

**PREVALENCE OF INTESTINAL PARASITES IN MECHE  
COMMUNITY OF JALTHAL VDC, JHAPA, NEPAL IN RELATION  
TO THEIR SOCIO-ECONOMIC STATUS**



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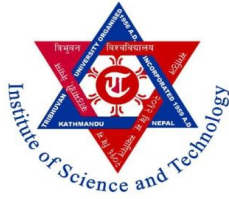
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## DECLARATION

I hereby declare that the work present in this thesis entitled “**Prevalence of intestinal parasites in Meche community of Jalthal VDC, Jhapa, Nepal in relation to their socio-economic status**” has been done by myself and has not been submitted elsewhere for the award of my degree. All the sources of information have been specifically acknowledged by references to all the author(s) or institution(s).

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## RECOMMENDATION

This is recommend that the thesis entitled “**Prevalence of intestinal parasites in Meche community of Jalthal VDC, Jhapa, Nepal in relation to their socio-economic status**” has been carried out by Nirmala Dhakal for the partial fulfillment of Master’s Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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## TABLE OF CONTENTS

DECLARATION .....	i
RECOMMENDATION.....	ii
LETTER OF APPROVAL .....	iii
CERTIFICATE OF ACCEPTANCE.....	iv
ACKNOWLEDGEMENT .....	v
TABLE OF CONTENTS .....	vi
LIST OF TABLES .....	viii
LIST OF FIGURES.....	viii
LIST OF PHOTOGRAPHS.....	viii
LIST OF ABBREVIATIONS.....	ix
ABSTRACT.....	x
1. INTRODUCTION.....	1
1.1 Background.....	1
1.2 Introduction to Intestinal Parasites.....	2
1.2.1 Intestinal protozoan parasites.....	2
1.2.2 Intestinal helminthes parasites: .....	4
1.3 Objectives .....	6
1.3.1 General objective.....	6
1.3.2 Specific objectives.....	6
1.4 Significance of the study .....	6
2. LITERATURE REVIEW .....	8
2.1 Histroy of Parasitology .....	8
2.2 Global context.....	8
2.3 National context.....	11
3. MATERIALS AND METHODS.....	15
3.1 Study area .....	15
3.2 Study duration.....	16
3.3 Study design .....	16
3.4 Sample size .....	17
3.5 Stool sampling .....	17
3.6 Materials.....	17
3.6.1 Equipments.....	17

3.6.2 Chemicals.....	18
3.6.3 Preparation of Potassium Dichromate: .....	18
3.6.4 Preparation of Normal Saline:.....	18
3.6.5 Preparation of Iodine Solution: .....	18
3.7 Laboratory work .....	18
3.8 Methods of stool examination .....	18
3.8.1 Unstained preparation of stool smear: .....	18
3.8.2 Stained preparation of stool smear: .....	18
3.8.3 Concentration methods .....	19
3.9 Eggs, Cyst and larva identification .....	19
3.10 Questionnaires .....	19
3.11 Data analysis and Interpretation .....	19
4. RESULTS .....	20
4.1 Results of stool examination .....	20
4.1.1 General Prevalence of Intestinal Parasites: .....	20
4.1.2 Prevalence individual intestinal parasites: .....	20
4.1.3 Distribution of Protozoan parasite:.....	20
4.1.4 Distribution of helminth parasite:.....	21
4.1.5 Sex-wise Prevalence of intestinal Prevalence .....	21
4.1.6 Age group-wise prevalence of Intestinal parasites .....	22
4.1.7 Concurrency of intestinal parasites: .....	22
4.2 Results of Questionnaire Survey Analysis .....	23
4.2.1 Knowledge of the interviewed people .....	23
4.2.2 Occupation wise Prevalence of Intestinal Parasites .....	23
4.2.3 Domestic Animals Ownership-wise Prevalence of Intestinal Parasites .....	24
4.2.4 Treatment Method-wise Prevalence of Intestinal Parasites .....	24
4.2.5 Prevalence of Intestinal Parasites on the basis of Food habit .....	24
4.2.6 Hand washing wise Prevalence of Intestinal parasites .....	25
4.2.7 Prevalence of Intestinal Parasites on the basis of water-treatment method.....	25
5. DISCUSSION .....	26
6. CONCLUSION AND RECOMMENDATIONS.....	29
ANNEX- 1 .....	44
ANNEX- 2.....	46



## LIST OF TABLES

Table 1: Sex-wise Prevalence of Intestinal Parasites .....	22
Table 2: Age group-wise Prevalence of Intestinal Parasites .....	22
Table 3: Knowledge towards intestinal parasites among Meche people.....	23
Table 4: Occupation wise prevalence of intestinal parasites .....	23
Table 5: Livestock and Domestic animals ownership-wise prevalence of intestinal parasites .....	24
Table 6: Treatment method-wise prevalence of intestinal parasites .....	24
Table 7: Prevalence of Intestinal Parasites on the basis of Food habit .....	25
Table 8: Hand washing wise Prevalence of Intestinal parasites .....	25
Table 9: Prevalence of Intestinal Parasites on the basis of water-treatment method .....	25

