ASSOCIATION OF HUMAN ABO AND RH (D) BLOOD GROUP SYSTEMS WITH SOME INTESTINAL PARASITIC INFECTIONS IN BABILE DISTRICT, EAST HARARGHE ZONE, OROMIA REGIONAL STATE, ETHIOPIA

MSc THESIS

WONDIMU HULUKA

JUNE 2017
HARAMAYA UNIVERSITY, HARAMAYA

Association of Human ABO and Rh (D) Blood Group Systems with Some Intestinal Parasitic Infections in Babile District, East Hararghe Zone, Oromia Regional State, Ethiopia

A Thesis Submitted to Department of Biology,
Postgraduate Program Directorate
HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN GENETICS

Wondimu Huluka

June 2017
Haramaya University, Haramaya

HARAMAYA UNIVERSITY
POSTGRADUATE PROGRAM DIRECTORATE

As Thesis research advisors, We hereby certify that we have read and evaluated this thesis prepared, under our guidance, by: Wondimu Huluka Dembel, entitled: **Association of Human ABO and Rh (D) Blood Group Systems with Some Intestinal Parasitic Infections in Babile District, East Hararghe Zone, Oromia Regional State, Ethiopia.** We recommend that it can be submitted as fulfillment of the Thesis requirement

Tamiru Oljira (PhD)	________________	______________
Major Advisor	Signature	Date
Sissay Menkir (PhD)	________________	______________
Co advisor	Signature	Date

As member of the Board of Examiners of the M Sc. Thesis Open Defense Examination, We certify that we have read and evaluated the Thesis prepared by: Wondimu Huluka Dembel and examined the candidate. We recommended that the Thesis is accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Biology (Genetics).

____________________	________________	______________
Chairman	Signature	Date
____________________	________________	______________
Internal Examiner	Signature	Date
____________________	________________	______________
External Examiner	Signature	Date

DEDICATION

This thesis is dedicated to my beloved family and friends for their love and partnership support in my academic success and life.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is the result of my own work and that all sources or materials used for this thesis have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirements for M.Sc. degree at Haramaya University and is deposited at the university library. I confidently declare that this thesis has not been submitted to any other institutions anywhere for the award of any academic degree, diploma, or certificate.

Brief quotations from this thesis may be used without special permission provided that accurate and complete acknowledgement of the source is made. Requests for permission for extended quotations from, or reproduction of, this thesis in whole or in part may be granted by the Director of Postgraduate Program Directorate when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author of the thesis.

Name: Wondimu Huluka Dembel Signature: ___________________

Place: Haramaya University, Haramaya

Date of Submission: June 12, 2017

BIOGRAPHICAL SKETCH

Mr.**Wondimu Huluka Dembel** was born in Meki Town, East Shewa Zone of the Oromia Region on May 15, in 1992. He attended his elementary and junior school in Bekele Oda Tuta Elementary School and Meki Elementary and Junior School, and attended his high school education in Oda Bokota Secondary and Preparatory School. After completion of his preparatory education, he joined Dilla University to attend the degree program in Biology (BSc) and graduated in July 2015. After graduation he was directly assigned by Ministry of Education in August 2015 to join Haramaya University, College of Natural and Computational Sciences, Department of Biology to pursue his M.Sc degree in Genetics.

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my heartfelt thanks to my major advisor Dr. Tamiru Oljira and Co advisor Dr. Sissay Menkir for their unreserved support and valuable advice in all aspect of my research work, with insight and guidance by devoting their precious time and energy to comment and improve the research work starting from proposal development to the completion of the research work and their provision of reference materials. They worked hard to keep me on the right pathway and accomplishment of the study.

My heartfelt gratitude also goes to Babile Town Haelth Office and Babile Wereda Health Office for their expert cooperation especially, to Mr. Tsegaye Gibe for his assistance in giving ideas. I am again thankful to Babile Health Center, Awsherif Health Center, Erer Valley Health Center and Wayu Health Center for their willingness to allow me use all the laboratory materials. I would like to extend my special thanks to laboratory technicians, Mr Ephrem Wondimu, Mrs. Zuhera Shafi, Mr. Filagot Kefiyalew and Mr. Mohamed Amin for their support throughout my laboratory session by preparing the whole reagents, organizing and documenting patients ethnicity records. I would also like to thank all management and staff members of Babile Health Center for their assistance on scholastic materials. Finally I would like to thanks Ministry of education and biology department for facilitate me to attend MSc program.

ABBREVIATIONS AND ACRONYMS

CDC	Center for Disease Control and Prevention
DNA	Deoxyribonucleic Acid
HDN	Hemolytic disease of the newborn
HLA	Human Leukocyte Antigen
ISBT	International Society of Blood Transfusion
IP's	Intestinal Parasite
NGOs	Non-Governmental Organizations
RBCs	Red Blood Cells
Rh	Rhesus factor
RPR	Rapid Plasma Reagin
SPSS	Statistical Package for Social Science
STH	Soil Transmitted Helminthes

TABLE OF CONTENTS

Continues…

Continues…

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES IN THE APPENDIX

Figure **Page**

Association of Human ABO and Rh (D) Blood Group Systems with Some Intestinal Parasitic Infections in Babile District, East Hararghe Zone, Oromia Regional State, Ethiopia

ABSTRACT

The ABO and Rh blood groups are the most important blood groups despite the long list of several other blood groups discovered so far. The ABO and Rh blood group frequencies vary worldwide and are not found in equal frequencies even among ethnic groups. In this study, a cross-sectional survey was used to assess the phenotypic, allelic and genotypic frequency distribution of ABO and Rh blood groups and possible associations with intestinal parasitic infections in Babile district, East Hararghe, Eastern Ethiopia A total of 420 study subjects were participated in this study. A total of 420 blood samples and 420 stool samples were collected from study individuals from November 2016 to January 2017 G.C. Stool examination was done by direct wet mount for detection of intestinal parasites. Blood samples were collected from each participant by open slide method and RPR card method. Data were analyzed by using SPSS version 20. Chi-square test (χ^2) was used to test whether the population is at Hardy Weinberg genetic equilibrium or not, and to assess the association between ABO blood groups and intestinal parasitic infections. Observed difference were considered to be significant at $P<0.05$.with 95% confidence interval (CI). Blood type O and Rh-positive had the highest frequency while blood type AB and Rh-negative had the lowest frequency in the study participants. The overall phenotypic frequencies of blood type O, A, B and AB were 48.6%, 28.6% and 17.4% and 5.5%, respectively. The overall allele frequencies were 0.693 I^O, 0.186 I^A and 0.121I^B and genotypic frequencies were I^OI (0.4802), I^AI^A(0.0346), I^AI^O(0.2578), I^AI^B(0.0450), I^BI^B (0.0146) and I^BI^O (0.1677) in the study participants. The frequency of Rh+ and Rh- were found to be 98.8% and 1.2%, respectively, in the overall sampl.e The allelic frequencies of I^D and I^d were 0.891 and 0.109, respectively and genotypic frequencies of Rh(D) blood group were I^DI^D (07938), I^DI^d (0.1942) and I^dI^d (0.0118) in the study participants. The distribution of overall observed frequencies of ABO blood group phenotypes do not differ from those expected under Hardy Weinberg equilibrium, which indicate that the population is at Hardy Weinberg genetic equilibrium. According to this study the prevalence of G. lamblia infection was 103 (24.5%) followed by 103 (24.5%) of E. histolytica 23(5.5%) of S. mansoni. 12(2.9%) of H. nana and 4(0.9%) multiple intestinal parasitic infection. This study shows that phenotypic, genotypic and allelic frequency of ABO and Rh(D) blood group system in the study participants and prevalence of intestinal parasitic infections do not show significant association in the study participants.

Key words: ABO and Rh(D) blood groups, Allele, Genotype, IP's, Oromo, Phenotype

1. INTRODUCTION

The ABO blood group system is a genetic polymorphism that leads to phenotypic polymorphism and is one of the classical molecular markers that are well studied since its discovery around the beginning of the 20^{th} century (1900). The discovery of the ABO blood group, over 100 years ago, caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood. As our understanding of the ABO group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different populations (Yamamoto, 2000).

Some of these antigens are also present on the surface of other types of cells and body secretions like saliva, sweat, semen, serum, tears, urine etc, which are used in forensic investigations. Several of these red blood cell (RBC) surface antigens that stem from one allele (or very closely linked genes) collectively form a blood group system. Blood groups are genetically determined and exhibit polymorphism in different populations. A total of 30 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT, 2008). In clinical practice, ABO and Rh (D) blood groups are the most important system among the 30 blood groups (Jaf, 2010).

Although about 400 blood grouping antigens have been reported, the ABO and Rh (D) are recognized as the major (clinically significant) blood group antigens. This system derives its importance from the fact that A and B are strongly antigenic and anti A and anti B occur naturally in the serum of persons lacking the corresponding antigen, these antibodies being capable of producing haemolysis in vivo. The ABO blood group system was the first human blood group system, while Rhesus blood group system was the fourth system, out of 15 most important systems discovered and yet it is the second most important blood group from the point of view of transfusion (Khan *et al*., 2004). According to the ABO blood group system, there are four different kinds of blood groups: A, B, AB and O. Blood group A has A antigens on the surface of RBC and B antibodies in blood plasma; blood group B has B antigens on the surface of RBC and A antibodies in blood plasma; blood group AB has both A and B antigens on the surface of RBC and no A and B antibodies at all in blood plasma, and blood group O has neither A or B antigens on the surface of RBC but it has both A and B antibodies in blood plasma (Daniels, 2005).

The Rh system is one of the most polymorphic of the human blood groups. More than 40 different antigens have been identified; five are commonly known as D, C, c, E and e. The Rh is genetically complex but it is simply described in terms of a single pair of alleles, *D* and *d*. Rh positive (Rh+) persons are *DD* and *Dd*, and Rh negative (Rh-) are *dd*. The Rh blood groups rank with ABO groups in clinical importance because of their relation to hemolytic disease of the newborn (HDN) and their importance in blood transfusion (Khan *et al.,* 2009). The phenotypic variation that are caused by genetic differences in a gene known as *H*, that was mapped to chromosome 19 and that encodes L-fucosyltransferase, the enzyme that adds the antigens to the surfaces of RBCs (Ahmed *et al*., 2007).

Intestinal parasitosis refers to a group of diseases caused by one or more parasitic species of protozoa, cestodes, trematodes and nematodes. These parasite groups are responsible for the major share of morbidity and mortality in those communities where there is over-crowding, poor environmental sanitation and personal hygienic practices, which make them a great concern for the developing countries. Intestinal parasitic infections caused by helminths and protozoa are the major causes of human diseases in most countries of the tropical region. It is estimated that about 3.5 billion people in the world are infected with intestinal parasites, of whom 450 million are ill (Keiser and Utzinger, 2010).

Prevalence of intestinal parasites has been studied in different areas of the tropics and subtropics (Leykun, 2001). Decades ago, a number of studies focusing on epidomology of intestinal parasites were done in different community groups such as preschool children, school children, camps and refugees in different parts of Ethiopia (Leykun, 2001, Mengistu, 2007).

Report from a literature survey indicated that Ethiopia has one of the lowest quality of drinking water supply and latrine coverage in the world (Kumie and Ali, 2005). This could be one reason for why intestinal parasite has been widespread in Ethiopia. In Ethiopia the prevalence of *Ascaris 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%); whereas *Trichuris trichiura* infection exhibited similar prevalence in all altitudinal regions (13% on the average) (Jemaneh, 2000). Mengistu *et al*. (2007) reported that *Entamoeba. histolytica/dispar, Giardia lamblia, Schistosoma, Strongyloids, Hymenopsis nana, Intestinal schistosome, Tenea saginata, Entrobis vermicularis* and hookworm with prevalence of 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.

The ABO blood type is hypothesized to be one among other genetic factors that affect susceptibility or resistance of individuals to parasitic infections (Berger *et al*., 1989). However, the epidemiological evidence to support this notion is varied. Several human blood group systems, such as ABO and Rh (D) have counterparts in non-human primates. The evolution of blood group diversity within populations may have been influenced by selective pressure for resistance to various endemic infectious diseases (Mohammad *et al*., 2001).

Although several factors were suggested to affect the severity of infection by *Schistosoma mansoni* in human, the ABO blood group of the human host; which may assist the S*chistosoma* to escape the host immune response is suggested to be the major one (Edward *et al*., 2004). Some studies documented increased risk of *S. mansoni* infection and related clinical outcomes in individuals with blood type A (Ndamba *et al*., 1997, Degarege *et al*., 2015). Ayele *et al*. (2014), showed a significant association between the ABO blood group and infection caused by *E. histolytica*. Blood group A is more susceptible for *E. histolytica infections* than O, B and AB, respectively in West Gojjam, Amhara regional state.

The present study area, Babille district was one of the district's in East Hararghe Zone which was included under the list of intestinal parasite endemic places in Oromia, although data related to the prevalence of these parasites was scanty for this district. There was no data on the relationship between intestinal parasites and ABO and Rh (D) blood group systems generated for this area. This study was conducted to know if there were significance association between ABO and Rh (D) blood groups and prevalence of intestinal parasitic infections in Babille district.

General Objectives

The general objective of this study was to evaluate the association of human ABO and Rh(D) Blood Group Systems with prevalence of some intestinal parasitic infections in Babile district, East Hararghe Zone of Oromia Regional State, Ethiopia.

The specific objectives of this study were:

- To determine allelic, genotypic and phenotypic frequencies of the ABO and Rh (D) blood group systems in the study population.
- To determine the prevalence of some intestinal parasitic infections (protozoan and helminths) in study population.
- To statistically test the association of ABO and Rh(D) blood groups with prevalence of some intestinal parasitic infections in the study population.

2. LITERATURE REVIEW

2.1. Blood Group Systems

The ABO blood group system is the most important in human blood transfusion. A complete blood type would describe a full set of 30 substances on the surface of RBCs, and an individual's blood type is one of the many possible combinations of blood-group antigens. Across the 30 blood groups, over 600 different blood-group antigens have been found, but many of these are very rare, some being found mainly in certain ethnic groups (ISBT, 2008).

Humans contain a series of glycoproteins and glycolipids on the surface of RBCs which constitute the blood group antigens. There are many blood group systems on the basis of different blood group antigens. ABO and Rh systems are important in clinical practice, (Mandal, 2002). According to the presence or absence of antigens human blood can be classified into different blood group systems, example ABO blood group, MN blood group, Rh blood group systems, etc. All blood groups in human are under genetic control, each series of blood groups being under the control of genes at a single locus or of genes that are closely linked and behave in heredity as though they were at a single locus, (Jaff, 2010). The human blood groups have been studied extensively for their involvement in incompatibility reactions.

2.2. The Genetics of the ABO and Rh Blood Group Systems

ABO blood group system is the most important blood group in transfusion and has been widely used in population studies. Several molecular techniques for ABO allele's detection are widely used for distinguishing various alleles of glycosyl transferase locus on chromosome 9 (Mohammad *et al*., 2012)

The ABO system consists of four main groups, A, B, AB and O which is determined on the basis of presence or absence of A and B antigens. These antigens are under control of three allelic genes, namely I^A, I^B and I^O which determine blood groups. I^A produces A antigen, I^B produces B antigen whereas I^O produces neither. I^A and I^B are mutant alleles and show co-dominances with each other but, both are dominant over the wild type allele I^O (Rai and

Kumar, 2011). The three alleles can produce six genotypes and four phenotypes of blood groups which are:

Phenotype	Genotype
A	I^AI^A
	I^AI^O
B	I^BI^B
	I^BI^O
AB	I^AI^B
O	I^OI^O (Mandal, 2002)

The antigens that determine ABO blood groups are oligosaccharide constituents of cell surface glycolipids and glycoprotein. These sugars are added to an existing chain of oligosaccharides (precursor molecule containing R chain to which N-acetylglucoseamine and D-galactose attached) which protrudes from the erythrocyte (RBC) membrane. The H antigen is produced by the addition of L-fructose to the terminal galactose of this precursor by an enzyme, L-fucosyltransferase, encoded by the *H* gene, located on chromosome 19 (Ahmed, *et al*., 2007).

The O group has only this H antigen. In people of blood group A, *N*-acetyl-D-galactosamine (A determining) is added to the terminal D-galactose of the H antigen with the help of *N*-acetylgalactosaminyltransferase enzyme which is encoded by the *IA* allele of the *ABO* gene located on chromosome 9 (9q34.2). A variant of this enzyme known as the D-galactosyltransferase, is encoded by the *B* allele of the *ABO* locus adds D-galactose to the terminal galactose of the H antigen, to form the B antigen. Both the A and the B antigens are found on the surfaces of the RBCs of individuals with AB blood group since they are heterozygote for the two alleles. The *O* allele does not produce a functional enzyme at this locus and there is no any addition to the H antigen. The *A* and *B* alleles are co-dominant while the *O* allele is recessive to both of them. The genotypes that determine the different phenotypes in the ABO system are shown in Table 1 (Ahmed *et al*., 2007).

The *ABO* locus contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the deletion that is found in most *O* alleles and results in a loss of enzymatic activity. The *A* and *B* alleles differ from each other by seven nucleotide substitutions, four of which translate into different amino acids in the gene product (Arg176Gly, Gly235Ser, Leu266Met, and Gly268Ala). The residues at positions 266 and 268 determine the A or B specificity of the glycosyl transferase they encode (Yamamoto *et al*., 1990). The *O* allele differs from the *A* allele by deletion of guanine at position 261. The deletion causes a frame shift and results in translation of an almost entirely different protein that lacks enzymatic activity. The *A* allele is sometimes subcategorized into two variant forms (*A1* and *A2*) (Yamamoto *et al*., 1990).

Table 1.The ABO and Rh blood groups, their genotypes, and antibodies produced in blood plasma

Genotype	Blood group	Comment	Antibodies in plasma
I^AI^A	A	The gene encoding the ‘A’ glycosyltransferase is present on both copies	Anti-B
I^AI^O	A	The gene encoding the ‘A’ glycosyltransferase is present only on a single copy.	Anti-B
I^BI^B	B	The gene encoding the ‘B’ glycosyltransferase is present on each copy	Anti-A
I^BI^O	B	The gene encoding the ‘B’ glycosyltransferase is present on only a single copy	Anti-A
I^AI^B	AB	One copy has the gene encoding the ‘A’ enzyme while the other has the gene encoding the ‘B’ enzyme	Neither anti-A or anti-B
I^OI^O	O	Neither the *‘A’* gene nor the ‘*B’* gene is present on either copies of the gene	Anti-A or anti-B
I^DI^D	Rh^+	Has a *RHD* gene encoding D-antigen	Anti –D
I^DI^d	Rh^-	Has a *RHD* gene encoding D-antigen	No anti –D

Source: (Ahmed *et al*., 2007).

