toilet use, and regular hand wash/not wash before meal and after toilet use, sources of drinking water, not boiling drinking water, habit of food consumption (raw meat, uncooked vegetables) and household waste disposal methods used by the interviewees (heads of household).

Table 11. Risk factors and intestinal parasite infection among the study populations in Borena, South Wollo, Ethiopia, 2008/09

Risks	Present no.(%)	Absent no.(%)	Total no.(%)
Sex: Male	15(60)	50(62.5)	65(61.7)
Female	10(40)	30(37.5)	40(38.3)
Education status: Illiterate	9(36)	8(10)	17(16.2)
Literate	16(64)	72(90)	88(83.8)
Toilet: present	7(20)	27(33.7)	34(31.4)
Absent	18(80)	53(66.3)	71(68.6)
Hand washed before meal and after toilet use: Yes	10(40)	64(80)	74(70.5)
No	15(60)	16(20)	31(29.5)
Sources of drinking water:			
Pond	0(0.0)	9(11.2)	9(4.8)
Spring	18(64)	48(60)	66(57.1)
River/stream	1(4)	4(5)	7(6.7)
Tap water	6(24)	19(23.8)	25(23.8)
Methods of use of drinking water: Direct	22(88)	49(61.3)	71(67.6)
Boiling	3(12)	31(38.7)	34(32.4)
Habit of eating raw meat: yes	12(48)	29(36.3)	41(39.0)
no	13(52)	51(63.7)	64(61.0)
Uncooked vegetables: Yes	16(64)	32(40)	48(45.7)
No	9(36)	48(60)	57(54.3)
Household waste disposal:			
Incineration/buried	12(48)	40(50)	52(49.5)
Open field	13(52)	40(50)	53(50.5)

The result of the simple bivariate analysis of risk factors for intestinal parasitic infection shown in (Table 13) implied that, being illiterate (OR=5.06, P=0.004), eating uncooked vegetables (OR=2.67, P=0.039), not washing hands before meal and after toilet use (OR=6.00, P=0.00) and direct use of (pond, river/ stream or tap water) (OR=7.84, P=0.002) can be considered as significant risks to intestinal parasite infection.

Risk factors such as absence of toilet, sources of drinking water, consumption of raw meat and open disposal of household wastes did not act as significant risk to intestinal parasitic infection (Table 12).

Using the bivariate analysis, whether each intestinal parasitic infection are associated or not associated with the risk factors outlined in Table 13, was tested. It was shown that the absence of the habit of hand washing before meal and after toilet was significantly associated with *G. lamblia* infection (OR=17.040, P=0.010) and *E. histolytica (*OR=5.104, P=0.001). But, the other risk factors such as the absence of toilet (present *vs* absent), sources of drinking water, types of drinking water, consumption of raw meat, uncooked vegetable consumption and household waste disposal methods were not associated with the risk of *G. lamblia* infection. There was no significant association between *A. lumbricoides* infection and the risk factors toilet (present *vs* absent), hand wash before meal and after toilet use, sources of drinking water (pond, spring, river/stream, tap water), methods of using drinking water, consumption of raw meat, uncooked vegetable consumption and household waste disposal. Similarly no significant association was seen between infection of Hookworm and *H. nana*, and any of the above risk factors.

Table 12. Factors associated with the risk of intestinal parasitic infection among the heads of household (n=105) of the study population in Borena, South Wollo, Ethiopia, 2008/09

Factor	No. (%)	OR(95%CI)
Sex: Female	10(40)	1.11(0.44-2.79)*
Male	15(60)	1.00
Education status: Illiterate	9(36)	5.063(1.69-5.14)**
Literate	16(64)	1.00
Toilet: a) absent	7(20)	0.76(0.28-2.05)*
Hand washed before meal & after toilet use: No	15(60)	6.00(2.28-15.82)***
Yes	10(40)	1.00
Sources of drinking water:		
Spring (spring *vs* others)	18(64)	1.71(0.64-4.57)*
River/stream (River *vs* others)	1(4)	0.79(0.08-7.43)*
Tap water (Tap water *vs* others)	6(24)	1.01(0.35-2.90)*
Types of drinking water: a) Direct	22(88)	7.84(2.12-29.00)*
b) boiling	3(12)	1.00
Consumption of: a) Raw meat	12(48)	1.62(0.66-4.03)*
b) cooked meat	13(52)	1.00
Uncooked vegetable consumption (uncooked *vs* cooked vegetables)	16(64)	2.67(1.05-6.77)**