## LIST OF FIGURES

Figure 1: Map of study area .....	16
Figure 2: Flowchart of research outline.....	17
Figure 3: Individual prevalence of intestinal parasites .....	20
Figure 4: Distribution of Protozoan parasite.....	21
Figure 5: Distribution of Helminth parasites .....	21

## LIST OF PHOTOGRAPHS

Photo 1: Examining under microscope .....	42
Photo 2: Slide preparation .....	42
Photo 3: With Meche people .....	42
Photo 4: Meche Basti .....	42
Photo 5: <i>Ascaris lumbricoides</i> .....	42
Photo 6: Hookworm .....	42
Photo 7: <i>Trichuris trichiura</i> .....	43
Photo 8: <i>Taenia solium</i> .....	43
Photo 9: <i>Entamoeba coli</i> .....	43

## LIST OF ABBREVIATIONS

gm	-	Gram
IPI	-	Intestinal Parasitic Infection
IP	-	Intestinal Parasite
KAP	-	Knowledge, attitude and practice
ml	-	Milliliter
mm	-	Millimeter
P-value	-	Probability value
rpm	-	revolution per minute
sp	-	species
STH	-	Soil transmitted helminth
TU	-	Tribhuvan University
VDC	-	Village Development Committee
WHO	-	World Health Organization
µm	-	Micrometer

## ABSTRACT

Intestinal parasitic infection possesses great morbidity among people with low socio-economic status as well as people living in poor hygiene and sanitary condition. This study was carried out to determine the prevalence of intestinal parasites in Meche community of Jalthal Village Development Committee of Jhapa district in relation to their socio-economic status during June to July 2017. A total of 150 stool sample were randomly collected from different age groups and sexes and were preserved in 2.5% potassium dichromate solution. The prevalence of the intestinal parasites was determined by stool examination using direct smear and concentration methods (sedimentation and flotation technique) in the Parasitology Laboratory of Central Department of Zoology, Kirtipur, Kathmandu whereas knowledge, attitude and practices (KAP) of the people were analyzed using prepared questionnaire. Out of 150 peoples, 41(27.33%) were found to be infected with one or two intestinal parasites among them prevalence rate in females were higher (32.47%) than males (21.91%). Statistically there was no significant difference in the sex-wise prevalence of the intestinal parasites ( $P>0.05$ ). The prevalence of the parasites was higher in the age group 21-40 years (47.22%) and lowest (20.51%) in the elder people above 41 years of age. However the difference was not statistically significant ( $P>0.05$ ). Altogether 5 species of the intestinal parasites were detected. Among them *Ascaris lumbricoides* (19.33%) topped the list followed by *Taenia solium* (2.67%), Hookworm (2.67%), *Entamoeba coli* (2.67%) and *Trichuris trichiura* (2%). The study revealed that the prevalence of single infection (92.68%) was higher than double infection (7.32%). Most of the people (70%) were unaware and (30%) people were aware about intestinal parasitic infection. Thus, the high prevalence of intestinal parasitic infection among Meche people seems directly related to unhygienic living condition, unsafe drinking water, unhygienic food, lack of health education, poor sanitary condition and low socio-economic status help to increase the burden of the infection in the community.

# 1. INTRODUCTION

## 1.1 Background

Those living organism which receive food and shelter from another organism are called parasites. Parasites may cause mechanical injury such as boring a hole into the host or digging into the other tissue, stimulate damaging inflammatory or immune response. Most parasites inflict combination of these conditions to their host (Taliaferro, 2009).

Intestinal parasites are cosmopolitan in distribution. Intestinal parasitosis still establishes one of the important causes of public health problem in world, especially in developing countries like Nepal. Around 3.5 billion people globally are estimated to be affected by intestinal parasitosis and 450 million are sick as a result of these infections, the majority being children (WHO, 2000). Intestinal parasitic disease is one of the global health burdens in many developing countries mainly due to fecal contamination of water, lack of adequate basic sanitation and environmental and socio-cultural factors enhancing parasitic transmission (Mordi and Ngwodo, 2007; Alli *et al.*, 2011).

More than one billion people are estimated to chronically infected with intestinal helminthes (WHO, 1998). Among the intestinal parasitic infections, Ascariasis, hookworm infection and trichuriasis are reported as the most common infection in the world, being responsible for considerable morbidity and mortality. Besides of causing morbidity and mortality, intestinal parasitic infections have been associated with physical weakness and low educational performance of the poor segments of the populations and intimately linked with low economic status, poor personal and environmental tropical climate and low altitude (Desta *et al.*, 2014).