The genomic sequence of known alleles have minor differences with prevalent alleles of ABO, and are mostly formed because of substations, hybrid alleles, base insertions, deletions and splice site mutation (Blumen feld and Patnaik 2004, Olsson and Chester 2001).

2.3. Distribution of ABO Phenotypes in Different Populations

All human population shares the same blood group systems inherited from common ancestor but they differ by their frequencies (Jorde and Wooding, 2004). For example, in Caucasians in the United States of America, the distribution was type O, 47%; type A, 41%; type B, 9%; and type AB, 3%. Among African American, the distribution was type O, 46%; type A, 27%; type B, 20%; and type AB; 7%. Among Western Europeans, 42% had group A, 9% group B, 3% group AB and the remaining 46% had group O (Laura, 2005). Genotypic and phenotypic frequencies depends on the frequency of the alleles and the mating system of a population (Reich *et al*,. 2001, Tishkoff and Kidd, 2004) and vary among different races (Chakraborty, 2011).

Many previous studies in sub-Saharan Africa reported that blood group O and Rh+ are the most frequent ABO and Rh blood groups, respectively, but the proportion varies by location (Hailu and Kebede, 2013, Sirina and Clement, 2013). Ilozumba and Uzozie, 2009, reported ABO blood group frequency distribution of 2.63%, 12.05%, 21.05%, and 63.83% for groups AB, B, A, and O, respectively. In another study, Sirina and Clement, 2013 have reported proportion of 3.2%, 18.52%, 20.82%, and 57.44% for groups AB, B, A, and O, respectively. Oladeinde *et al.* 2014 also reported that blood groups O (57%) and AB (3.2%) were with the highest and lowest distribution in Benin City of Nigeria, respectively.

Among Ethiopians, the distribution is that type O, was 42%; type A 30%; type B 22%; and type AB 6 %, (http://rhesusnegative.net). In population of south west Ethiopia, at Gilgel Gibe Field Research Center, the frequency of O, A, B and AB phenotypes were 42%, 31%, 21% and 6% respectively among a total of 1965 study participants (Abraham *et al*., 2012). The phenotypic frequency of O, A, B, and AB blood groups of Sidama ethnic group was found to be 51.3%, 23.5%, 21.9% and 3.3% , respectively (Tewodros *et al*., 2011). Hailu Tadesse and Kebede Tadesse (2013) reported the distribution of ABO blood groups was O (46%), A (27.1%), B (23.1%), and AB (3.8%), in northwest Ethiopia. Similar study in West Gojam, Ethiopia (Ayele *et al,*.2014) showed frequency distributions of 60%, 26%, and 14% for blood groups of O, A, and B, respectively. Table 2 shows the distribution of ABO blood types of different racial and ethnic lines.

Table 2. Racial and ethnic distribution of ABO (without Rh) blood types

People group	O (%)	A (%)	B (%)	AB (%
Abyssinian	42	30	22	6
African American	46	27	20	7
Ainu (Japan)	17	32	32	18
Albanians	38	43	3	6
Grand Andamanese	9	60	23	6
Arabs	34	31	29	6
Armenians	31	50	13	6
Asian (in USA general)	40	28	27	5
Austrians	36	44	13	6
South Africans	45	40	11	4
Basques	51	44	4	1
Belgians	47	42	8	3
Blackfoot (N. Indian)	17	82	0	1
Bororo (Brazil)	100	0	0	0
Sudanese	62	16	21	0
Bulgarians	32	44	15	0
Burmese	36	24	33	7
Buryats (Siberia)	33	21	38	8
Bushmen	56	34	9	2
Caucasians (USA)	47	41	9	3
Chinese-Canton	46	23	25	6
Kikuyu (Kenya)	60	19	20	1
Nigeria				

Source:ISB,2008,C:\Users\Administrator\Desktop\(http:\www.bloodbook.com\worldabo.htm)

2.4. Frequency of Rh blood type in different population

Rh negative blood types are much less in proportion of Asian populations (0.3%) than they are in White (15%). In Table 3, the presence or absence of the Rh antigens is indicated by the + or - sign, so that for example the A- group does not have any of the Rh antigens (Cummings, 2000).Of the Rhesus blood group system, the gene *D* which gives rhesus positive status is at its lowest in Europe. It increases in frequency east and south to approximately 80% over almost all of Africa south of the Sahara. In eastern Asia, Australia and Indonesia it often attains 100% .The same holds for American indigenous populations in many of whom the D frequency is 100 % (Reddy *e t al*., 2008).

Table 3 Allele frequency of Rh blood types studied in different population across the world

Population	**Rh+**	**Rh-**	**References**
Ethiopia	0.94644	0.05356	Seifu and Kifle, 1985.
Germany	0.9500	0.0500	Akbas *et al*., 2003
Kenya	0.8030	0.1970	Lyko *et al*., 1992
Lagos (Nigeria)	0.9400	0.0600	Adeyemo &Soboyejo. 2006.
Mandi Bahauddin (Pakistan)	0.9140	0.0860	Anees *et al*., 2007.
Nigeria	0.9430	0.0570	Falusi, *et al*., 2000.
Ogbomoso (Nigeria)	0.9670	0.0330	Bakare *et al*., 2006.
Port Harcourt (Nigeria)	0.9677	0.0323	Jeremiah, 2006.
Red Indians (USA)	1.00	0	Reddy *et al*., 2008
Saudi Arabia	0.9300	0.0700	Khattak *et al*., 2008.
U.S.A	0.8500	0.1500	Khattak *et al*., 2008.

Source: ISBT, 2008 (http://www.bloodbook.com/world-abo.html

2.5. Intestinal Parasitic Infections

Endoparasites are parasites that live in the internal organs of animal, especially in the gastrointestinal tracts of humans and other animals (Loukopoulos, 2007). Intestinal parasites can live throughout the body, but most prefer the intestinal wall (free encyclopedia, 2009). Intestinal parasites of humans are cause of important health problems in the most communities, especially those situated in tropical and subtropical area (Kia *et al*., 2008). They can cause significant morbidity and mortality throughout the world. At least one-third of the world's population is infected with intestinal parasites (CDC, 2007. The major Intestinal parasitic infections (IPI's) of global public health concern are the protozoan species: *Entamoeba histolytica* and *Giardia* species, and the soil transmitted helminthes: *Ascaris lumbericoides, Hymenolepis nana* and *Trichuris trichiura* (WHO, 2000).

Giardia lamblia (Protozoan**)** is a flagellate protozoan reproduces by binary fission. This is a type of reproduction in which one cell divides into two new cells by mitosis during the growth cycle. The components of the cell multiply so that each daughter cell is a complete copy of the parent cell. This parasite has a simple direct life cycle consisting of an infective cyst and a vegetative trophozoite. The cyst form is resistant to drying and other environmental effects and is infectious. Infection is limited to the small intestine and /or biliary tract. It is transmitted through food and water contaminated by sewage, by food handlers with poor hygiene, and through other fecal-oral routes (Figure 1). Infection is more common in children than in adults. Patients with clinical illness may develop acute watery diarrhea with abdominal pain, or they may experience a protracted, intermittent disease which is characterized by passage of foul-smelling diarrhea or soft stool associated with flatulence, abdominal distention, and anorexia (Minnesota Refugee Health Provider Guide 2013).

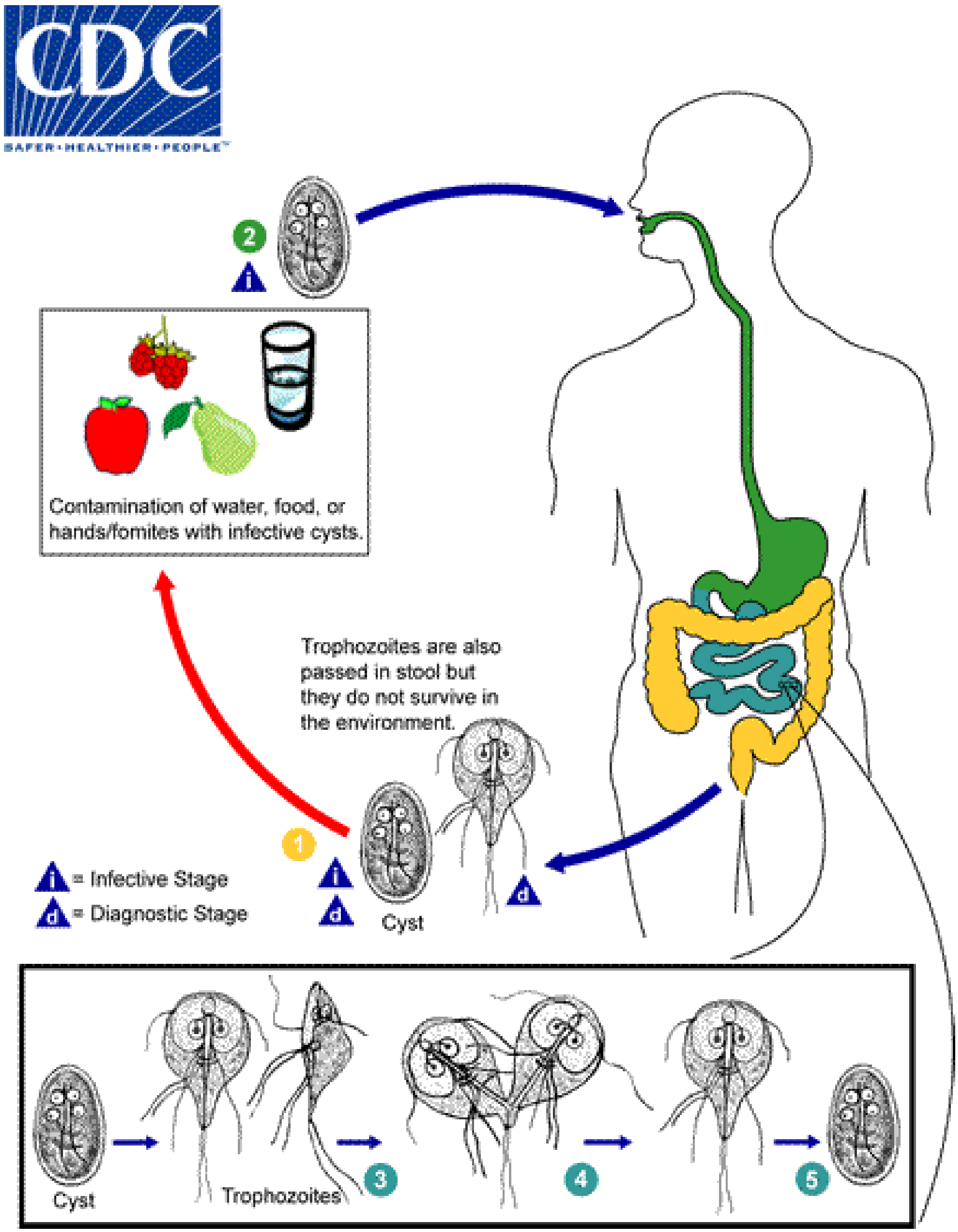


Figure.1 Life cycle of *Giardia lamblia*

Source: CDC, 2009

Entoameba histolytica (protozoan) is an intestinal parasite that characterized by possessing clear protoplasm which form pseudopodia. These pseudopodia are the means by which the organisms move and use for feeding purposes. The two species *Entamoeba histolytica* and *Entamoeba dispar* are morphologically identical but pathologically distinct (WHO, 1997).

However, only *E. histolytica* is capable of causing disease (medically important) does not require any intermediate host. Mature cysts are passed in the feces of an infected human. Another human can get infected by ingesting them in fecal contaminated water, food or hands (Figure 2). If the cysts survive the acidic stomach, they turn into trophozoites in the small intestine. Trophozoites migrate to the large intestine where they live and multiply by binary fission. Both cysts and trophozoites are sometimes present in the feces. Cysts are usually found in formed stool, whereas trophozoites are found in loose stool. Only cysts can survive longer periods (up to many weeks outside the host) and infect other humans. If trophozoites are ingested, they are killed by the gastric acid of the stomach. The *E. histolytica* cell differentiation takes place inside the colon, during this process the trophozoite stops its pseudopods formation, the nuclei starts division, the characteristically irregular shape is lost and the cell takes a spherical form at the time that a thick cystic wall appears. The trophozoite differentiation concludes with the tetranucleated cyst formation (Martínez-Palomo and Espinosa-Cantellano, 1998).

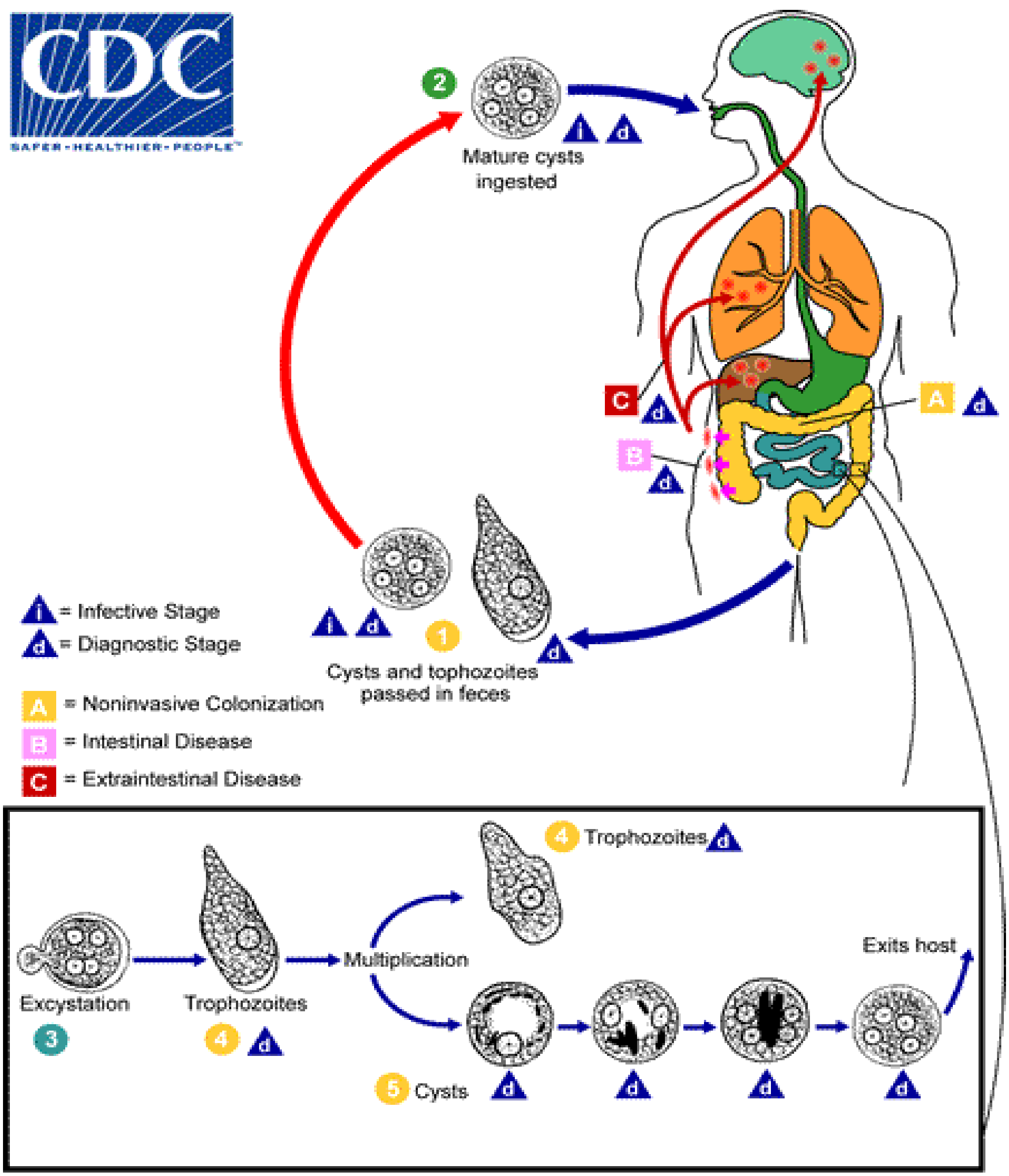


Figure 2 Life cycle of *Entoamoeba histolytica*

Source: CDC, 2009.

Cryptosporidiosis is a parasitic disease caused by *Cryptosporidium*, a protozoan parasite in the phylum Apicomplexa. It affects the intestines and is typically an acute short-term infection. It is spread through the fecal-oral route, often through contaminated water (CDC, 2009); the main symptom is self-limiting diarrhea in people with intact immune systems.

Cryptosporidiosis is typically an acute short-term infection but can become severe and non resolving in children and immune compromised individuals. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue. Infection is through contaminated material such as earth, water, uncooked or cross-contaminated food that has been in contact with the feces of an infected individual or animal. Contact must then be transferred to the mouth and swallowed. It is especially prevalent amongst those in regular contact with bodies of fresh water including recreational water such as swimming pools. Other potential sources include insufficiently treated water supplies, contaminated food, or exposure to feces (CDC, 2009).

Hymenolepis nana **(**Helminths**)** eggs are immediately infective when passed with the stool and cannot survive more than 10 days in the external environment. When eggs are ingested by an arthropod intermediate host, they develop into cysticercoids, which can infect humans or rodents upon ingestion and develop into adults in the small intestine. A morphologically identical variant, *H. nana* var. fraterna, infects rodents and uses arthropods as intermediate hosts. When eggs are ingested (in contaminated food or water or from hands contaminated with feces), the oncospheres contained in the eggs are released. The oncospheres penetrate the intestinal villus and develop into cysticercoid larvae. Upon rupture of the villus, the cysticercoids return to the intestinal lumen, evaginate their scoleces, attach to the intestinal mucosa and develop into adults that reside in the ileal portion of the small intestine producing gravid proglottids. Eggs are passed in the stool when released from proglottids through its genital atrium or when proglottids disintegrate in the small intestine. An alternate mode of infection consists of internal autoinfection, where the eggs release their hexacanth embryo, which penetrates the villus continuing the infective cycle without passage through the external environment (Figure 3). The life span of adult worms is 4 to 6 weeks, but internal autoinfection allows the infection to persist for years (CDC, 2009).