Key: OR= odds ratio,
-- = no OR
* $P > 0.05$, ** $0.05 > P \geq 0.01$, *** $P<0.01$

Table 13. Factors associated with the risk of *G. lamblia*, *A. lumbriciodes* and *E. histolytica/dispar* infection

Risk factor	*G. lamblia*			*A. lumbricoides*			*E. histolytica/dispar*		
	P(%)	OR (95%CI)	P	P (%)	OR (95%CI)	P	P(%)	OR(95%CI)	P
Sex (Female *vs* Male)	2(1.9)	3.32(0.29-37.8)	0.334	6(5.7)	1.28(0.30-5.42)	0.741	3(2.9)	0.58(0.144-2.32)	0.44
Education (Illiterate *vs* Literate)	4(3.8)	4.21(1.04-6.95)	0.043	1(0.95)	2.69(0.23-31.43)	0.43	6(5.7)	9.06(2.36-34.69)	0.001
Toilet(present *vs* Absent)	2 (1.9)	4.18(0.37-47.79)	0.250	6(5.7)	4.94(0.98-17.87)	0.053	11(10.5)	2.933(0.61-14.05)	0.799
Hand washed before meal & after toilet use (yes *vs* no)	6 (5.7)	17.04(1.95-148.57)	0.010	2(1.9)	5.034(0.44-57.67)	0.194	7(6.7)	5.104(1.38-18.98)	0.001
Sources of drinking water:									
Pond (pond *vs* others)	0	---	---	0	---	---	0	--	--
Spring (spring *vs* others)	1 (0.95)	0.285(0.03-3.25)	0.312	7(6.7)	2.195(0.43-11.14)	0.343	10(9.5)	6.786(0.83-55.22)	0.07
River/stream (River *vs* others)	1 (0.95)	12.04(1.95-148.57)	0.059	0	--	--	0	--	---
Tap water (Tap water *vs* others)	1 (0.95)	1.625(0.14-18.71)	0.697	2(1.9)	0.907(0.18-4.67)	0.907	1(1)	0.292(0.04-2.40)	0.25
Methods of using drinking water	8 (7.6)	2.032(0.41-10.13)	0.387	0	--	--	10(9.5)	5.410(0.66-44.13)	0.1
Consumption of Raw meat*	2 (1.9)	1.043(0.17-6.53)	0.946	5(4.8)	2.083(0.53-8.27)	0.29	5(4.8)	1.343(0.38-4.72)	0.646
Uncooked vegetable	0	---	---	7(6.7)	4.695(0.93-23.79)	0.06	8(7.6)	3.60(0.90-14.43)	0.071

Key: OR= odds ratio

-- = no OR, P=prevalence

* Raw meat *vs* cooked meat

P(%) =prevalence in %

5. Discussion

The retrospective data analysis indicated that the seasonal malaria transmission had been a major cause of outpatient morbidity in Borena Dstrict and imposed heavy burden on the health services for the last six years. Although there is no documented information on the nature of annual maximum and minimum temperature, rain fall and relative humidity in the District, the extension of heavy rainy season until November may have been creating conducive environmental condition for malaria transmission in the District. This is consistent with the report from malaria prevalence and associated risk factors that variations in weather condition act as a basic cause for the occurrence of malaria epidemics (Abeku *et al.,* 2004).

Analysis of the clinical data record from Mekane-Selam Health Center showed that malaria prevalence was reduced (25.0%) in 2004 compared to that in 2003 (33.1%). This may have been due to the control intervention measures following the 2003 malaria epidemic. The escalation of malaria prevalence to 30.1% by 2005 may have been associated with discontinuation of the control intervention measures as reported elsewhere (Bayoh and Lindsay, 2003). Thenafter, the prevalence of malaria has been successibly reduced from 2006 onwards following resumption of integrated prevention and control measures through the global Roll Back Malaria (RBM) initiatives (Personal information, Yoseph Mulugeta, Malaria and Other Infectious Diseases Control Unit, February 2009, Mekane-selam). Such reduction in malaria prevalence following control measures is reportedly known in other African countries such as Eritrea, Namibia, South Africa, Zambia Zanzibar (WHO, 2009), and several regions such as Tigray, Oromia, SNNPR (Shargie *et al*., 2008) including Amhara (Otten *et al*., 2009) where the present study was conducted.