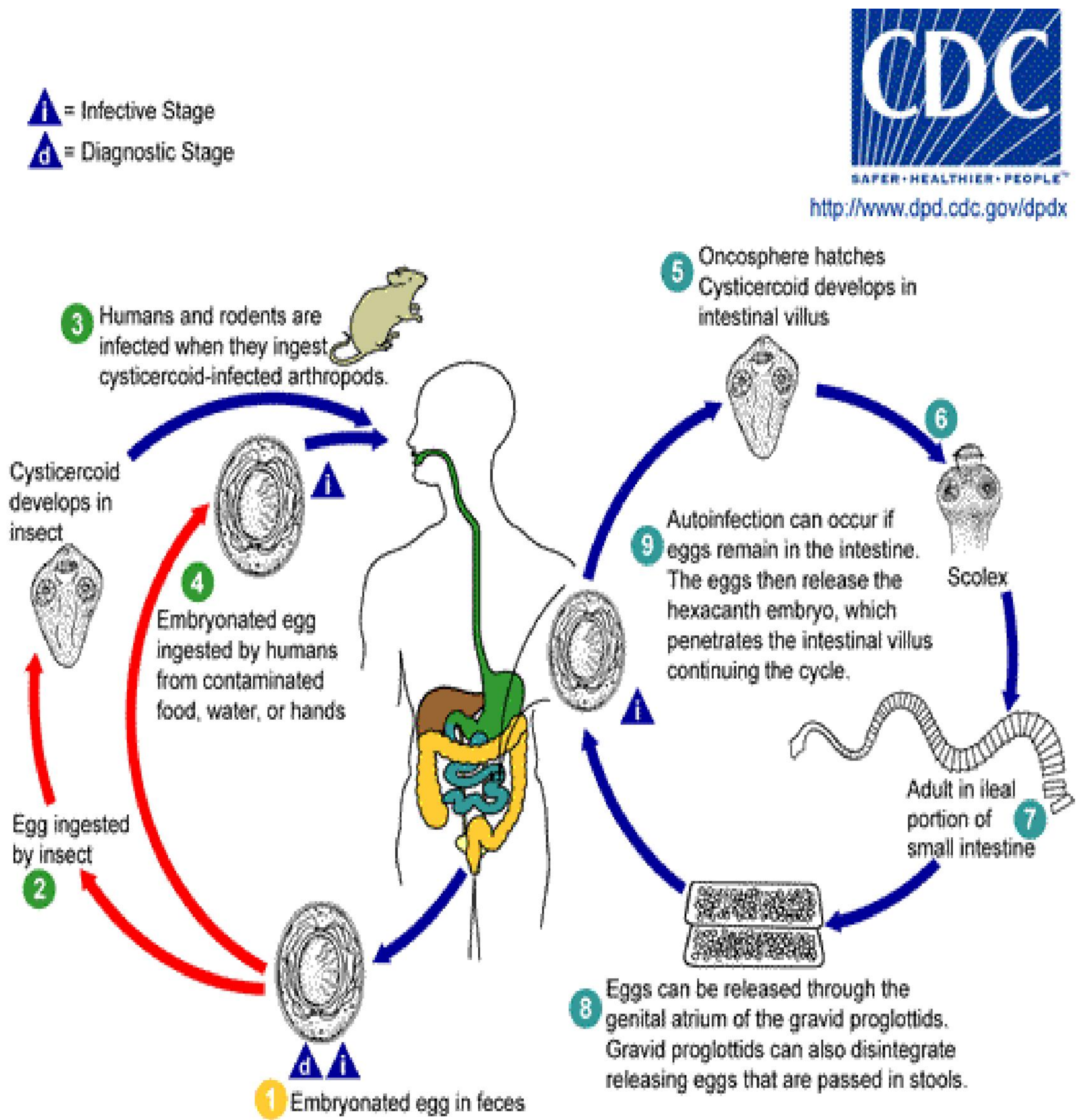


Figure 3 Life cycle of *Hymenolepis nana*

Source: CDC, 2009.

Schistosoma species (Fluke) encompasses three distinct phases of clinical manifestations and on a worldwide scale is one of the most common causes of hematuria. Individuals exposed to various *Schistosoma* spp will initially produce a pruritic papular dermatitis after penetration of the skin by cercariae. With non-human pathogen species, this is referred to as “swimmer’s itch,” and can be contracted from fresh and salt water (Minnesota Refugee Health Provider Guide, 2013).

Human pathogenic species include the following: *S. mansoni, S. japonicum, S. haematobium, S. mekongi, and S. intercalatum.* These species rely on the presence of a fresh water snail as intermediate host (Figure 4) and have various geographic distributions. *S. mansoni is* found mainly in tropical Africa, Latin America, the Caribbean and the Arabian Peninsula. *S. haematobium is* found mainly in Africa and the Eastern Mediterranean area. *S. mekongi* and S. *japonicum*, as their names reflect, are found mainly in the Mekong River delta and in parts of China, the Philippines, and Indonesia, respectively. After skin penetration, the organism migrates through the blood stream via the lungs before ultimately lodging in the venous plexus draining the bladder (haematobium) or the colon. After four to six weeks, an acute illness characterized by fever, malaise, cough, rash, abdominal pain, nausea, diarrhea, lymphadenopathy, and eosinophilia occurs and is termed “Katayama Fever.” With heavy gastrointestinal infections, bloody diarrhea and tender hepatomegaly may occur (Minnesota Refugee Health Provider Guide, 2013).

Chronic disease reflects the worm burden and fibrosis with inflammation at the sites of deposited eggs. Infected individuals may be asymptomatic with light infestations. Heavy colon involvement may cause chronic bloody, mucoid diarrhea, abdominal pain, hepatosplenomegaly, ascites, and esophageal varices (due to portal hypertension). Bladder symptoms related to inflammation and fibrosis may include dysuria, terminal hematuria (microscopic or gross), secondary UTIs, and pelvic pain (Minnesota Refugee Health Provider Guide, 2013)

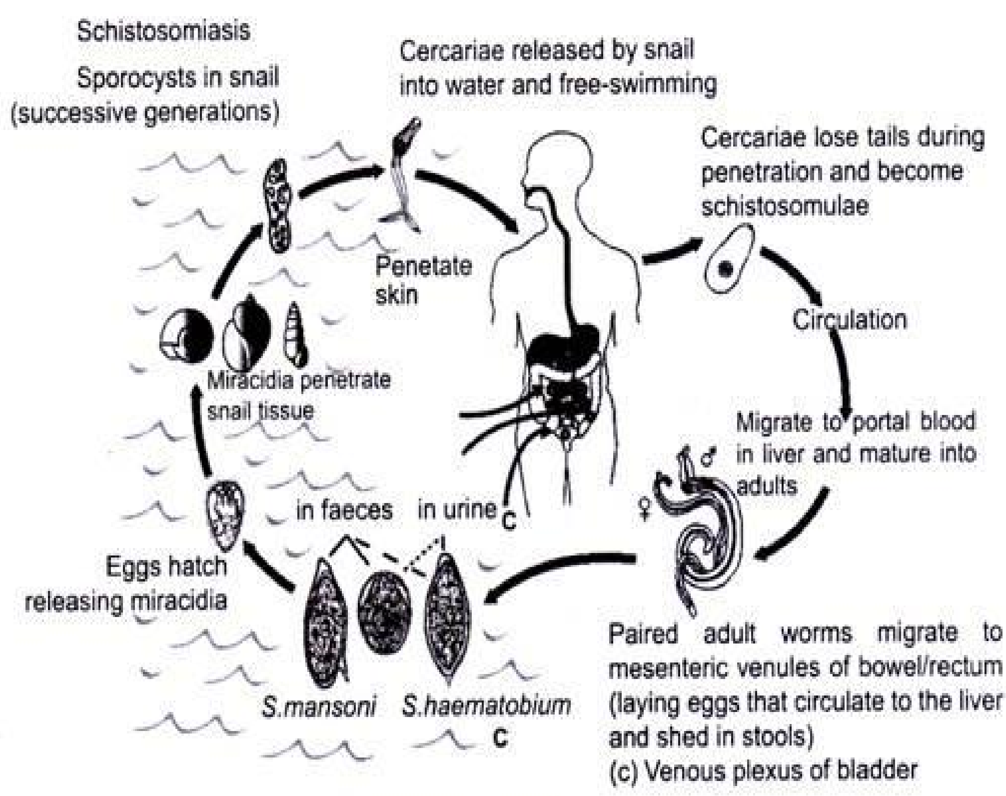
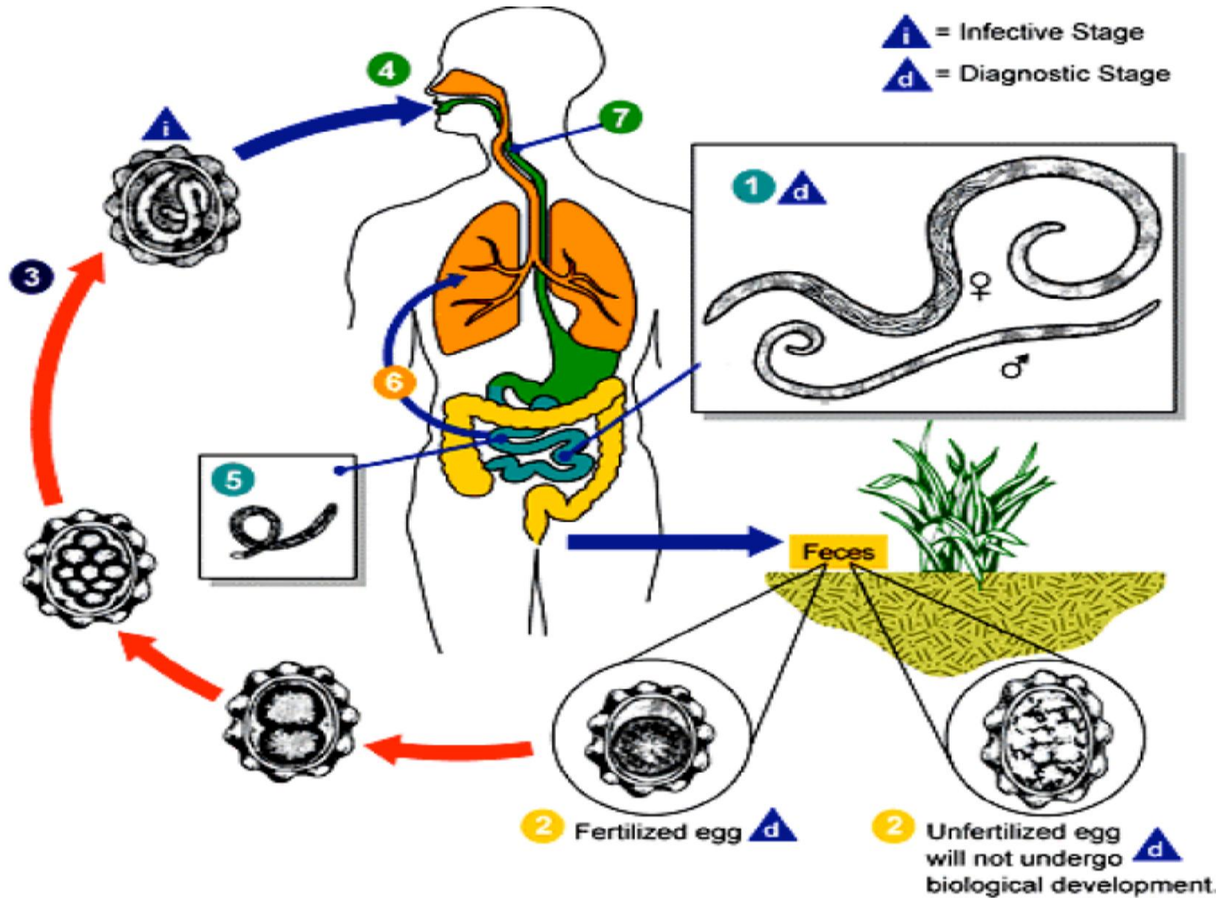


Figure 4 Life Cycle of Schistosoma species

Source: www.google.com.

Ascaris lumbricoides is the most common and important soil-transmitted helminth. It is the largest and the most common helminthes parasitizing the human intestine and currently infects about 1 billion people worldwide (CDC, 2006). It is estimated that 25% of the world population harbors the parasite. Hand to mouth transmission is most common; it is found in association with poor personal hygiene, poor sanitation, and in places where human feces are used as fertilizer. Consumers of uncooked vegetables and fruits grown in or near soil fertilized with sewage are most at risk for acquiring infection. Water is rarely implicated as a source of Ascaris (Bogitsh *et al.,* 2005). *A. lumbricoides* have been shown to play a significant role in childhood malnutrition, which leads to growth retardation, cognitive impairment, and poor academic performance, resulting in a poorer quality of life and less ability to contribute to society (Drake *et al*., 2000).The infection occurs by ingestion of food contaminated with infective eggs which hatch in the upper small intestine. The larvae (250 x 15 micrometers) penetrate the intestinal wall and enter the venules or lymphatics. The larvae pass through the liver, heart and lung to reach alveoli in 1 to 7 days during which period they grow to 1.5 cm. They migrate up the bronchi, ascend the trachea to the glottis, and pass down the oesophagus to the small intestine where they mature in 2 to 3 months (WHO, 2002). Its life cycle is presented in Figure 5.



1. Adult worm 2. Unfertlized egg 3. Fertelized egg become infective 4. Infective stage swallowed 5. Larvae hatch

Figure 5: Life cycle of *Ascaris lumbricoides*

Source: CDC, 2009.

Infection with *Trichuris trichiura* (*trichuriasis*) is the third most common helminth infections of humans (Peters and Pasvol, 2005). The distribution of trichuriasis is worldwide, being most abundant in the warm moist regions of the world. It is spread via fecal-oral transmission as shown in Figure 6 (life cycle of the parasite) and high prevalence occurs in areas with tropical weather and poor sanitation practices (Bethony *et al*., 2006).The parasite commonly occurs together with *A. lumbricoides* and likewise mainly affects children. According to CDC (2010 and 2011), trichuriasis is transmitted when the infective eggs of the whipworm are unintentionally ingested, usually through consuming soil that has been contaminated with human feces via dirt covered food or hands. The spread of human whipworm eggs usually occurs in areas where outside defecation takes place or human feces is used as fertilizer. Children may be heavily infected and constitute important reservoirs.

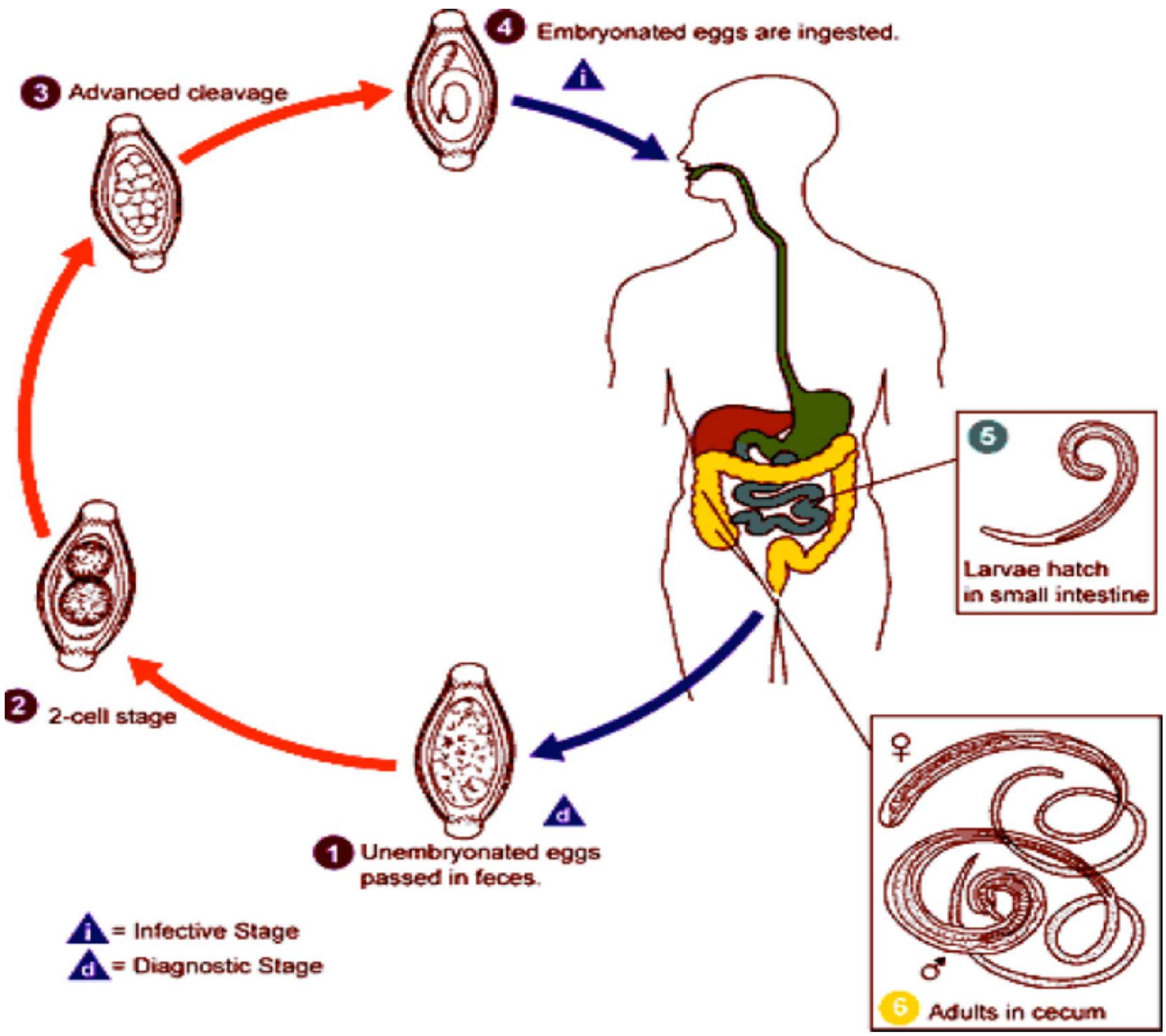


Figure 6: Life cycle of whipworms

Source: CDC, 2009.

2.6. Epidemiology and Geographic Distribution of Intestinal Parasites

2.6.1. Global Epidemiology and Geographic Distribution of Intestinal Parasites

Intestinal parasitic infections are among the most common infections in the world and are responsible for considerable morbidity and mortality (Kongs *et al*., 2001). The epidemiology of intestinal parasitic infections shows that these parasites are found in every group and in both sexes. However, the incidence is high in some areas and in some age groups. Human intestinal parasitic infections have a worldwide distribution, with the greatest incidence and intensity occurring in developing countries (Mccarhty *et al*., 2004).

Intestinal protozoan parasite infections enjoy a wide global distribution. They are estimated to affect 3.5 billion people. Majority are children and young ones residing in developing countries. The major intestinal protozoan parasites of global public health concern are *Entoameba histolytica, Giardia lamblia* and *Cryptosporidium* species (WHO, 2000).

Intestinal protozoan parasitic infections are endemic worldwide and have been described as constituting the greatest single worldwide cause of illness and disease. Poverty, illiteracy, poor hygiene, lack of access to potable water and hot and humid tropical climate are the factors associated with intestinal parasitic infections. Intestinal parasitic protozoa and helminthes are responsible for some of the most devastating and prevalent diseases of humans. Intestinal parasitic protozoan infections constitute a global health burden causing clinical morbidity in 450 million people, many of these women of reproductive age and children in developing countries (Quihui *et al*., 2006).

Invasive amoebiosis is prevalent in certain areas of the world including West and South-east Africa, China and Mexico. In Tehran province, the highest infection rate (41.5%) was related to protozoan parasites. *Entomoeba histolytica* has been recovered worldwide and is more prevalent in the tropics and sub- tropics than in colder climates. However, in poor sanitary conditions in temperate and colder climates, infection rates have been found to equal that seen in the tropics. In a related study in Ardabil Iran, a total of 10 species were identified with *Giardia lamblia* (14%), *Blastocystis hominis* (10%) and *Entoamoeba histolytica* (4.1%) being the most common parasites (Aksoy *et al*., 2005).

Gardia lamblia also has a worldwide distribution with an incidence rate or between 11% and 30%. In the united state of America, it is now considered to be the most common intestinal parasite of man and the leading cause of diarrhea due to protozoan infections in human. It is also the most frequently reported intestinal parasite in Peru (Beltran *et al*., 2004). *Giardiasis* is one of the most common parasitic infections having a worldwide distribution and occurring both in developed and developing nations. In Africa, Asia and Latin America about 200 million cases have been estimated to occur annually.

Soil transmitted helminthes infections are widely distributed in tropical and sub tropical areas and since they are linked to a lack of sanitation, occur where ever there is poverty. STHs are with the greatest public health burden occurring in developing countries, particularly in sub-Saharan Africa (Bethony *et al*., 2006).

More than 1.5 billion people or 24% of the world's population are infected with STH infections worldwide. Infections are widely distributed in tropical and sub tropical areas .with greatest number occurring in Sub-Saharan Africa, Americans china and East Asia. Over 270 million preschool age children and over600 million school age children live in areas where these parasites are intensively transmitted and one in need of treatment and preventive interventions (WHO, 2016).

2.6.2. Epidemiology and Geographical Distribution of Intestinal Parasites in Ethiopia

Dawit Ayalew (2006) reported that the higher prevalence of cryptosporidiosis, giardiasis and amebiasis has been found among children below 14 years old with an average prevalence rate of 11.9%, 38% and 33.7%, respectively in Dire Dawa.

Nineteen communities located in the southern part of the Ethiopian Rift Valley were surveyed for soil-transmitted helminth parasites of man (Birrie *et al*., *1994).* Parasites encountered included *A. lumbricoides* (1.2%), *T. trichiura* (10.3%), hookworms (25.3%), *Taenia* sp. (8.1%), *Strongyloides* sp. (2.9%), *H.nana* (0.8%) and and *E. vermicularis* (0.1%). In some communities the prevalence of hookworms, *A. lumbricoides* and *T. trichiura* reached 70%, 66.6% and 60%, respectively. According the study conducted in 6 districts in the South Gondar Zone of the Amhara National Regional State, 2279 school children were examined for helminthes (Jemaneh, 2000). The overall prevalence rates were 28.9%, 9.5% and 12.9% for *A. lumbricoides*, *T. trichiura* and hookworms, respectively.

In 2002, a parasitological survey was made to determine the magnitude of soil transmitted helminthiasis for Wondo Genet area, southern Ethiopia. The prevalence of infection for *Ascaris lumbricoides* and *Trichuris trichiura* among school children was 83.4% and 86.4%, respectively (Erko and Medhin, 2003). Legesse and Erko (2004) also reported high helminthiasis prevalence in primary school children in southeast of Lake Langano.

2.7. Diagnosis of Intestinal Parasitic Infections

Diagnosis of intestinal parasites is confirmed by the recovery of protozoan trophozoites and cysts, helminthes eggs and larvae in the clinical parasitological laboratory. Microscopic examination of feces is essential for the recognition and identification of intestinal parasites. Due to the low density of the parasites in the feces, direct microscopy is useful for the observation of motile protozoan trophozoites and the examination of cellular exudates, is not recommended solely for the routine examination of suspected parasitic infections. It is essential to increase the probability of finding the parasites in fecal samples to allow for an accurate diagnosis. Therefore, a concentration method is employed. (Direct wet mount examination should not be entirely excluded as the trophozoites are usually destroyed during the concentration procedure and therefore, microscopic examination of wet mounts should be performed). The concentration procedure requires the use of ether or ethyl acetate as a lipid removing agent and formalin as a fixative. Oocysts of the intestinal coccidians can be seen in a fecal smear by using a modified Ziehl-Neelsen method (Arcari *et al*., 2000).

In protozoan cysts, the number of nuclei and the presence of inclusions are the aid identification of protozoa. In trophozoites, the presence of red cells in amoebae is diagnostic of *E. histolytica* and flagella also aid identification of some protozoan trophozoites. In helminthes eggs, the shape of the egg, the thickness of the shell, the color of the ovum and the presence or absence of features such as an operculum, spine or hook lets are diagnostic pointers to the identity of the parasite (Arcari *et al*., 2000).

Trophozoites and cysts of the intestinal amoebae, flagellates and ciliates can be found and identified best in permanently stained fecal smears. Trophozoite stages are most often found in watery or diarrheic fecal specimens and usually cysts are not seen in such specimens. On the other hand, cysts are the stage typically found in formed fecal specimens. A mixture of trophozoites and cysts may occur in softer and semi-formed feces. In direct smears of feces in saline, motile trophozoites may be found (WHO, 2004).

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)

2.8. Prevention and Control of Intestinal Parasitic Infections

The prevention of intestinal parasitic infection depends upon the erection of barriers to the spread of parasites through the practical application of biologic and epidemiological knowledge. Almost every parasite at sometime in its life cycle is susceptible to several special exterminate measures. Thus, for human barriers such as sanitary excreta deposal may be established to break the link in the life cycle. The prevention of parasitic infection in man include the following stages; reduction of the source of infection by therapeutic measures, education is a personal prophylaxis to prevent dissemination of infection and reduce opportunistic for exposure, sanitary control of water, food living and working conditions, and waste disposal, destruction or control of reservoir hosts and vectors, erection of biological barriers to the transitions of parasites (Neva and Brawn, 1994).

2.9 Association between ABO Blood Group System and Intestinal Parasites

2.9.1. Association between ABO blood groups and Intestinal Protozoan Parasitic Infections

There is no doubt that genetic factors can play a major role in the protection of the body against some protozoan infection. This role of genetic factors is especially obvious in blood parasites; for example, Duffy blood group system confers resistance of RBCs to penetration by *Plasmodium vivax* (Marcela, and Anatole, 1999), and the presence of significance linkage of symptomatic visceral leishmaniasis to a region of chromosome 22q12 in a genome (Bucheton *et al*., 2003). This importance of genetic factor is not so distinct in intestinal protozoa. Various studies have sought to establish an association between the ABO blood types and intestinal parasites. These studies have however been unable to establish link between the ABO blood groups and the prevalence and incidence of intestinal parasites.

Study by Al-Taie *et al*., 2011, shows that an increase in number of AB and B groups and decrease in number of A and O groups. These variations in results might be indicated that there is no association between ABO and Rhesus blood groups and *G. lamblia* infection. Ayele *et al*. (2014) reported a significant association between the ABO blood group and infection caused by *E. histolytica* in West Gojjam, Amhara regional state. Blood group A is more susceptible than O, B and AB, respectively. According to Degarege *et al.* (2014) intestinal helminth-infected individuals with blood group AB had a significantly lower mean haemoglobin level compared to helminth-infected individuals with blood type O. Although statistically not significant, intestinal helminth-infected individuals with blood group A and B showed a trend towards a lower mean haemoglobin level compared to intestinal helminths infected individuals with blood type O. The study showed that no significant relation between ABO blood group systems and giardiasis.

2.9.2. Association between ABO blood groups and Intestinal Helminthes Parasitic Infections

In addition, *A. lumbricoides* extracts from individuals with blood type A and B prohibit agglutination of anti-A antibodies and anti-B antibodies, respectively (Deleon *et al.,* 2000; Deleon and Valverdeh, 2003). This suggests that, *A. lumbricoides* mimics A and B antigens of infected hosts. Thus, immune responses of individuals with blood type A, B and AB against the parasites may not be as effective leading to exacerbation of morbidity related to helminths infection, such as anaemia (Haseeb *et al*., 2008),

Degarege *et al. (*2015) reported that children with blood type A are associated with increased odds of helminth infection and a related reduction in mean haemoglobin level. The findings suggest that the ABO blood type might be linked with helminth infection and related clinical outcomes. In light of this, children with blood type A may be more susceptible to helminth, particularly hookworm, infection and related reduction in haemoglobin level, while children with blood type O could be partially protected against the infection and related anaemia. Different helminth species such as Hookworms, *S. mansoni* and *A. lumbricoides* contain polysaccharides that may resemble molecularly the substances in blood type A (Haseeb *et al.,* 2008).

Indeed, anti-A antibodies failed to recognize *A. lumbricoides* antigens isolated from individuals with blood type A (Deleon *et al*., 2000; Deleon and Valverdeh, 2003). This supports the notion that *A. lumbricoides* may have molecules that resemble the antigen of blood type A, or that the parasite may adsorb blood type A antigens. In addition, the increased odds of helminth infection among children with blood type A might be due to the possibility that blood type A antigens could contain an increased amount of glycolipid substances that serve as adhesion receptors for *hookworm, S. mansoni* and *A. lumbricoides* antigens (Berger *et al*., 1989). This will help the parasites to survive and multiply easily within the host, causing severe intestinal damage and high blood loss.

Previous studies have also documented high prevalence or intensity of *S. mansoni* in children with blood type A (Ndamba *et al*., 1997; Degarege *et al*., 2015).. Degarege *et al*., (2016) suggest that children with blood type A are at increased risk of helminth infection, especially hookworm, and related reduction in haemoglobin level.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

Babile district is one of the districts in the East Hararge Zone of the Oromia Regional state, eastern Ethiopia. The district is located at 560 km east of Addis Ababa and 30 km east of Harar and 54.1 km from Haramaya University. Babile district, has a latitude and longitude of 9°13′N -9°22′ N to 42°20′ E - 42°33′ E. The elevation is in ranges of 950-2000 m.a.s.l, with mean annual rain fall 410 – 800mm and mean annual temperature 24^{o}C – 28^{o}C. It is bordered on the south and east with Somali region, on the west with Fedis distinct and on the north with Gursum distinct. The district has currently an estimated population of 118,072 of whom 59,444 were men and 58,615 were women. Out of these.95.71% of the population is Muslim and 3.4% of them are orthodox. The three largest ethnic group reported in the *woreda* were Oromo (84.44%), Somali (10.77%), and Amhara (3.76%). All other ethnic groups composed of the remains 1.03% of the population as estimated from the 2007 national census report (https://www.google.com.et/**)**. The district has one Hospital and four Health centers namely Babile Health Center, Awsherif Health Center, Erer valley Health Center and Wayu Health Center in at which the present study was conducted.

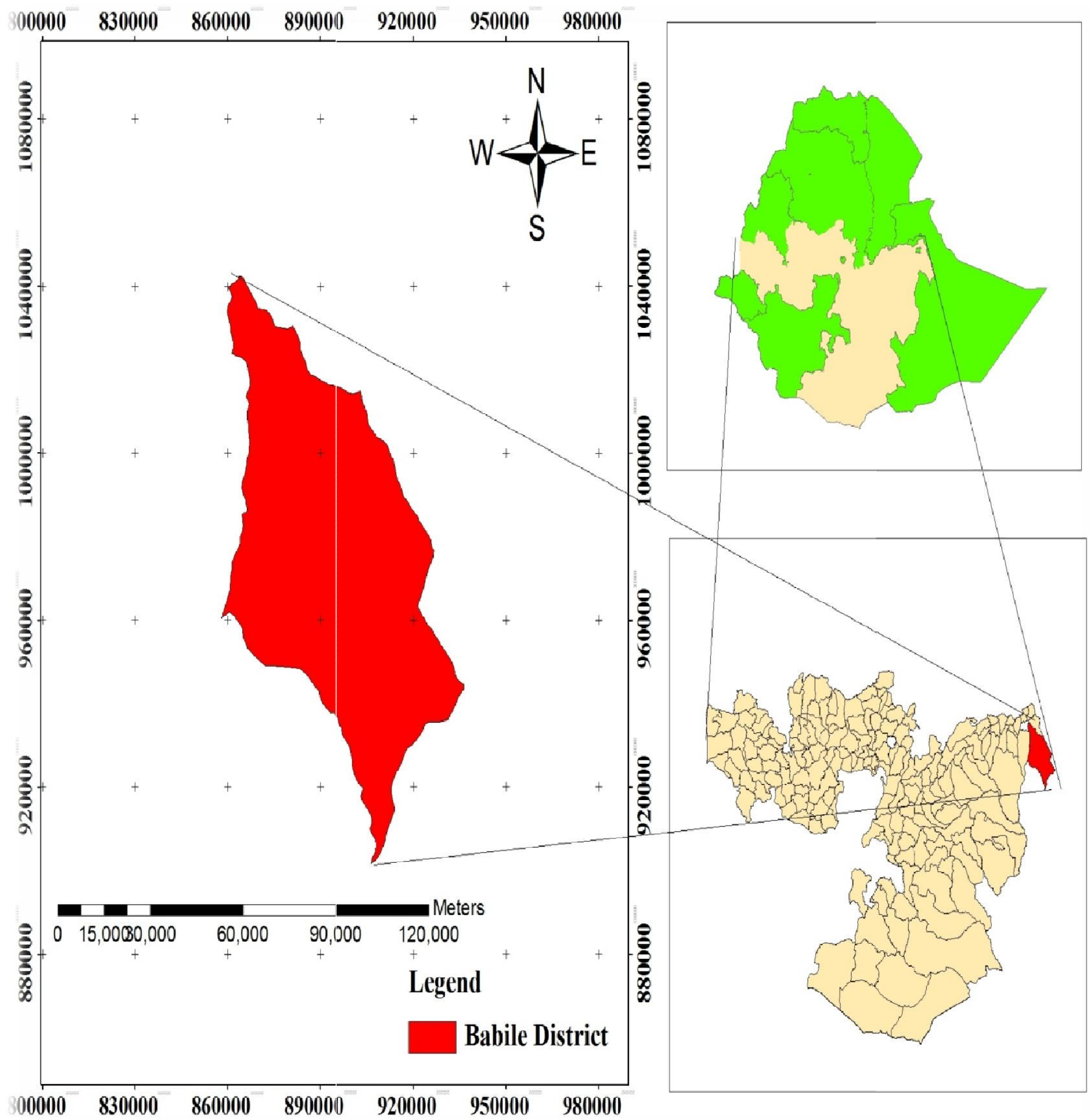


Figure 8: Map of the study area
Source: GIS

3.2. Study Design

A cross sectional survey was used to assess the prevalence of blood group systems (ABO and Rh-D) and intestinal parasitic infections and the association between the ABO and Rh(D) blood groups and intestinal parasitic infections among study participants in Babile Health Center, Awsherif Health Center, Erer Valley Health Center and Wayu Health Center of Babile district from November 2016 to February 2017.

3.3. Study Population

The background population for the study was permanent residents of Babile district. Patients of all ages groups who were visiting the district's health Center for Stool examination from November 2016 –February 2017, were considered as study population.

3.4. Inclusion-Exclusion Criteria

3.4.1. Inclusion Criteria

Individuals included in the study were permanent residents of the district, a member of a dominant (in terms of population size) Oromo ethnic group in the district, willing to give a signed consent to participate in the study.

3.4.2. Exclusion Criteria

Participants who took anti-parasitic drugs within the last two weeks before the date of the test were excluded. The Somali (n= 20(, the Amhara (n= 9) and the Gurage (n= 2) samples were excluded from this analysis due to their small sample size.

3.5. Sample Size Determination and Sampling Methods

The sample size for the study were estimated by using the following statistical formula used for determination of sample size in studies involving qualitative variables *(*Naing *et al.,* 2006

$$N = \frac{Z^2 p(1-p)}{d^2}$$

Where, n = required sample size

P = Prevalence of the disease

Z= 95% confidence interval, at 1.96 standard value

d= Absolute error or precision (usually= 0.05)

Since the overall prevalence of intestinal parasitic infections were not known for this study area, a prevalence of 50%was taken in the estimation. Inserting the values in to the formula was gave the minimum sample size of 384.

$$N = \frac{Z^2 p(1-p)}{d^2};\ N = \frac{(1.96)^2(0.5)(1-0.5)}{0.05^2} = 384$$

With 10% contingency the total sample size was over 420 participants.

In this study all of the four Health Centers found in the district were selected for their availability of laboratory equipments for data collection. The study participants were selected by serial random when they are coming to the health center for examination and treatment.

3.6. Data Collection

3.6.1. Socio Demographic Data

Socio demographic data were collected via questionnaire administered though face to face interview. The questions were used in order to capture data on socio-demographic characteristics (age, sex, ethnicity, and address), history of intestinal parasitic infections and environmental information relevant to the diseases.

3.6.2. Clinical and Laboratory Diagnosis

All the clinical data including the blood groups were collected following standard procedures by clinicians, nurses, and laboratory technicians.

3.6.2.1. Stool collection and examination

Participants were provided with a plastic stool container and asked to bring approximately 10g of their own stool. Stool examination was done by direct wet mount and Formol-ether concentration methods.

Direct wet mount method: a drop of saline was placed on a slide; approximately 0.05 g of the stool was added using an applicator stick; and mixed in a drop of saline and covered by cover slip. Finally the sample was examined microscopically at low power (10x objective) and high power (40 x objectives) magnifications for detection of intestinal parasites (Zhou *et al*., 2007).

Formol-ether concentration method: according to this method by using a wooden applicator stick, 1 gram of stool specimen was added to 8ml of 10% formalin in a small beaker and thoroughly emulsified, and brought into suspension. Next the suspension was strained through a double layer of wet gauze directly into a 15 ml centrifuge tube and the gauze was then discarded.

Then 3ml of diethyl ether was added to the suspension in the tube, stopper was closed and shaken vigorously for 10 seconds. The content was centrifuged by using Swinging bucket at 2000rpm for 3 minutes; the supernatant (comprising the top 3 layers) was decanted; and then the deposit/sediment was re-suspended with a disposable Pasteur pipette. A few drops of the suspension was transferred onto a microscope slide and covered with a cover slip. Finally the preparation was examined using the low power (X10) objective, and in a systematic manner as to observe the entire cover slip area. If an organism or suspicious objects are seen, the higher magnification (X 40 objective) was used to observe its detailed morphology (WHO, 1997).