According to the clinical record, malaria prevalence peak was observed in February (29.8%), March (35%) and December (31%) in 2004. In 2005, high malaria prevalence was detected almost throughout the year. The malaria prevalence peak in December is likely to occur, but the prevalence peaks in February and March by 2004 and throughout the year by 2005 are unlikely occurrences and hence these could be to some extent due to relapses of *P. vivax* on patients and unreliable data of the Health Center.

In the present study, the 0.96% malaria prevalence, based on a cross-sectional survey for Borena District, is much lower than those reported from several cross-sectional studies in Ethiopia. For example, a malaria prevalence of 5.8% was reported from Metekel resettlement area, North-western Ethiopia (Kidane and Teklehaimanot, 1988); an average prevalence of 4.6% reported from the Amhara Regional state (Endeshaw *et al*., 2008) and a higher overall prevalence (2.4%) of malaria in Oromia and SNNP regions, 5.4% from SNNPR (Shargie *et al*., 2008). However, Shargie et al. (2008) reported nearly similar prevalence (0.9%) to that of the present study from the Oromia Regional state. Environmental variation, sample size, nature of population, and method diagnosis may contribute for the difference for the different studies.

Furthermore, there was discrepancy between the retrospective average annual malaria prevalence (25.0%) based on clinical record from Mekane-Selam Health Center, and the prevalence (0.96%) of malaria from the present study based on a cross-sectional survey. The existence of discrepancy between the retrospective and cross-sectional prevalence values is a well-established phenomenon and has been reported in different malaria epidemiological studies. For instance, a study conducted in malaria prevalence in the community of Mekong Delta region (Vietnam) showed that the retrospective malaria prevalence (62%) is higher than the cross-sectional malaria prevalence determination (2.4%) in the same survey period (Erhart *et al*., 2004). This can be explained by the fact that the clinical records are based on patients that have been reporting to the Health Center seeking treatment while the cross-sectional study is based on a random probability sampling.

In most of the study sites, there were perennial water sources like rivers, streams and springs along the sides of which small traditional irrigated farming is practiced. Most irrigation farms are close to the human dwellings. Hence, these water sources are likely to support mosquito populations that could be responsible for malaria transmission in the communities of the nearby villages. Moreover, people work on their irrigated farms during early part of the nights and early in the morning when the malaria vectors are particularly active in search of blood meal and are likely to bite.

In Borena District, there is no significant influx of population from malarious into non-malarious areas and the epidemiological condition observed from the study sites showed that

the study area is characterized by very low, seasonal and unstable malaria transmission whose prevalence oscillates between the wet and the dry seasons.

According to the information obtained from the Mekane-Selam Health Office, indoor residual spray is being applied against malaria vector mosquitoes in selected Kebeles, based on repeated severe incidence of malaria epidemic, once every year. The IRS must have contributed to the reduction of malaria transmission together with other intervention measures such as ITN and drug treatment of cases. From 2005 onwards, malaria prevention and control intervention in the District has been supported with the distribution of at least 1 (rarely 3) ITNs per family) (based on the size of family) and prompt treatment based on RDTs and/ or microscopic diagnosis of malarial patients. This anti-malaria measures and increased awareness of the community about malaria must have been responsible for the low malaria prevalence determined in the cross-sectional study.

Information from the clinical record of Mekane-Selam Health Center showed high cumulative annual prevalence of intestinal parasitic infection (46.5%) over the last six years; even with no significant decreasing trend for the past six years. This may be attributed to the lack of environmental sanitation, personal and food hygiene because of less consideration given by health authorities to the prevention and control of intestinal parasitic infections (Maneeboonyang *et al*., 2008). Comparison of malaria with other parasitic diseases showed that malaria was the most prevalent disease among amoebiasis, giardiasis, helminthiasis and malaria during the years 2003, 2004 and 2005 (Fig.3). After 2005, amoebiasis due to *E. histolytica/dispar* became the most prevalent infection among the four parasites compared. This can be explained by the fact that the integrated malaria prevention and control measures being implemented in the study area had reduced malaria prevalence while the low level of environmental sanitation, personal, food and water hygiene increased amoebiasis.

Among the widely prevalent intestinal parasites in developing countries (Rao *et al*., 2003), seven were detected on microscopic diagnosis of the stool specimens collected in the present study. The total prevalence (24.6%) of intestinal parasites detected in the present study was lower than the average prevalence (46.5%) of the retrospective clinical record obtained from Mekane-Selam Health Center and other studies conducted in different parts of Ethiopia. For example, Roma and Worku (1997) had reported an infection prevalence of 89.4% among

schoolchildren in Wondo-Genet Zuria, Southern Ethiopia and Mengistu *et al.* (2007) had reported a prevalence of 83% among urban dwellers in Southwest Ethiopia. However, the present finding is comparable to Tadesse (2005) (27.2%) from the study conducted among schoolchildren in Babile town, Eastern Ethiopia. The high overall prevalence of intestinal parasites might be due to the climatic and environmental conditions of the area along with poor water supply and sanitation facilities which could be favorable for their transmission (Ali *et al.*, 1999).

In the present study, multiple parasite infections were identified in 3.1% of the survey population and in 12.5% of the participants who were found infected by intestinal parasites. The level of polyparasitism detected in the present study (3.1%) was much lower than that reported by Wadood *et al.* (2005) (18%) from Pakistan from a study conducted in a children's Hospital Quetta and that of Mengistu *et al*. (2007) (56.7%) from urban dwellers in Southwest Ethiopia. The multiple parasitism detected in the present study was similar to that reported by Tadesse (2005) (3.0%) from the study done on schoolchildren in Babile town, Eastern Ethiopia. These differences in multiple parasitic infections in the different studies may be due to variation in sample proportion, nature of population and method employed for stool processing (Mengistu *et al*., 2007). Malaria and intestinal parasitic infection co-infection was not detected in the present study. This may be due to the low malaria infection cases that reduced the probability of co-infection occurrence.

The most prevalent intestinal parasitic infection detected in the present study was due to *E. histolytica/dispar* infection (10.83%). This is much lower than that reported by Ali *et al*. (1999) (21.9%) from the study conducted on students of Asendabo Elementary and Junior Secondary school, Southwest Ethiopia and that of Mengistu *et al.* (2007) (17.1%) among urban dwellers in southwest Ethiopia. Much lower *E. histolytica/dispar* infection was reported by Woldemichael *et al.* (1999) (0.5%) from a study conducted in Western Abaya, Ethiopia. However, it is nearly similar to that reported by Legesse and Erko (2004) (12.7%) in a study conducted among schoolchildren in a rural area close to the Southeast of Lake Langano.

A. lumbricoides was the second most prevalent intestinal parasitic infection in this study with a prevalence of 7.6%, which is much lower than that reported by several studies in Ethiopia. For example, Jemaneh (1998) in a survey of schoolchildren in several communities of three

altitudinal regions in Ethiopia had reported a prevalence of 29% in the highlands, 35% in the temperate areas and 38% in the lowlands. Merid *et al.* (2001) had reported a prevalence of 76.9% among children around Lake Awassa area and Woldemichael et al. (1999) reported a prevalence of 10% in a study conducted from Western Abaya. On the other hand, Tadesse (2005) also reported a lower prevalence of A. lumbricoides (3.9%) than the present study among schoolchildren in Babile town, Eastern Ethiopia. This variation in the distribution of *A. lumbricoides* most probably is an indication of the variations in the local environments with regard to soil type, temperature, etc., that determine the transmission of the parasite.

H. nana with a prevalence of 3.6% in the present study was lower in prevalence than that reported by Tadesse (2005) (10.1%) among schoolchildren in Babile town, eastern Ethiopia, but nearly similar to that reported by Mengistu *et al.* (2007) (5%) among urban dwellers in South West Ethiopia. This can be explained by the fact that both the present study and that of Mengistu *et al.* were based on the general population while the study from Babile town was on children who are known to be at higher risk to *H. nana* infection.

The variation in the findings among studies can be because of the variation in geography, socio-economic conditions, cultural practices, the category of the survey population, the method employed for stool examination, the time of study (Tadesse, 2005) and the preventive and control measures implemented in the study areas.