3.6.2.2. ABO blood group and Rhesus tests

Blood samples were collected from each participant by open slide test method. The ABO and Rh (D) blood groups were typed using commercial kits (Jiangsu Huinda Medical Instruments Co., Ltd, China.) based on serological test of agglutination following the Health Centers recommended procedures. The ABO blood group of each subject was determined using cell grouping Antisera (A, B and D). Three drops of each subject blood sample is placed on separate slides or RPR (Rapid Plasma Reagin) card. A drop of antisera A, B and D was placed beside the blood and thoroughly mixed to obtain a homogenous mixture with the aid of applicator stick and the slide or RPR card will be rocked gently to ensure uniform mixing. The mixtures were carefully observed to determine blood group by the presence of agglutination or not. Antiserum D were used to determine the Rhesus factor and the blood of participants were grouped in to A+,A, B+, B-, AB+, AB-, O+ and O- (Simon-Oke *et al*., 2016).

3.7. Data Quality Control

The collected data were checked and rechecked for quality at all stages of the research; from data collection by entering them into excel for analyses. Particularly ethnicity was strictly considered, as populations may vary in the frequencies of the blood types, to make sure that population structuring were confound the association. The clinical data; history of intestinal parasitic infections and ABO and Rh blood group typing and identification of parasite type were collected by trained personnel (physician, nurses and laboratory technicians respectively) to have reliable data.

3.8. Ethical Consideration

This study followed the ethical standards and requirements set by the country in general and those by Haramaya University, College of Natural and Computational Science. Before starting data collection, explanation about the objectives and the procedure of the study was given by the researcher and a written informed consent form was obtained from each participant (or from parents/caretakers in the case of children under 18). Ethical clearance was obtained from Haramaya University, Ethical Review Committee.

3.9. Data Analysis

Data were entered in to Microsoft Excel, checked for its correctness, and exported to and analyzed using SPSS version 20. Chi-square test was used to assess the association between ABO blood groups and Intestinal parasitic infections. Observed difference were considered to be significant at $P<0.05$.with 95% confidence interval (CI.

In this study, the phenotypic distribution of blood groups among participants was expressed in simple percentages and frequencies. Gene frequency was calculated from phenotypic frequencies by considering two alleles at the same locus for Rh system and three alleles at the same locus for ABO system using Hardy-Weinberg equilibrium equations.

3.9.1. Phenotypic Frequency Determination of ABO and Rh-D Blood Groups

$$\text{Observed frequency} = \frac{\text{Observed number}}{\text{Total number}} \times 100$$

$$\text{Observed percentage} = \frac{\text{observed number}}{\text{expected number}} \times 100$$

3.9.2. Allelic Frequency Determination of ABO and Rh(D) Blood Groups

Frequency of the three ABO blood group alleles (p, q, and r,) were determined as follows

$r = \sqrt{O}$

$p = 1 - \sqrt{B+O}$

$q = 1 - \sqrt{A+O}$, (p, q, r denote allele frequencies and A, B, O denote observed frequencies of blood types A, B and O).

A correction factor (d) was calculated as

$d = 1 – p – q – r$.

The final allele frequencies were then calculated as follows:

$p1 = p\ (1 + d/2)$;

$q1 = q\ (1 + d/2)$;

$r1 = (r + d/2)\ (1 + d/2)$, where P1, q1, and r1 denote corrected allele frequencies.

Frequencies of the two Rh(D) blood group alleles (p and q) were determined as follows

$q = \sqrt{Rh}$, $P = 1 – q$, where p and q are allele frequecies

3.9.3. Genotypic Frequency Determination For ABO and Rh(D) Blood Groups

The frequencies of the genotypes at equilibrium were computed by trinomial expansion of the allelic frequencies. $(p + q + r)^2 = p^2(I^AI^A) + 2pq\ (I^{AB}) + q^2(I^BI^B) + 2pr\ (I^AI^O) + 2qr\ (I^BI^O) + r^2\ (I^OI^O)$ (Griffith *et al*., 2008)

The genotypic frequencies of ABO blood groups are calculated as follows:

- Genotype $I^AI^A = p^2$
- Genotype $I^AI^O = 2pr$
- Genotype $I^BI^B = q^2$
- Genotype $I^BI^O = 2qr$
- Genotype $I^AI^B = 2pq$
- Genotype $I^OI^O = r^2$

The genotypic frequencies of Rh (D) blood group are calculated as follows:

- Genotype $I^DI^D = p^2$
- Genotype $I^DI^d = 2pq$
- Genotype $I^dI^d = q^2$

3.9.4. The Chi-Square Test for the Goodness of Fit of ABO and Rh Blood Group Distribution

The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square test to check whether population is at Hardy- Weinberg genetic equilibrium or not (Yassin, 2013).

$$\text{Chi-square } (\chi^2) = \sum \frac{(O_f - E_f)^2}{E_f}$$

Where :

$\chi 2$= chi-square

df = degree of freedom

dev = deviation

dev^2 = deviation square

Expected phenotypic frequencies were calculated as:

Ef = Genotypic frequency X number of total sample

- For A blood group Ef = frequency of (AA + AO) X number of total sample
- For B blood group Ef= frequency of (BB + BO) X number of total sample
- For AB blood group Ef = frequency of AB X number of total sample
- For O blood group Ef = frequency of OO X number of total sample

4. RESULTS AND DISCUSSION

4.1. Socio Demographic Characteristics of Participants

A total of 420 participants were enrolled in the study. All 420 participants gave stool and blood samples and interviewed standardized and pre-tested questionnaires. Out of 420 participants, 245 (58.3%) were males and 175(41.7%) were female, 162 (38.6%) were in the age group of >10 years, all 420 participants were from Oromo ethnic group. In case of marital status, 267(63.6%) were unmarried, 146(34.8%) were married, 5 (1.2%) were divorced and 2 (0.5%) were widowed (Table 4).

Table 4: Socio-demographic characteristics of participants in Babile district (n = 420).

Characters		Frequency	Percent %
Sex	Male	245	58.3
	Female	175	41.7
Age	< 10	162	38.6
	11-20	105	25
	21-30	87	20.7
	31-40	40	9.5
	41-50	10	2.4
	51-60	10	2.4
	>61	6	1.4
Ethnicity	Oromo	420	100
Marital status	Unmarried	267	63.6
	Married	146	34.8
	Divorced	5	1.2
	Widowed	2	0.5

4.2. Distribution of ABO and Rh-D Blood Group Phenotypes

The frequency distribution of ABO and Rh(D) blood groups among the study participants in Babile district are shown in Table 5. Phenotypic frequencies of ABO and Rh(D) blood groups of 420 Oromo sample are expressed in simple percentages as indicated in the Table 5.

There are large differences among phenotypic percentage distribution of the ABO blood group of Oromo in the study area. Blood type "O" has the highest frequency while blood type "AB" has the lowest frequency distribution (Table 5). In the present study , the proportion blood type "O" was 48.6%, followed by blood type A", 28.6%, blood type and "B"17.4%. The least percentage was that of blood type "AB" (5.5%), (Table 4).

Many other studies of the world have shown that blood type "O" was the most common blood type and blood type "AB" was the least common blood type in different ethnic groups (Nwauche and Ejele, 2004). Among the Caucasians in the United States of America, the frequency of blood type "O", "A", "B" and "AB" were 47.0%, 41.0%, 9.0% and 3.0 %, respectively (Adeyemo and Soboyejo, 2006) which is in agreement with this study.

This study was also in agreement with ABO frequency in Tigrean, Kunama, Saho and Blen people where blood type O was 43.47 %, 41.09 %, 44% and 41.5%, respectively, followed by blood type A, 26.51, 23.9%, 27%, and 27.5% and blood type B was 24.78%, 26.03%, 24% and 25.5% in Tigrean, Kunama, Saho and Blen ethnic groups, repectively and the least percentage frequency was that of blood type AB in the three ethnic groups which is 5.21%, 5.48%, 5%, and 5.5% in, Tigrean, Kunama, Saho and Blen Ethnic people respectively (Zelalem, 2014)

The frequency distribution of Rh (D) blood group in Babile district is also shown in Table 5. Rh (D) positive blood type had higher frequency than Rh(D) negative blood type. As shown in Table 5, the frequency distributions of Rh phenotypes among Oromo ethnic people vary. The frequency distribution of Rh positive blood type among Oromo was 98.8%, and the frequency of Rh negative blood type was 1.2 %.

This result was consistent with previous findings of Ethiopian populations (Abraham *et al*., 2012). In addition, the findings of this study was in agreement with report from previous similar studies in different parts of the world where the Rh(D) positive was found to be higher in the population sampled than the Rh (D) negative (Adeyemo and Soboyejo, 2006, Bakare *et al.,* 2006, Ahmed *et al*., 2007, Ahmed *et al*., 2009; Tekleab ,2014). This finding is in agreement with the previous studies in Nigeria the frequency of Rhesus D positive antigen was found in 98.4% and Rh (D) negative was 1.6% of all the children (Onanuga and Lamikanra, 2016). Jeremiah (2006); Pennap *et al*., (2011) reported 94 - 98% of Rhesus D positive antigen in adult individuals in south India. About 95% of African-Americans are Rh-positive (Chavhan *et al.*, 2010 and Abraham et *al*., 2012).

Table 5 also shows the combined frequency distributions of ABO and Rh blood group phenotypes in the overall sample. The percentage of the ABO phenotypes linked with Rh positive phenotypes was O+ (48.3%) and its frequency is relatively higher than the others. Blood type A+ was 28.3%, blood type B+ was obtained to be 16.9%,. Blood type AB+ was 5.2%, its frequency was relatively lower than the other (Table 5).

The percentage of blood type A- was 0.2%, the frequency of individuals with blood type B- was 0.5% and its frequency was relatively higher than the other blood type, the frequency of individuals with blood type AB- was 0.2%, the distribution of O-blood in the present study was 0.2 and its frequency was relatively lower in the study population than the rest blood types. Thus, apart from the importance of ABO and Rh (D) blood group systems and the variations in blood type frequencies in Oromo sample, there is a need to have information on these blood type frequencies in any population of different ethnic group. The relevance of having knowledge about the blood group systems among different ethnic groups in any population is enormous.

Table 5: Distribution of ABO blood group phenotypes among Oromo ethnic group

Sex	Rh blood group	ABO Phenotypes								Total
		A		B		AB		O		
		No	%	No	%	No	%	No	%	
Male	Positive	45	25.7	32	18.3	10	5.7	85	48.6	175
	Negative	1	0.6	2	1.1	0	0	0	0	
Female	Positive	74	30.2	39	7.75	12	4.9	118	48.2	245
	Negative	0	0	0	0	1	0.4	1	0.4	
Total	Positive	119	28.3	71	16.9	22	5.2	203	48.3	415(98.8%)
	Negative	1	0.2	2	0.5	1	0.2	1	0.2	5 (1.2%)
		120	28.6	73	17.4	23	5.5	204	48.6	420

4.3. Estimation of Allelic and Genotypic Frequencies of ABO and Rh Blood Groups among Individuals in the study area

4.3.1. Allelic Frequencies of ABO and Rh Blood Groups in the Study Area

Allele frequencies of ABO blood groups of the participants of Oromo ethnic group are presented in Table 6. The frequencies of alleles I^A, I^B, and I^O were calculated according to the modified Hardy -Weinberg Law of equilibrium based on data presented in Table 2. The allele frequencies of the ABO blood groups in the overall data were 0.186 I^A, 0.121 I^B, and 0.693 I^O (Table 6). As indicated in Table 6, the frequencies of ABO blood group alleles of the participants were 0.186 I^A, 0.121 I^B, and 0.693 I^O. The order of allele frequencies of ABO blood group in the overall sample were $I^O > I^A > I^B$. Previous studies among various segments of the world population have documented similar pattern of allelic frequencies. For instance, studies by Hussain *et al*. (2001) among Baluchistan in Pakistan, Yan *et al.* (2005) on Chinese populations, Bakare *et al*. (2006) in Ogbomoso, South-west Nigeria, and Iyiola *et al*. (2011) in Ilorin, Kwara State of Nigeria all found the allelic frequencies to occur in $I^O>I^A>I^B$ order.

The allele frequencies of Rh-D blood group were calculated according to the Hardy-Weinberg equation using the data presented in Table 6. The frequency of allele *D* and *d* are found to be 0.891 and 0.109 respectively in the overall sample (Table 6). This shows that allele *D* has higher frequency than allele *d*. This also agrees with many studies where Rh positive has higher incidence than Rh negative in different populations and ethnic groups (Nwauche and Ejele, 2004; Bakare *et al*., 2006)

4.3.2. Genotypic Frequencies of ABO and Rh Blood Groups in the Study Area

The genotypic frequencies for ABO blood groups of 420 participants were calculated based on estimated allele frequencies according to the Hardy-Weinberg Law. Table 6 presents the frequencies of the various genotypes and allele frequencies in the ABO blood group system of the Participants in Babile district.

Table 6 also, presents the frequencies of the various genotypes in the ABO and Rh systems. So, that I^OI^O = 0.4802, I^AI^A = 0.0346, I^AI^O= 0.2578, I^AI^B = 0.0450, I^BI^B = 0.0146 and I^BI^O =

0.1677 in the study area. The frequencies of the genotypes for Rh blood group were I^DI^D = 0.7938, I^DI^d = 0.1942 and I^dI^d = 0.0118 in the study participants. The study also agreed with the suggestion of Bakare *et al.,* (2006), that the predominance of "O" allele may also be as a result of the fact that many "A‟s" and "B‟s" may have been heterozygous carrying "O" allele silently thereby maintaining "O' allele in the heterozygous population.

Table 6. Genotypes and alleles Frequencies of ABO and Rh blood groups blood groups among population of Babile district

ABO blood type	Gene(allele)	Allele Frequency	Genotype	Genotype Frequency	Phenotype	Phenotype Frequency
A	$I^A(p)$	0.186	I^AI^A (p^2)	0.0346	A	29.24
			I^AI^O (2pr)	0.2578	A	
B	$I^B(q)$	0.121	I^BI^B (q^2)	0.0146	B	18.23
			I^BI^O (2qr)	0.1677	B	
AB			I^AI^B (2pq)	0.0450	AB	4.5
O	$I^O(r)$	0.693	I^OI^O (r^2)	0.4802	O	48.02
Rh-D Factor						
D	I^D	0.891	I^DI^D (p^2)	0.7938	D	98.8
			I^DI^d (2pq)	0.1942	D	19.42
D	I^d	0.109	I^DI^d (q^2)	0.0118	D	1.2

4.3.3. The Chi-Square Test for the Goodness of Fit of ABO and Rh Blood Group Distribution

In this study, the ABO blood group distribution is compared with the population of Ethiopia by using the Chi-square test at P value < 0.05, 95% confidence level. The ABO blood group distribution of Ethiopia is given in Table 2 which is blood group O 42%, A 30%, B 22% and 6% AB.

Table 7 below shows observed versus the expected values of ABO blood group phenotypes. The deviations between the distributions of observed and expected values in the Hardy-Weinberg equilibrium were tested using chi-square. The distribution of the overall observed frequencies of ABO blood group phenotypes do not differ significantly from those expected under Hardy Weinberg equilibrium (Goodness of fit χ^2 = 1.1431, df = 3, p> 95%)

(Table 7). This shows that the population is at genetic equilibrium. This might be due random intermarriage in the population.

Table 7. Observed versus expected frequency of ABO blood groups phenotypes of participants in the total sample

Blood groups	Observed	Expected	Dev	Dev^2/ Expected
A	12O	122.808	2.808	0.0642
B	73	76.566	3.566	0.1660
AB	23	18.9	4.1	0.8864
O	204	201.684	2.316	0.0265
Total	420			

The chi-square test for Rh was compared with Ethiopia's population in general at P value <0.05, 95% confidence level (Table 3). The Rh blood group phenotypic distributions in the study participants is given in Table 5 as Rh positive, 98.8% and Rh negative.1.2%.

Table 8 shows observed versus the expected values of Rh-D blood group phenotypes in the total sample. The variation of distribution of the overall observed frequencies of Rh-D blood group phenotypes from those expected under Hardy-Weinberg equilibrium were also in significant (Goodness of-fit $\chi 2 = 1.5\times10^{-9}$, df = 1, p > 95%) (Table 8).

Table 8: Observed versus expected frequency of Rh-D blood groups phenotypes of participants in the total sample

Blood groups	Observed	Expected	Dev	Dev^2/ Expected
Rh-D +ve	415	414.96	0.04	0.00000385
Rh-D –ve	5	4.956	0.044	0.00039
Total	420			

4.4. Prevalence of Intestinal Parasites among Population of Babile district

In the present study, microscopic stool sample examination by direct mount techniques showed that infections with various intestinal helminthes and protozoan parasites were common among children in the study areas. The prevalence of infection with different intestinal helminthes and protozoan parasites was shown in Table 9.

Intestinal parasites including protozoans and helminthes were detected. The prevalence of *G. lamblia* infection 103 (24.5%) followed by *E. histolytica* 103 (24.5%), *S. mansoni* 23 (5.5%) and *H. nana* 12(2.8%) and multiple intestinal parasites 4(0.9%) was recorded in study participants. For both sexes, the proportion of infections was higher for protozoa compared to helminthes (Table 9).

According to the present study, infection with *G. lamblia* and *E. histolytica* were more prevalent among both male and female study participants, while multiple intestinal parasite was least prevalent in both sexes .The result of parasitological investigations showed that, from 420 specimens of the Oromo participants, 245 (58.3%) were positive for one or more intestinal parasites. Of these, 118 (48.2%) were male and 127 (51.8%) were female participants positive for one or more intestinal parasites. The prevalence rate of intestinal parasites in terms of sex was *G. lamblia* 22% and 28%, *E. hisolytica* 19.6% and 31.4%, *S. mansoni* 4.5% and 6.8% , *H. nana* 1.6% and 4.6% and multiple infection 0.4% and 1.7% for male and female, respectively.

Table.9: The prevalence (%) of Intestinal Parasitic infections, by sex in 420 study participants attending of Babile district Eastern Ethiopia.