There was very low hookworm prevalence (1.99%) in this current study compared to that reported by Erko *et al*. (1995) (40.0%) from Bahir Dar; Jemaneh (1998) (24%) from lowlands, 15% from temperate and 7% from highland areas in school children from several communities in Ethiopia; Tadesse (2005) (6.7%) among school children in Babile town, Eastern Ethiopia and Mengistu *et al.* (2007) (17.5%) among urban dwellers in Southwest Ethiopia. This can be explained by the relatively high proportion of the population wearing shoes, the climatic condition, and the nature of soil type in the present study.

The significant difference between males and females infected by *G. lamblia* (P=0.048) may be due to the fact that females are more often involved in food processing and handling activities than males and hence water and food contamination which is the most common mode of transmission (WHO, 1987) would account for this sex associated infection.

In the test for possible association of risk factors like sources of drinking water, household waste disposal, availability of toilet and food habit with intestinal parasite infection, access to toilet was not significantly associated. This may indicated that, although latrines may have been constructed, they are not utilized. This finding was in contradiction with what was found by Meike *et al*. (2006) from the study conducted on the prevalence and risk factors of intestinal parasites among Cuban children**.** However, it was in agreement with the findings of Tadesse (2005) from the study done on schoolchildren in Babile town, Eastern Ethiopia. Illiteracy was found to be associated with the risk to intestinal parasitic infection in the current study, which is similar to that reported by Tadesse (2005) and Meike *et al.* (2006). Thus, the findings both from Ethiopia and Cuba point to the fact that literacy of the population is a critical factor for use of toilets; which would protect against intestinal parasite infection.

The identification of consumption of uncooked vegetables as a risk factor to intestinal parasitic infection by the study participants is a good indication that if poverty is alleviated and the livelihood of the population is improved most of the parasitic infections that are transmitted through contamination of food items can be avoided.

The habit of not washing hands before meal and after toilet use was found to be associated with the risk of intestinal parasitic infections. This is because eating food without washing hands before meal and after toilet use will increase the chance of pathogen contaminating the food consumed.

On average, use of anti-malarial drugs and ITN/LLIN for preventing malaria transmission was reported by a significant number of heads of household (78.1%). This can be accounted for the contribution of the health extension workers in mobilizing the community against malaria in particular and all diseases in general. Whereas the awareness towards the use and knowledge of malaria prevention and control measures such as ITN/LLIN and anti-malaria drug treatment was high among the heads of household, the reported use and knowledge of the heads of household towards treatment against intestinal parasitic infection and the health impact of intestinal parasitic infections was low in the community. This can be explained by the fact that most intestinal parasitic infections particularly geo-helminthiasis does not show immediate clinical signs and symptoms on patients.

Limitations of the study

We selected sample Kebeles (sites) for convenience of sampling and accessibility, due to logistic and time constraints. Stool sample testing to be conducted at the spot of collection was delayed at times by 6 to 24 due to lack of diagnostic facility at the sampling sites. Because of this limitation, some of trophozoite stages of some protozoan parasites could not be identified with certainty.

6. Conclusions

- The two *Plasmodium* species namely *P. falciparum* and *P. vivax* were the species that cause malaria in Borena District.

- The integrated malaria control intervention measures that have been in operation in the study area are associated with the low malaria prevalence.

- Even though the retrospective and cross-sectional studies showed high level of reduction in malaria prevalence, it is possible that resurgence of malaria can occur if the control activities are not sustained.

- The high overall prevalence of intestinal parasites in the present study signifies the need for mass deworming in the community and establishment of good personal hygiene and environmental sanitation.

7. Recommendations

♠ IRS and ITN/ILLN provision must be continued in a sustainable manner particularly in those areas where small traditional irrigation systems are in practice.

♠ Regular health education must provide to raise individual and community awareness about the mode of malaria transmission prevention and control.

♠ Awareness creation through education and demonstration to bring about behavioral change towards use of toilet, maintenance of personal and food hygiene must be instituted to protect the population from infection with intestinal parasites.

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Appendix 1. **Written consent form.**

I am conducting a study to assess public health importance of malaria and intestinal parasites in order to understand the prevalence and health consequences of afore mentioned parasites on the community.