Sex	No examined	*G. l*	*E. h*	*S. m*	*H. n*	Multiple infection
		No %	No %	No %	No %	No %
Male	245	54 (22%)	48 (19.6%)	11 (4.5%)	4 (1.6%)	1(0.4%)
Female	175	49 (28%)	55 (31.4%)	12 (6.8%)	8 (4.6%)	3(1.7%)
Both sexes	420	103(24.5%)	103(24.5%)	23 (5.5%)	12 (2.8%)	4(0.9%)
Chi-square		5.489	1.368	0.727	0.353	0.462
P- value		0.019	0.252	0.394	0.552	0.497

Key: *G.l Giardia lamblia*, *E.l Entoameba histolytica, S.m Schistosoma mansoni* and *H.n Hymenolepis nana*

According to the data on Table 10, highly significant association was found between *Gardia lamblia* and age group of participants (p = 0.000). The prevalence of *Gardia lamblia* species by age of the study individuals; 37.6%, 15.2%, 17.2%, 10%, 20%, 40% and 16.7% were in the age group of less than/equal to 10, 11-20, 21-30, 31-40,41-50, 51-60, and greater than/equal to 61 years old, respectively had infection. Similarly, 20.3%, 26.7%, 27.6%, 22.5%, 40%, 40% and 16.7% were in age group of less than/equal to 10, 11-20, 21-30, 31-40, 41-50, 51-60, and greater than/equal to 61 years old had *Entoameba histolytica* infection, respectively.

On the other hand, 4.9%, 5.7%, 6.9%, 5% and 10% were at age groups of less than/equal to 10, 11-20, 21-30, 31-40 and 41-50 years old had infected by *Schistosoma mansoni*, respectively. 5.5%, 0.9%, 2.5% and 10% were in age group of less than/equal to 10, 11-20, 31-40 and 41-50 had *H. nana* infection, respectively. and 1.2%, 0%, 1.1%, 10%, 0% and 0% were in age group of less than /equal to 10, 11-20, 21-30, 31-40, 41-50, 51-60 and greater than/equal to 61 years old had multiple parasitic infections, respectively.

In the study area the prevalence of infection decreased with an increase in age of the participants. According to this study the prevalence of parasitic infections were high in children less than 10 years old and lower in adults. The finding of 90% and 80% prevalence in individuals with age group of 41-50 and 51-60 adults respectively in the study area is difficult to interpret as only 10 individuals in each age group had examined. The prevalence of parasitic infections was higher in the age group of <10, years (69.1%), children. This was because younger people have lower resistance to parasitic infections as compared to adults since many of the defense systems are not fully developed in children and children usually do not take care of their personal hygiene. For instance, they play in contaminated outdoor environments, in and around disposal sites (which can certainly cause serious health problems), face problems of absence of latrine and lack of basic life skills, such as washing hands before and after meals (Abu, 2004).

Table 10: The prevalence (%) of intestinal parasitic infections, relative to host age, in 420 patients attending Babile district Eastern Ethiopia

Age group (years)	No examined	G.l	E.h	S.m	H.n	Multiple infections
		No %	No %	No %	No %	No %
< 10	162	61(37.6%)	33(20.3%)	8(4.9%)	9(5.5%)	1(1.2%)
10-20	105	16(15.2%)	28(26.7%)	6(5.7%)	1(0.9%)	0(0%)
21-30	87	15(17.2%)	24(27.6%)	6(6.9%)	-	1(1.1%)
31-40	40	4(10%)	9(22.5%)	2(5%)	1(2.5%)	1(2.5%)
41-50	10	2(20%)	4(40%)	1(10%)	1(10%)	1(10%)
51-60	10	4(40%)	4(40%)	-	-	0(0%)
≥ 61	6	1(16.7%)	1(16.7%)	-	-	0(0%)
All age groups	420	103(24.5%)	103 (24.5%)	23(5.5%)	12 (2.3%)	4 (0.9%)
Chi-square		30.438	6889	1.781	10.509	11.086
P- value		0.000	0.441	0.971	0.162	0.135

Key: *G.l Giardia lamblia*, *E.l Entoameba histolytica, S.m Schistosoma mansoni* and *H.n Hymenolepis nana*

4.5. Association of ABO and Rh-D blood group Systems with Intestinal Parasitic Infections

The present study assessed all the data of the parasitic infections which was found in 58.3% of the total population and its association with ABO and Rh(D) blood group systems. The results of this study showed no significant association between *E. histolytica* and A blood type (p = 0.542), B blood type (p = 0.977), AB blood type (p = 0.750) and O blood type (p = 0.500) in the present data on Table 11. Blood group O is more susceptible than A, B and AB for *E. histolytica*. The frequency of ABO blood groups in patients with *G. Lamblia* does not differ significantly from population data with A blood type (p=0.084), B blood type (p =0.569), AB blood type (p=0.618) and O blood type (*p*=0.826). Despite the absence of significance difference in frequency of ABO between *G. lamblia* infected and non infected participants in the present study blood type A is more susceptible than O, B and AB for *G. lamblia*(Table 11).

This study shows there was a no significant association between *S. mansoni* infection and, ABO blood type. However, blood type A and B were more susceptible than O and AB for *S. mansoni*. It has been hypothesized that blood type A individuals are at an increased risk for *S. mansoni* infection due to the absence of anti-A antibodies. Haseeb *et al*., (2008) reported evidence that chemo-attraction between adult flukes likely involves N-acetyl-D-galactosamine (GalNAc) epitopes on females. Anti-A may provide protection against *S. mansoni* by blocking GalNAc structures necessary for fluke mating.

Previous studies have also documented high prevalence or intensity of *S. mansoni* infection in children with blood type A (Ndamba *et al*., 1997; Degarege *et al*., 2015). The number of children infected with *S. mansoni* (n= 23) in the present study was small. This could be the reason why the difference in the prevalence of infection with A. *S. mansoni* was not significant when compared across the different blood types in the current study. This was in agreement with study conducted in Tikur Wuha Elementary School in north-western Ethiopia (Degarege *et al*., 2016). This variation in results could be due to nature of the study participants. Variation in socioeconomic and other genetic factors (such as human leucocyte antigen, glyoxalase I system) that may affect an individual's susceptibility for parasitic infection may contribute to the unseen difference in the risk of parasitic infection between the different blood types.

The result of this study indicates that there was no significant association between *H. nana* infection and ABO blood groups, with A blood type (p= 0.749), B (p = 0.138), AB blood type (p = 1.000) and O blood type (p = 0.383). But frequency of *H, nana* was high among patients of blood type "B" and low among blood type "AB". Present study also indicated the lack of significant association between ABO blood groups and multiple intestinal parasites. Some studies reported a lack of association between ABO blood type and prevalence of helminthes infection (Gabr & Mandour, 1991; Cooper *et al.,* 1993; Degarege *et al*., 2014). This could be due to the nature of the study participants. The study by Degarege *et al*. (2014) involved patients who might be immunologically compromised, and the study by Cooper *et al*., (1993) was conducted among adults, who might have a stronger immune status than children. As a result, the effect of ABO blood type on helminthes infection might have been underestimated.

In addition, variation in socioeconomic and other genetic factors (such as human leucocyte antigen, glyoxalase I system) that may affect an individual's susceptibility for parasitic infection may contribute to the unseen difference in the risk of parasitic infection between the different blood types in previous studies (Gabr & Mandour,1991; Cooper *et al*., 1993; Degarege *et al*., 2014). Furthermore, a small size (n= 420) might also have resulted in the absence of significant difference in prevalence of parasitic infection across different blood types (Gabr & Mandour, 1991.

The ABO blood group individuals infected with *G. lamblia* was A (26.7%), O (25%). B(23.3%) and AB (17.4%). The percentage of infected ABO blood group individuals with *E. histolytica* were O (26 %), B (24.7%), A (22.5%) and AB (21.7%). Individual ABO blood group infected with *S. mansoni* were A (8.3%), B (8.2%), O (3.4%) and AB (0%). Infected ABO blood group individuals with *H. nana* were B (5.5%), A (3.3%), O (2%) and AB (0%)and also Infected ABO blood group individuals infected with multiple intestinal parasite were AB(4.3%), B(1.4%), A(0.8%) and O (0.5%) in study participants (Table11).

Table 11: Association of ABO blood group with some intestinal parasitic infection in the study area

Blood group	Number Examined (no = 420)	Intestinal Parasite type		χ2, P
		G. lamblia Positive	*G. lamblia* negative	
A	120	32(26.7%)	88 (73.3%)	0.417, 0.519
B	73	16(23.3%)	57(76.7%)	0.324, 0.569
AB	23	4(17.4%	19(82.6%)	0.669, 0.618
O	204	51(25%)	153(75%)	0.049, 0.826
		E. histolytica positive	*E. histolytica* Negative	
A	120	27(22.5%)	93(77.5%)	0.372, 0.542
B	73	18(24.7%)	55(75.3%)	0.001, 0.977
AB	23	5(21.7%)	18(78.3%)	0.102, 0.750
O	204	53(26%)	151(74%)	0.455, 0.500
		S. mansoni Positive	*S. mansoni* Negative	
A	120	10(8.3%)	110(91.7%)	2.649, 0.104
B	73	6(8.2%)	67(91.8%)	1.284, 0.257
AB	23	0(0%)	23(100%)	1.410, 0.627
O	204	7(3.4%)	197(96.6%)	3.204, 0.073
		H. nana Positive	*H. nana* Negative	
A	120	4(3.3%)	116(96.7%)	0.137, 0.749
B	73	4(5.5%)	69(94.5%)	2.189, 0.138
AB	23	0(0%)	23(100%)	0.716, 1.000
O	204	4(2%)	200(98%)	1.148, 0.383
		Multiple IP Positive	Multiple IP Negative	
A	120	1(0.8%)	119(99.2%)	0.025, 1.000
B	73	1(1.4%)	72(98.6%)	0.163, 0.535
AB	23	1(4.3%)	22(95.7%)	2.974, 0.202
O	204	1(0.5%)	203(99.5%)	0.898, 0.624
Total	420	245(58.3%	175(41.7%)	20.788, 0.144

The proportions of the Rh positive individuals affected with Intestinal parasite infections were 100 % (*H. nana*), 100%, (*S. mansoni*), 100% multiple intestinal parasite, 98% (*E. histolytica*) and 97.1 % (*G. lamblia*) of the total 420 individuals of the study site for each category of the intestinal parasite infections. Among Rh negative individuals with intestinal parasite infection, (2.9%) *G. lamblia*, (2%) *E. histolytica*, (0%) *H. nana*, (0%) *S. mansoni* and (0%) multiple intestinal parasite was recorded. Also, there was no significant difference in the frequency of Rh factor between infected and non-infected population (P = 0.340), 98.8% patients were typed as Rh positive and 1.2 % subjects were Rh negative. These variations in results indicated that there is no correlation between ABO and Rhesus blood groups and Intestinal parasitic infections (Table 12).

Table 12: Association of Rh- factor with some intestinal parasitic infection in study population

Rh blood group	No. Examined (n = 420)	Intestinal Parasite type		χ2, P
		Rh- Positive	Rh- Negative	
G. lamblia positive	103	100(97.1%)	3(2.9%)	3.441, 0.097
G.lamblia negative	317	315(99.4%)	2(0.6%)	
		Rh positive	Rh- Negative	
E. histolytica positive	103	101(98%)	2(2%)	0.655, 0.600
E. histolytica negative	317	314(99%)	3(1%)	
		Rh-Positive	Rh-Negative	
S. mansoni positive	23	23(100%)	0(0%)	0.293, 1.000
S. mansoni negative	397	392(98.7%)	5(1.3%)	
		Rh- Positive	Rh- Negative	
H. nana positive	12	12(100%)	0(0%)	0.162, 1.000
H. nana negative	408	403(%)	5(%)	
		Rh- positive	Rh-Negative	
Multiple IP's positive	4	4(100%)	0(0%)	0.049, 1.000
Multiple IP's negative	416	411(98.8%)	5(1.2%)	
Total	420	415(98.8%)	5(1.2%)	5.669, 0.340

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study aims at providing information on the distribution pattern of the phenotypes, genotypes, and the allelic frequencies of the ABO and Rh blood group and its association with intestinal parasitic infections among study participants in Babile district.

A total of 420 study subjects were participated in this study. A total of 420 blood samples were collected from study individuals from November 2016 to January 2017 G.C. Blood types were determined by open slide test and RPR card method. A drop of each of the anti sera, anti A, anti B and anti D was added and mixed by applicator stick for 60 minutes with each blood sample and rocked gently to observe agglutination. Stool examination was done by direct wet mount; a drop of saline was placed on a slide; approximately 0.05 g of the stool was added using an applicator stick; and mixed in a drop of saline and covered by cover slip. Finally the sample was examined microscopically at low power (10x objective) and high power (40 x objectives) magnifications for detection of intestinal parasites

In this study, the percentage frequency distribution of blood type O is the highest with percentage frequency of 48.6%, followed by blood type A (28.6%, and blood type B (17.4%, and the least percentage frequency is that of blood type AB (5.5%. With respect to allelic , allele I^O records the highest frequencies (0.693), followed by allele I^A (0.186), while allele I^B records the least frequency (0.121), in the study participants.

In addition, it was observed that Rh (D) positive has the highest percentage frequency (98.8%), while Rh-negative has the lowest percentage frequency (1.2%) as observed among the participants in the study area. The frequency of I^D allele was (0.891), while the frequency of I^d allele was (0.109) for study participants.

From this findings, it was true that the proportion and allele frequencies of individuals belonging to blood type O in the studied population were most predominant. The indication of this is that blood type O is the most commonly available blood group in the study area which is more advantageous for the population in the event of blood transfusion.

According to this study the prevalence of *G. lamblia* infection was 103 (24.5%) followed by 103 (24.5%) of *E. histolytica* 23(5.5%) of *S. mansoni*. 12(2.8%) of *H. nana* and 4 (0.9%) of multiple intestinal parasitic infections, respectively. For both sexes, the proportion of infections was higher for protozoa compared to helminthes. The prevalence of *G. lamblia* and *E. histolytica* were more prevalent among both male and female population, while multiple intestinal parasitic infection was least prevalent in both sexes. The proportions of the Rh positive individuals affected with intestinal parasite infections were high in *H. nana* and *S. mansoni* while among Rh negative individuals with intestinal parasite infection; *G. lamblia was* higher than the rest.

5.2. Conclusions

The distribution of the overall observed frequencies of ABO and Rh (D) blood group phenotypes do not differ significantly from those expected under Hardy Weinberg equilibrium. This indicated that, the study population is under Hardy Weinberg equilibrium.

In the study area the prevalence of infection decreased with an increase in age of the participants. According to this study the prevalence of parasitic infections were high in females and children less than 10 years old. Phenotypic, genotypic and allelic frequency of ABO blood group system in the studied population does not show significant association with prevalence of intestinal parasitic infections.

Generally, this study would be helpful to the researchers in the field of population genetics to explore the factors responsible for the observed distribution patterns of these genetic markers in this part of Eastern Ethiopia. The study would generate data on prevalence of intestinal parasites and frequency of blood groups [ABO and Rh (D)] in Babile district. These data would help partly in designing appropriate prevention and control strategies against intestinal parasite infections in the area. The blood group data would help in planning blood donation campaigns. People with more severity blood types may be warned to take necessary precautions in the area.

5.3. Recommendation

- ✓ Studies of similar kind should be carried out in other populations too, so as to have better information about the distribution of ABO and Rh blood group alleles among different ethnic groups in the country
- ✓ Further study at molecular level would definitely reveal the degree of genetic proximity of IP's and ABO and Rh(D) blood groups in study population
- ✓ The sample size may not represent the population to test if there is, strong association between ABO blood group and intestinal parasites, further study with more sample size is needed.

6. REFERENCES

Abraham Haileamlak, Ayalew T. Muluneh, Fessahaye Alemseged, Fasil Tessema, Kifle Woldemichael, Makonnen Asefa, Yoseph Mamo, Solomon Tamiru, and Gemeda Abebe. 2012. Hemato-immunological profile at Gilgel Gibe Field Research Center, south west Ethiopia. *Ethiopian Journal of Health Science,* 22 (1): 39-50.

Abu Mourad T.A, 2004. Palestinian refugee conditions associated with intestinal parasites and diarrhoea: Nuseirat refugee camp as a case study. *Journal of Public Health*, 118: 131-142.

Adeyemo, A.F. and Soboyejo O.B. 2006. Frequency distribution of ABO, RH blood groups and blood genotypes among the biology and genetics students of university of Lagos, Nigeria. *Afri. J biotechnology,* 5 (22): 2062-2065.

Ahmed, N., M. Dawson, C. Smith, and E. Wood. 2007. *Biology of Disease.* Taylor and Francis Group. New York

Ahmed, S.G., Ibrahim, U.A; and Hassan, A.W. 2007. Adequacy and pattern of blood donations in northeast Nigeria: The implications for blood safety. *Annual Tropical Medical Parasitological,* 10(1): 725-731.

Ahmed, S.G., Kangu, M.B. and Abjah, U.A.M. 2009. The role of du testing in scaling down the burden of Rhesus-D negative transfusion in Northern Nigeria. *International Journal of Third World Medicine,* 8(2): 983-1006.

Akbas, F., M. Aydin and A. Cenani, 2003. ABO blood subgroup allele frequencies in the Turkish population. *Anthropol. Anz*., 61:257-60.

Akhigbe, R.E., Ige, S.F., Afolabi, A.O., Azeez, O.M. Adegunlola, G.J. and Bamidele, J.O. 2009. Prevalence of hemoglobin variants ABO and rhesus blood groups in Ladoke Akintola University of Technology, Ogbomoso, Nigeria. *Trends Medical Research,* 4(3): 24-29.

Aksoy, U. Akisu, C. Tuneay, S. Delibas, S.B. Iceboz, T. Over, .L. Oral, A M, 2005. An Outbreak of Intestinal Protozoa Associated with drinking Water. *Journal of Science and Medicine,* 73:163-174.

Albonico, M, Crompton, D.W, Savioli L, 1999. Control strategies for human intestinal nematode infections. *Adv. Parasitol*ogy, 42: 277-341.

Al-Taie, Lazim, H. Kasim Sh. Al-Mayah, Abdul-Jalil Thweni, 2011.The association between human giardiasis and ABO and Rhesus blood groups. *Iraqi Journal of Science,* 52(1): 132-134.

Arcari, M., A. Baxendine and C.E. Bennett, 2000. Diagnosing Medical Parasites through Coporological Techniques. University of Southampton (Industrial Liaison).

Ayele Mandefro, Musin Kelel and Gary Wessel, 2014. Association of ABO Blood Group and Rh Factor with Malaria and Some Gastrointestinal Infectious Disease in a Population of Adet and Merawi, Ethiopia. *Global Journal of Biotechnology and Biochemistry,* 9 (4): 137-142.

Bakare, A.A., M.A. Azeez and J.O. Agbolade, 2006. Gene frequencies of ABO and rhesus blood groups and hemoglobin variants in Ogbomosho, South-West Nigeria. *Afr. J. Biotechnology,* 5:224-229.

Barnes, G.L, Kay, R, 1977. Blood groups in giardiasis. *Lancet,* 1: 808.