You are being to participate in this study. If you agree, I would like to obtain finger prick blood sample and stool specimen, in filter paper and plastic sheet, respectively, from you/or your child(ren), which would be used only to detect the presence of malarial and intestinal parasites. You will not get any risk in participating but you may experience a small pain during finger pricking. When you or your children are found positive for either both of the above parasites, you will receive standard drugs free of charge. The information in your records is strictly confidential.

Your participation in this study is completely voluntary and you can refuse to participate or free to withdraw yourself from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits.

Do you understand what has been said to you? If you have questions, you have the right to get proper explanation.

I am informed to my satisfaction about the purpose of this study nature of laboratory investigation. I am also aware of my right to opt out of the study at any time during the course of the study without having to give reasons for doing so.
This consent form has been readout to me in my own language (Amharic language) and I understand the content and I am voluntarily consent to participate in the study.

Study code no. _____

Name _____Signature _____ Date _____

Wittiness Name_____Signature_____Date_____

Investigator Name _____ Signature _____Date_____

Appendix-2. Questionnaire

Addis Ababa University Science Faculty, questionnaire format for collection of information on the prevalence of intestinal parasites and malaria in Borena District, South Wollo, North-Central Ethiopia, 2008/09.

Part-one: Demographic information **Code no._____**

Village----------Kebele----------Huose no.----------

House hold name----------sex---------Age-------

Occupation; Farmer/gov. employee; salary----------.

Educationstatus: illiterate, read&write, elementary/dropuot, high school/ dropout, 10/12 complete.

Part-two: Questionnaire on malaria

Fill in the blank space with'×' for/choose-the correct response.

1. Have you ever contracted malaria? Yes----. No-------
2. Did you take anti-malaria treatment (drug) for this? Yes--------. No------
3. What kind of drug? Coartm----? Chloroquin----? Qc-pyrimethamine----?
4. Have you been moved to other malarious areas before this week? Yes---- No-----
5. Have you used mosquito control and preventive measures? Yes---.No--.
   a) ITNs (LLINs)--? B) IRS---? C) Other-----
6. If yes, a) how frequent? Every day-------? Often-------?
   b) All family members? Yes--------.No-------
7. Have you contracted malaria after Bed net use? Yes---No-----
8. Who supplied you the ITNs/LLINs? Gov't------NGOs--------
9. Has IRS been implemented? Yes----No------
10. If yes, how frequent? ----------
11. Do you keep your cattle out door at night? Yes-----No-----
12. Have you been in malarious area outside Borena District?
13. Where do you get treatment services for malaria infection? Government Health Center---, Clinics---, Health post----, Private clinics and pharmacies---, others---

Part-3: Questionnaire on intestinal parasites

Village----------Kebele----------Huose no.----------

House hold name----------sex---------Age-------

Occupation; Farmer/gov. employee; salary----------.

Education status (underline): illiterate, read&write, elementary/dropuot, 9/10/10+1/10+2, 10/12dropout, 10/12 complete.

Fill in the blank space with'×' for/choose-the correct response

1. Have you ever encountered abdominal pain-----, cramp-----, bloating----- and nausea and Vomit---- during intestinal discomfort?
   And observed signs and symptoms, like a) Bloody diarrhea----b) Non-bloody diarrhea----?
   C) Loose / fatty stool------ when you defecate?
2. Did you take treatment for this discomfort? Yes-----No-----
3. Do you wash your hands always before meal and after latrine use? Yes----. No----
4. Do you wear shoes always? Yes------No------
5. Do you have toilet? Yes------No-----
6. Where do you get drinking water from? Pond----, Spring----, River-----, Pipe-------.
7. How do you use drinking water? Boiling----, Filtering-----Direct-----,Chlorine treated--
8. How do you dispose house hold wastes? Burry underground/Incinerate-----, open Field--
9. Have you ever eaten raw meat? Yes-------No------
10. What about uncooked vegetables? Yes-------No------
11. Where do you get treatment services for intestinal parasitic infections? Government Health Center---, Clinics---, Health post----, Private clinics and pharmacies---, others----

Declaration

I, the under signed declare that this thesis is my original work and has not been presented for a degree in any other university.

Name_____

Signature: _____

Date_____

This thesis has been submitted for examination with my approval as a University advisor:

Beyene Petros (Prof)

Date_____

Signature_____

Place and date of submission: Addis Ababa University, Department of Biology, June 2010.