Beltran, M, Garaycochea, M, Bellido, N, Garcia J, RIOS l, Bermui, G, Gonzales, R, 2004. Prevalence of Ameobiasis by *Entoameoba histolytica* in three region of peru. *National Institute of Health*, 8: 316.

Berger, S.A., Young, N.A. & Edberg, S.C. 1989. Relationship between infectious diseases and human blood type. *European Journal of Clinical Microbiology and Infectious Disease,* 8: 681–689.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D. and Hotez, P.J. 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis and hookworm.relation to environmental, socioeconomic, and behavioral determinants of exposure to infection in children from southeast Madagascar. *J. Parasitol*ogy, 84: 480–484.

Birrie, H., Erko, B. and Tedla, S. 1994. Intestinal, helminthic infections in the southern Riftvalley of Ethiopia with special reference to schistosomiasis. *East Afr Med J*., 71 (7): 447- 452.

Blumenfeld. O.O and Patnaik. S.K. 2004. Allelic genes of blood group antigen: a source of human mutation and cSNPs documentation in the Blood Group Antigen Mutagen Data base. *Hum. Mutat*., 23: 8-16.

Bogitsh, B.J., Carter, C.E. and Oeltmann, T.N., 2005. Human Parasitology. 3rd ed. Elsevier Academic Press. London. pp. 348-353.

Bucheton, B.;Abel, L.and El-Safi, S. 2003. A major susceptibility locus on chromosome 22q12 plays a critical role in the control of kala-azar. *Am. J. Human Genet*ics, 73:1050 –1060.

Cappello, M., 2004. Global health impact of soil-transmitted nematodes. *Pediatr Infect Dis J.,* 23 (7):663-664.

CDC (Center for disease control and prevention), 2006. Laboratory Identification of Parasites of Public Health Concern Center. Atlanta.

CDC (Center for disease control and prevention), 2007. Department of human health and human services. http://www.cdcinfocdc.gov.

CDC (Center for Diseases Control), 2009. *Parasitology life cycle*. Available: http: //www.dpd.cdc.gov/dpdx.

Chakraborty, S., 2011. Heterozygosity and Fixation Index for ABO Gene in Barak Valley populations. *Not. Sci. Biol.,* 3(1): 7-11.

Chavhan, A; Pawar, S; and Baig, M. 2010. Allelic frequency of ABO and Rh D blood group among the Banjara backward caste of Yavatmal District, Maharashtra, India. *Nature Proceedings,* 5(1):65-76.

Cooper, P.J., Guevara, A. & Guderian, R.H. 1993. Intestinal helminthiases in Ecuador: the relationship between prevalence, genetic, and socioeconomic factors. *Revista da Sociedade Brasileira de Medicina Tropical,* 26: 175–180.

Crompton D, Nesheim M. 2002. Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu. Rev. Nutr,* 22:35-59.

Daniels, G. 2005. The Molecular Genetics of blood group polymorphism, *Transplantation Immunology,* 14:143-153.

Dawit Ayalew, 2006. Association of *Cryptosporidium Parvum, Giardia Lamblia Andentamoeba Histolytica*/Dispar Infection with drinking water sources among children in rural part of Dire-Dawa, Eastern Ethiopia. Thesis Presented on the school of graduate studies of Addis Ababa University, Addis Ababa, Ethiopia.

De Silva, N. R., Brooker, S., Hot ez, P. J., Montresor, A., Engles, D. and Savioli, L. 2003. Soil Transmitted Helminth Infections: Updating the Global Picture. Trends *Parasitol*., 19: 547–551.

Degarege, A. Yimam, Y., Madhivanan, P and Erko, B, 2016. The relationship between helminthinfections and low haemoglobin levels inEthiopian children with blood type A. *Journal of Helminthology,* 10:1- 6.

Degarege, A., Animut, A., Medhin, G., Legesse, M. and Erko, B. 2014. The association between multiple intestinal helminth infections and blood group, anaemia and nutritional status in human populations from Dore Bafeno, southern Ethiopia. *Journal of Helminthology,* 88:152–159.

Degarege, A., Hailemeskel, E. and Erko, B. 2015. Age-related factors influencing the occurrence of under nutrition in northeastern Ethiopia. *BMC Public Health,* 15:1-7.

Deleon, P.P. and Valverdeh, J, 2003. P System antigenic determiners expression in *Ascaris lumbricoides*. Revistado Instituto de Medicina *Tropical de Sao Paulo,* 45: 53 – 54.

Deleon, P.P., Foresto, P., Zdero, M. & Valverdeh, J. 2000. Preliminary studies on antigenic mimicry of *Ascaris lumbricoides*. Revista do Instituto de Medicina *Tropical deSao Paulo,* 42: 295– 296.

Drake, L., Jukes, M., Sternberg, R.J. and Bundy, D.A.P. 2000. Geo-helminthiasis (Ascariasis, Trichuriasis and hookworm): cognitive and developmental impact. Semin Pediatr vaccine for human hookworm disease: the Human Hookworm Vaccine Initiative. *Int J. Parasitol*., 33:1245-1258.

Edward, J.P, Colleen, M.K, Jie, S, 2004. The response polarization during infection with the helminth parasite *Schistosoma mansoni. Immunological Reviews,* 201: 117-126.

Erko,B. and Medhi n, G. 2003..Human, helminthiasis in Wondo Genet, southern Ethiopia, with emphasis on geo-helminthiasis. *Ethiop. Med. J.,* 41:333-344.

Falusi, A.G., O.G. Ademowo, C.A. Latunji, A. C. Okeke and P.O.Olatunji and T.O. Onyekwere, 2000. Distribution of ABO and Rh genes in Nigeria. *Afr. J. Med. Sci.,* 29:23-26.

Gabr, N.S. & Mandour, A.M.(1991) Relation of parasiticinfection to blood group in El Minia Governorate, Egypt. *Journal of Egyptian Society of Parasitology,* 21: 679–683.

Garcia, L. S. 2001. *Diagnostic Medical Parasitology* 4th ed. ASM Press, Washington, D.C.

Griffith, A.F, S.R, Wessler, R.C, Leewontin., and S.B. Carroll., 2008. *Introduction to Genetic* Analysis. 9th edition. W.H. Freeman and company. New York. p838.

Guyatt H.L.2000. Do intestinal nematodes affect productivity in adult hood? *Parasitol* Today, 16(4):153-158.

Hailu Tadesse. and. Kebede Tadesse, 2013. Assessing the association of severe malaria infection and ABO blood groups in northwestern Ethiopia. *Journal of Vector Borne Diseases,* 50(4): 292–296.

Haque, R., 2007. Human intestinal parasites*. J. Hlth. Pop. Nutr,* 25(4): 387-391.

Haseeb, M.A., Thors, C., Linder, E.and Eveland, L.K.2008. *Schistosoma mansoni*: Chemoreception through N-acetyl-D-galactosamine-containing receptors in females offers insight into increased severity of schistosomiasis in individuals with blood group A. *Experimental Parasitology,* 119: 67 – 73.

Hussain, A., Sheikh, S.A., Haider, M., Rashied, R., and Malik, M.R. 2001. Frequency distribution of ABO and Rhesus blood groups in population of Baluchistan, Pakistan. *Armed Forces Medical Journal*,51: 22-26.

Ijagbone, I.F and Olagunju, T.F, 2006. Intestinal Helminths Parasites in School Children in Iragbiji, Boripe Local Government, Osun State, Nigeria. *African J Biomedical Resesrch,* 9(1):63-65.

ISBT (International Society of Blood Transfusion). 2008. Table of blood group systems; URL:http://ibgrl.blood.co.uk/isbt, accessed June 7-12.

Iyiola, O.A., Igunnugbemi, O.O., Raheem, U.A., and Anifowoshe, A.T. 2011. Gene frequencies of ABO and Rh (D) blood group alleles in Ilorin, North-Central Nigeria. *World Journal of Biological Research,* 4(2): 633-743.

Jaff, M.S. 2010. ABO and rhesus blood group distribution, in Kurds*. Journal of Blood Medicine,* 1:143-146.

Jemaneh, L. 2000. The epidemiology of *Schistosoma mansoni* and soil-transmitted helminthes in elementary school children from the South Gondar Zone of the Amhara National Regional State, Ethiopia. *Ethiop. Med. J*., 38:105-118.

Jeremiah, Z.A. 2006. Abnormal haemoglobin variants, ABO and Rh blood groups among students of African descent in Port Harcourt, Nigeria. *African Health Sciences,* 6 (3): 177-181.

Jorde, L. and S. Wooding, 2004. Genetic variation, classification and 'race'. *Nat. Genet.,* 36: 28-33.

Keiser, J, Utzinger, J, 2010. The drugs we have and the drugs we need against major helminth infections. *Adv. Parasitology,* 73: 197–230.

Khan, M. S., Bakhshi, A., Akhtar, M.S., and Amin-ud-Din, M. 2009. Distribution of ABO and Rh D blood groups in the population of Poonch District, Azad Jammu and Kashmir; E. *Mediterranean Health Journal,* 15(3):717-721.

Khan, M. S., Subhan, F., Tahir, F., Kazi, B. M., Dil, A. S., and Sultan, S. 2004. Prevalence of blood groups and Rh factor in Bannu region NWFP (Pakistan); *Pakistan Journal of Medical Research,* 3(1): 8–10.

Khattak, I.D., T.M .Khan, P .Syed, A. M. Shah, S.T. Khattak and A. Ali, 2008. Frequency of ABO and rhesus blood groups in district Swat, Pakistan. *J. Ayub Med Coll Abbottabad*, 20 (4):127-129.

Kia, E., M. Hosseini, M. Nilforoushan, A. Meamar and M. Rezaeian, 2008. Study of Intestinal

Knight, J. C. 2009. Human Genetic Diversity: Functional Consequences for Health and Disease. *Lancet,* 367:1521-1532.

Kongs, A, MarKs, G, Verle, p, Van Der, Stuyft, P, 2001. The unreliability of the kato-katz technique limits its usefulness for evaluating *S. mansoni* infections. *Tropical medicine and international health,* 6:163 -169.

Kumie A, Ali A. 2005. An overview of environmental health status in Ethiopia with particular emphasis to its organization, drinking water and sanitation: a literature survey. *Ethiop. J. Health Dev*., 19(2):89-103.

Laura Dean, M.D. 2005. Blood Groups and Red Cell Antigens; *National Center for Biotechnology Information*, United States Government, 861-967.

Legesse, M. and Erko, E. 2004. Prevalence of intestinal parasites among school children ina rural area close to the southeast of Lake Langano, Ethi opia. *Ethiop J Health Dev*.,18:116- 120.

Leykun. J, 2001. Soil transmitted helminthic infections and *S. mansoni* in school children from Chilga District, North West Ethiopia. *Ethiop J Health Science*,11:79-87.

Lindblade, K.A, Odhiambo, F, Rosen, D.H and De Cock, K.M., 2003. Health and nutritional status of orphans <6 years old cared for by relatives in Western Kenya. *Tropical Medicine and International Health,* 8 (1): 67-72.

Loukopoulos, P., A. Komnenou, E. Papadopoulos and V. Psychas, 2007. Lethal Ozolaimus megatyphlon infection in a green iguana (Iguana iguana rhinolopa). *J. Zoo and Wildlife Medicine,* 38: 131-134.

Lyko,J. H.Gaertner., J. N.Kaviti., M.W. Karithi, and B.Akoto., 1992. The blood groups Antigens ABO and Rh in Kenyans. *Hamdard Medicus,* 35:59-67.

Mandal, S. 2002. *Fundamentals of Human Genetics*, India.2nd edition. New Central Book Agency (P) LTD, New Delhi.

Marcela, C. and Anatole, L. 1999. Antigens in human blood. In: Hoff brand, A. V, Lewis, S. M. and Tuddenham, E. G.(eds.), *Post Graduate Hematology*. Fourth Edition. Butterworth Heinemann, Boston, USA. pp 412-413.

Martinez-Palomo, A. and Espinosa-Cantellano, M, 1998. Intestinal amoebae. In: Cox, F.E.G.,Kreier, J.P., Wakeline, D., eds. *Topley and Wilson s Microbiology and Microbial Infections,* 5 (9). 157-177.

McCarty,J. and Moor, T. A, 2007. Prevalence of intestinal protozoan parasite. *International journal for Parasitology*. Parasitol Today, 13: 438–443.

Mengistu, A., 2007. Prevalence of intestinal parasitic infections among urban dwellers in southwest Ethiopia. *Ethio. J. Health Dev*., 21(1):12–17.

Mohammad Nojavan, Karim Shamsasenjan, Ali Akbar Movassaghpour, Parvin Akbarzadehlaleh, Seyd Esmail Torabi and Morteza Ghojazadeh, 2012. Allelic Prevalence of ABO Blood Group Genes in Iranian Azari Population. *Bio. Impacts,* 2(4): 207-212.

Mohammad, A, Tanvir, A, Mohammad, A.R, Fahima, C, Rasheduzzaman, R,.2001. Individuals with Le (a+b) Blood Group Have Increased Susceptibility to Symptomatic Vibrio cholerae Infection. *Journal of Neglected tropical disease,* 5: 1-9.

Naing, L., Winn, T. and Rusli, B.N. 2006. Practical Nyantekyi, Liza A., Mengistu Legesse, Mulugeta Belay, Konjit Tadesse, Kebreten Manaye, O'donoghue, P.J., 1995. Cryptosporidium and cryptosporidiosis in man and animals. *Int. J.* Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation. Issues in calculating the sample size for Msambweni area, Coast Province, Kenya. Am. *J. Trop. Med*. Hyg., 70:449-456.

Ndamba, J., Gomo, J.E., Nyazema, N., Makaza, N. & Kaondera, K.C.1997. Schistosomiasis infection in relation to the ABO blood groups among school children in Zimbabwe. *Acta Tropica, 65*:181–190.

Neva, F.A. and H.W. Brown, 1994. *Basic Clinical Parasitology*, 6th ed. Norwalk, Connecticut: Appleton and Lange.

Nicholas, J. White/Joel, G. Breman, Harrison, 2008. *Principles of internal medicine*, seventeenth edition (p 1448).

Nwauche, C.A. and O.A. Ejele, 2004. ABO and rhesus antigens in a cosmopolitan Nigeria population. *Niger Journal of Medicine,* 13(3): 263-266.

Oladeinde, B. HOmoregie, R. Osakue, E. O. and Onaiwu, T. O., 2014. Asymptomatic malaria among blood donors in Benin City Nigeria. Iranian *Journal of Parasitology,* 9(3):415-422.

Olsson, M.L and Chester, M.A., 2001. Polymorphism and recombination events at the ABO locus: a major challenge blood grouping strategies. *Tranfus. Med*., 11: 295-313.

Onanuga, A and Lamikanra, A., 2016. Association of ABO blood group and Plasmodium falciparum malaria among Children in the Federal Capital Territory, Nigeria. *Afr. J. Biomed. Res.,* 19(1):1-6.

Ilozumba P. C., and. Uzozie, C. R, 2009. Prevalence of malaria parasitaemia and its association with ABO Blood Group in Odoakpu Area of Onitsha South Local Government Area, Anambra State Nigeria. *Nigerian Annals of Natural Sciences,* 8(2): 1–8.

Park, K. 2004. *Text Book of Preventive and Social Medicine*. 18th Ed. Bandarsi das Bhanot Publishers, Jabalpur, India.

Partnership for Child Development, 1999. A situation analysis: a participatory approach to building programs that promote health, nutrition and leaning in school. pcd, oxford, uk.

Pennap, G.R., Envoh, E. and Igbawua, I. 2011. Frequency Distribution of Haemoglobin Variants, ABO and Rhesus Blood Groups among Students of African Descent. *British Microbiology Research Journal,* 1 (2): 33-40.

Peters,W. and Pasvol, G. 2005. *Atlas of Tropical Medicine and Parasitology*: 6th ed. Mosby Elsevier pp. 429.

Quihui, L., M. E Valencia, D. W. Crompton, S, Philips, P. Hagan G. Morales and S. P.Diaz-Camacho, 2006. Role of the employment status and education of mothers in the prevalence of intestinal parasitic infections in Mexican rural school children .*BMC Public Health* 6:225. Regional state.

Rai,V., and, Kumar, P. 2011. Genetic analysis of ABO and Rh blood groups in Brahmin Population of Uttar Pradesh, India. *Nature Proceedings,* 21(4): 126-232.

Reddy, V.M., M. Daniel, E. Bright, S.R. Broad and A.A. Moir, 2008. Is there an association Rhesus blood groups and hemoglobin variants in Ogbomosho, South west Nigeria. *Afr. J. Biotechnol*ogy, 5: 224-229.

Reich, D., M. Cargill, S. Bolk, J. Ireland and P. Sabeti, 2001. Linkage disequilibrium in the humangenome. *Nature,* 411: 199-204.

Seifu Seyoum and Kifle Dagne, 1985. ABO and Rhesus Blood Type Frequencies In Data From Hospitals and the Red Cross in Ethiopia. *Ethiopia Med. J.,* 23:1-40.

Simon-Oke, I. A, Afolabi, O. J. and Itansanmi, A. I., 2016. Prevalence of ABO Blood Groups and Its Relationship with Malaria Parasitemia among Students of Federal University of Technology, Akure, Ondo State. *International Journal of Tropical Disease and Health,* 17(1): 1-8.

Sirina, M and Clement, O, 2013. The prevalence of malaria parasitaemia and predisposition of ABO blood groups to Plasmodium falciparum malaria among blood donors at a Ghanaian Hospital, *AU Journal of Technolog,y,* 16(4): 255-260.

Stephenson, L.S, Latham, M.C, Ottesen, E.A, 2000. Malnutrition and parasitic helminth infections. *Parasitology,* 121: 23-38.

Stoltzfus, R.J, Chway, H.M, Montresor, A, Tielsch, J.M, Jape, J.K, Albonico, M, 2004. Low dose daily supplementation improves iron status and appetite but not anemia, whereas quarterly ant helminthic treatment improves growth, appetite and anemia in Zanzibari preschool children. *J Nutr.*, 134: 348-56.

Tekleab Mesfin, 2014. Genotypic and Allelic Frequencies at the ABO and Rh (D) Loci Among the Somali Tribes in the Ogaden Zone, Ethiopia A Thesis Submitted to The Department of Biology, School of Graduate Studies, Haramaya University, Ethiopia.

Tesfa-Yohannes, T.M, Kloos, H. 1988. Intestinal parasitism. pp 214-230. In: Zein A.Z and Helmut Kloos (Eds). *The ecology of health and disease in Ethiopia*, Ministry of Health, Addis Ababa.

Tewodros Zerihun, Abraham Degarege, and Berhanu Erko. 2011. Association of ABO blood group and Plasmodium falciparum malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pacific Journal of Tropical Biomedicine,* 1(4):289-294.

Tishkoff, S. and K. Kidd, 2004. Implications of biogeography of human populations for 'race' and medicine. *Nat. Genet*., 36: 21-27.

Wang, L.D, Guo, J.G, Wu .X.H, Chen, H.G, Wang, T.P, Zhu, .S.P, Zhang, Z.H, Steinmann, P, Yang, G.J, Wang, S.P, 2009. China's new strategy to block *Schistosoma japonicum* transmission: experiences and impact beyond schistosomiasis. *Trop. Med. Int. Health,* 14: 1475–1483.

WHO (World Health organization) 1997. Report of a Consultation of Experts on Amoebiasis (WHO/PAHO/UNESCO). WHO Weekly Epidemiological record No.14. Geneva, World Health organization.

WHO (World Health Organization), 2000. Control of tropical diseases. WHO, Geneva.

WHO (World Health organization), 2002. Expert Committee. Prevention and control of schistosomiasis and soil transmitted helminthiases. *WHO Technical Report Series,* 912:1-57.

WHO (World Health Organization), 2004. Training Manual on Diagnosis of Intestinal Parasites: Schistosomiasis and Intestinal Parasites Unit Division of Control of Tropical Diseases. Geneva based on the WHO Bench Aids for the diagnosis of intestinal parasites.

WHO (World Health organization). 2010. Neglected Tropical Diseases - PCT Databank.

Yakubu, N, Musa,G, Yakubu, S.E, 2003. Seasonal changes in the distribution and infection rate of Schistosoma intermediate hosts in River Kubanni and its tributaries. *Bio. Res. Com*., 15:207-214.

Yamamoto, F., 2000. Molecular genetics of ABO blood groups. *Vox. Sang,* 78:91-103.

Yamamoto, F., H. Clausen, T. White, J. Marken, and S. Hakomori. 1990. Molecular genetic basis of the histo-blood group ABO system. *Nature,* 345: 229-233.

Yan, L., Zhu, F., Fu, Q., and He, J. 2005. ABO, Rh, MNS, Duffy, Kidd, Yt, scianna, and Colton blood group systems in indigenous Chinese. *Immuno-hematology,* 21:10-14.

Yassin Woliye Datu, 2013. Frequency of ABO and Rhesus (RhD) Blood Group Alleles Among Students of Oromo Ethnic Group Belonging to Arsi, Guji, and Borena Clans in Robe College of Teachers Education, Ethiopia. A Thesis Submitted to School of Graduate Studies of Haramaya University, Ethiopia.

Zelalem Alemu, 2014. Allelic And Genotypic Frequencies of ABO And Rh Blood Groups Among Tigrean, Kunama, Saho And Blen Ethnic People Inhabiting Western Tigray; Ethiopia A Thesis Submitted to the School of Graduate Studies, Department of Biology, Haramaya University, Haramaya, Ethiopia.

Zhou, XN, Guo, J.G, Wu, X.H, Jiang, Q.W, Zheng, J, 2007. Epidemiology of Schistosomiasis in the People's Republic of China. *Emerging Infectious Diseases,* 13: 1470-1476.

7. APPENDICES

APPENDEX- I

CONSENT FORM

Association of ABO and Rh(D) Blood Group Systems with Prevalence of Some Intestinal Parasitic Infections in Babile District. East Hararghe Zone, Oromia Regional State, Ethiopia

The purpose of the study is to evaluate the distribution of the ABO and Rh (D) blood group systems and the relationship between, ABO and Rh-D blood group systems with *Gardia lamblia, Entoamoeba histolyitica, Hymenoslepis nana, Schistosoma mansoni,* and *Trichuris trichuria* in understanding their co-evolution in Babile district, East Hararghe Zone, Oromia Regional State, Ethiopia. Therefore, I will ask you some questions related to Intestinal parasite and examine you for the signs and symptoms of Intestinal parasites and I will take stool and blood sample to examine for Intestinal parasite and blood type respectively. Please be assured that the information will be confidential since participation is based on your willingness. However, your kindly participation would play key role in the success of this study. In addition, no personal identification will be written and we assure you that whatever information you are providing will only be used for the research purpose and the data will be handled only by the researcher. Are you willing to participate in the study?

Answer: Yes ____________ No ______________

For Parents / Care takers of Children Less than 18 years

Here I am intending toevaluate the relationship between Intestinal parasite particularly,*Gardia lamblia, Entoamoeba histolyitica, Hymenoslepis nana, Schistosoma mansoni,* and *Trichuris trichuria* and the distribution of the ABO and Rh (D) blood group systems in understanding their co-evolution in Babile district. For this, I need stool and blood sample from your children, which would be used only to detect the presence of intestinal parasite infections and to determine the ABO blood type. It will be my pleasure, if you are volunteer to let the children participate in this particular study. I also want to inform you that there is no any health related risk in participating.

This consent form has been readout to me in my own language, and I understand the content and I am voluntarily consent to participate in the study.

Study Area ____________________

Witness Name __________________________Signature __________Date __________

Investigator Name ____________________________Signature _________ Date ________

CONSENT FORM (AFAN OROMO VERSION)

Guca Walii Galtee

Barbaachisummaan qorannoo kanaa, faffaca'iinsa Sirna gartuulee dhiigaa ABO FI Rh (D) fi akkasumas walitti dhufeenya Sirna gartuulee dhiigaa ABO fi Rh (D 'n Jaardiyaa, Ameebaa fi Bilarziyaa waliin qabu madaaluf kan yaadameedha. Kanaafuu, gaaffilee muraasaa maxxantoota mar'imaan keessaa waliin wal qabatan isin qoradha, amaloota fi mallattoolee maxxantoota mar'imaan keessaa isin qoradha. Akkasumas, gosa maxxantuu fi gartuu dhiigaa keessan adda baaffachuuf boolii xiqqoo fi dhiigaa xiqqoo qorannoof ta'u isin irraa fuudha. Hirmaannaan kessan fedhii keessan irratti kan hundaa'ee waan ta'eef odeeffannoon kun amanamaa ta'uu qaba. Hirmaannaan isin gootaan milkaa'ina qorannoo kanaa gahee guddaa taphata. Itti dabaluunis, odeeffannoon enyummaa dhuunfaa kamiiyyuu hin barreeffamu. As irraatti wanti isin hubachiisuu barbaadu, odeeffannoon isin kennitan kamiiyyuu faayidaa qorannoo kanaatif ta'uu fi harka qoratichaa qofatti kan hafu ta'uu isaati. Kanaafuu, qorannoo kana irratti hirmaachuu ni feetaa ?

Deebii: Eeyyen ______________ Lakki ______________

Maatii /guddistoota Ijoollee umrii 18 gadi jiraniif

Ani asitti walitti dhufeenya maxxantoota mar;imaanii keessumaayyuu, Jaardiyaa, Ameebaa fi Bilaarziyaa madaaluuf akkasumas, faffaca'iinsa Sirna gartuulee dhiigaa ABO FI Rh (D) qorachuufin deema. Kanaaf, gosa maxxantootaa fi gartuu dhiigaa adda baaffachuuf boolii xiqqoo fi dhiigaa xiqqoo qorannoof ta'u mucaa keessan irraa barbaada. Mucaa keessan qorannoo kana irratti hirmaachisuuf heeyyamamaa osoo taatanii natty tola. Kana irratti wantin isinii ibsuu barbaadu qorannoo kana waliin wal qabatee rakkoon fayyaa kamiyyuu hin jiru.

Gucni walii galtee kun afaan ani danda'uun naaf dubbifame, anis qabiyyee isaa hubadheera, kanaaf, fedhii kiyyaan qorannoo kana irratti hirmaachuuf walii galeera.

Iddoo qorannoo ________________________

Maqaa wabii ________________________ Mallattoo ____________ Guyyaa ____________

Maqaa qorataa ________________________ Mallattoo ____________ Guyyaa ____________

APPENDIX II. QUESTIONNAIRE

Haramaya University

Postgraduate Program Directorate

Masters of Science in Genetics, 2017

Questionnaire to be answered by study individuals selected in Babile district to collect data for the research. Thank you for your genuine reply.

I) General study individual's information

1. Sample code______________________

2. Age: ________

3. Gender: Male ☐ Female ☐

4. Village: ________________________

5. Ethnicity______________

6. Marital status: Unmarried ☐ Married ☐ Widowed ☐ Divorced ☐

II) Intestinal parasite and environment

7. Have you caught by intestinal parasite before? A) Yes B.) No

8. Have you used any anti-intestinal parasitic infection drugs before (Please tick):

Yes ☐ No ☐

III) Results of Laboratory Diagnosis

9. ABO/Rh blood group (Please tick): A+ve ☐ B+ve ☐ AB+ve ☐ O+ve ☐

A-ve ☐ B-ve ☐ AB-ve ☐ O-ve ☐

10. Intestinal parasite test results: Positive ☐ Negative ☐

11. Intestinal Parasite type(s) detected (if positive): ____________________________

APPENDIX III

FIGURES

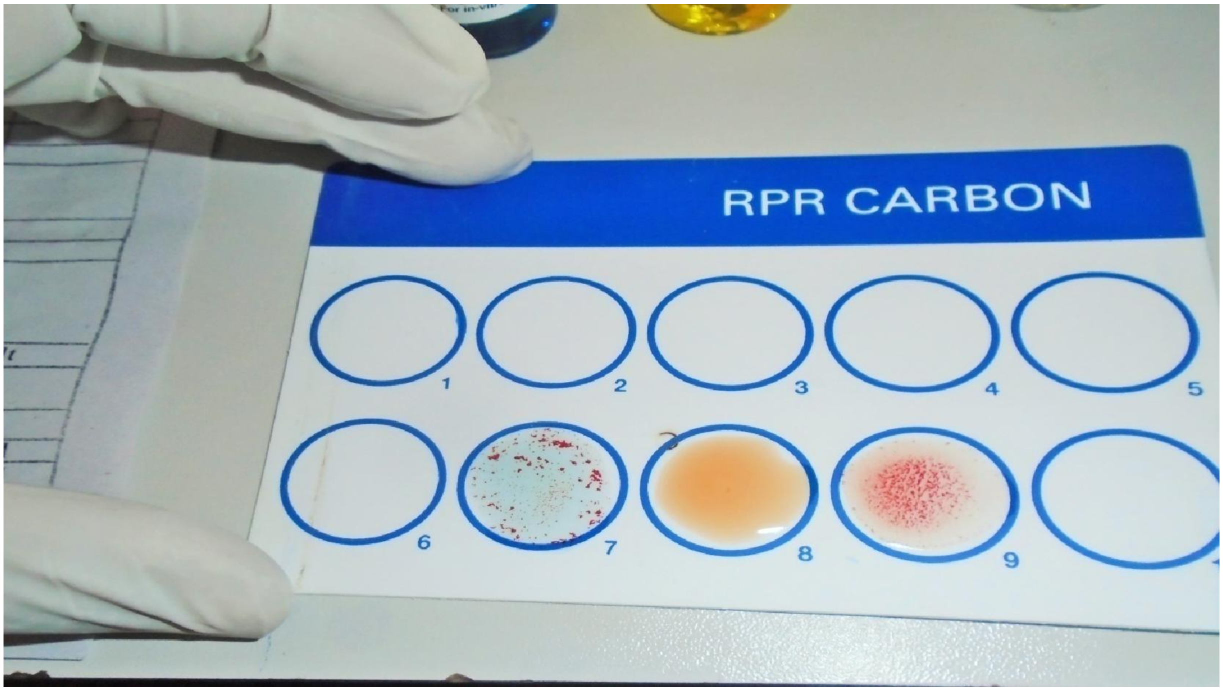
1. Figure showing researcher during obtaining consent form from patients

2. Figure showing laboratory technicians during identification of intestinal parasite in the stool.

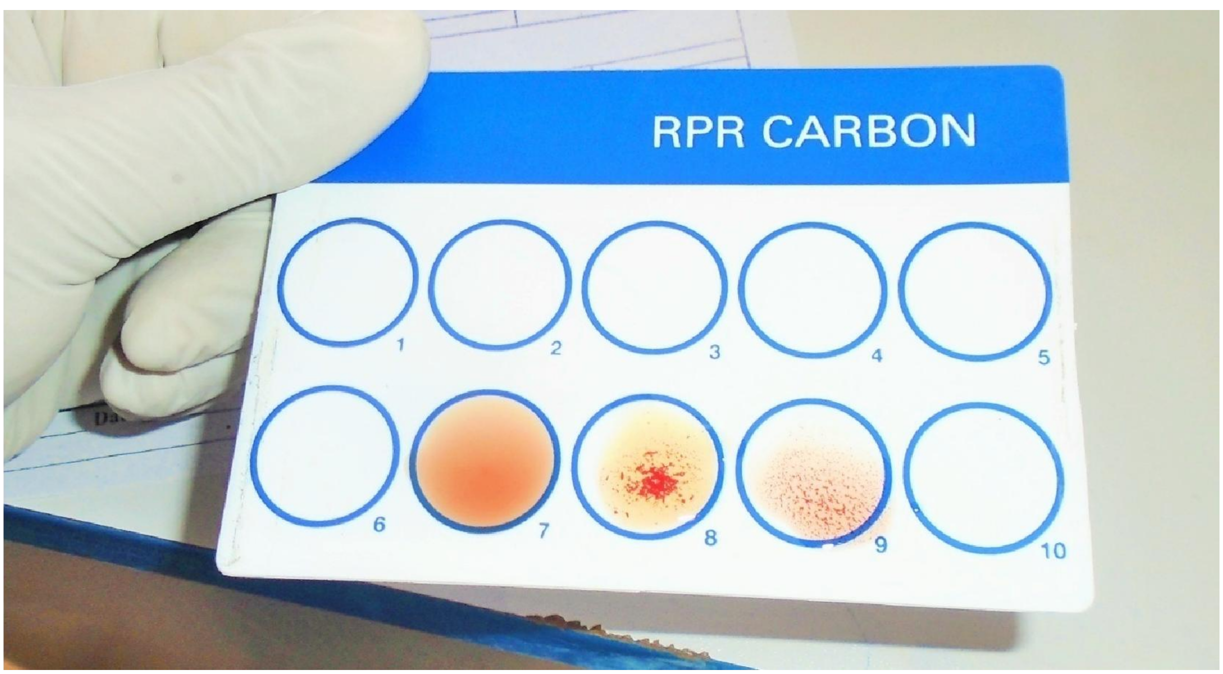
3. Figure showing laboratory technicians during blood sample taking for ABO blood typing

4. Figure showing the three different antiserums (anti-A, anti-B & anti-D)

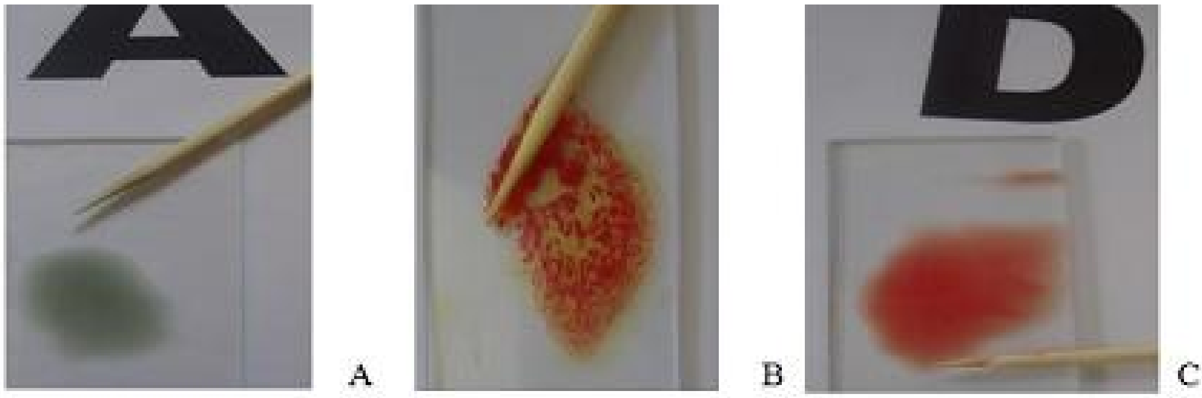
5. Figure showing dropping and mixing the ant-serum with blood sample



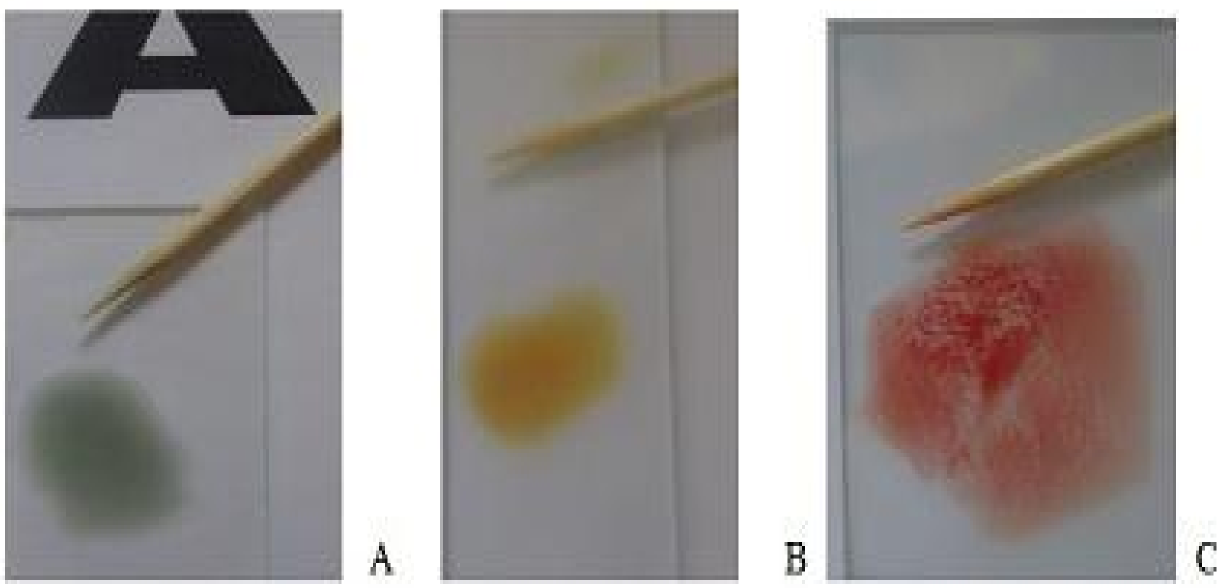
6. Figure showing the difference of coagulation results during the blood typing (showing blood type A +ve)



7. Figure showing blood type “B‟ with positive Rh factor



8: Figure showing blood type “B‟ with negative Rh factor



9: Figure showing blood type “O‟ with positive Rh factor