Developing countries are located mainly in warm or hot and relatively humid areas that is associated with poverty, malnutrition, high population density, unavailability of potable water and low health status, provide optimum condition for the growth and transmission of intestinal parasites (Sayyari *et al.*, 2005). Hot and humid climate during rainy season in tropical and subtropical region accelerates the infection rate owing to their ubiquity and despite their high rate of infection in this region, physician and public health authorities show little interest in their control (WHO, 1981). More frequently poor populations are victimized by parasites and diarrhea (Rai *et al.*, 2004). At least 750 million of diarrheal cases occur per year in developing countries that results in 5 million deaths (Shrestha *et al.*, 2012). People are more susceptible to infection of parasitic disease due to the unhygienic management, mal-nutrition, poverty, poor water supply, inadequate health facilities, unsanitary condition, and ignorance about the complicated life cycle of the parasites.

Nepal is small and impoverished country situated in the south Asia, with infectious disease, including intestinal parasitosis, with high prevalence rate (Rai *et al.*, 2004). Particularly in rural area of Nepal, open air defecations are common that enhance the parasites to invade into individual. In Nepal, over 70% morbidity and mortality rate concern with intestinal parasitic infection and is also reflected in "top ten disease of Nepal"(Rai *et al.*, 2011). *Ascaris* has remained as a leading human parasite in Nepal (Rai

and Rai, 1999). Diarrhea is caused by variety of etiological agents but of them intestinal parasitic infection alone contributes to a great extent in the cause of diarrhea and is one of the most common public health problems in Nepal (Tandukar *et al.*, 2013).

Due to soil transmitted helminthes, more than 610 million children of school age are at risk of morbidity (WHO, 2011). It is reported that the prevalence of the intestinal parasites in Nepal varies considerably from one study to another study and reaches nearly 100% in some tropical areas (Estevez *et al.*, 1983; Rai and Gurung, 1986). In Nepal, children are more commonly infected than adults (Rai and Gurung, 1986; Regmi, 2012). The intestinal helminth commonly reported from Nepalese children is *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* (Thapa *et al.*, 2011). The agents spread faeco-orally through contaminated sources.

Intestinal parasitosis appears as one of the major economic burden to the developing countries like Nepal. Jhapa is the eastern most district of Nepal and lies in the fertile Terai plains. It contains highly developed areas to remote places. The resident of the developed city areas are more educated than rural areas.

## **1.2 Introduction to Intestinal Parasites**

Gastrointestinal parasites are the organisms that inhabit the gastrointestinal region of human or other organisms. Helminthes and protozoa are two common intestinal parasites of small and large intestine of human.

### **1.2.1 Intestinal protozoan parasites**

Protozoan parasites consist of single cell like unit which is morphologically and functionally complete (Chaterjee, 2009) and are widely distributed. About 6,500 species of protozoa are reported, of which 10,000 are parasites. The protozoa perform the reproduction either by asexual or by asexual method. They harm the human causing serious health problem. Some common intestinal protozoan parasites are *Giardia lamblia*, *Entamoeba histolytica*, *Trychomonas hominis* and other.

#### ***Giardia* and Giardiasis**

*Giardia intestinalis*, now also known as *Giardia lamblia*, *Giardia duodenalis* or *Lamblia intestinalis*. It was observed by Leeuwenhoeck in 1681 in his own stool. It is distributed throughout the world with highest prevalence in the tropics and subtropics area. It is reported in all age groups but highly prevalent in children. It is generally lodge in duodenum and the upper part of jejunum in man. It is flagellated protozoan and exists in two morphological forms: trophozoite and cyst. The size of trophozoite is about 10-20µm in length, 6-15µm in width and 1-3µm in thickness. It is actively feeding stage. Trophozoite is bilaterally symmetrical, pear shaped with two nuclei, which appeared as a typical monkey face structure in microscopic examination. It reproduces by binary fission in the intestine. When the environmental conditions are unfavorable, trophozoite gets converted into cyst. It is oval in shape with four nuclei measuring 8-14µm in length and 6-10µm in width. Cysts are transmitted through water sources and are the infective stages to man (Chatterjee, 2009).

### ***Entamoeba* and Amoebiasis**

*Entamoeba histolytica* was 1<sup>st</sup> discovered by Losch in 1875 in stool of Russian suffering from dysentery. It is invasive intestinal pathogenic parasitic protozoan that causes amoebiasis. About 40-50 million people are suffering from clinical amoebiasis each year, resulting upto 100,000 death (Singh *et al.*, 2009). Clinical manifestation of amoebiasis are diarrhea, dysentery, cramping, flatulence etc. More severe disease is characterized by presence of blood and mucous in the stool. Morphologically, it can be categorized into trophozoite, precyst and cyst. Trophozoite is feeding and growing stage which measures 15-30µm in size. The shape of the parasite is not fixed because it changes its position constantly. Precyst is larger than cyst but smaller than trophozoite. It is colorless, round or oval shape. Its size ranges between 10-20 µm. Cyst is rounded and surrounded by smooth cyst wall. Initially cyst is unicellular and then develops into a binuclear after binary fission. Transmission of amoeba occurs by fecal-oral route. The trophozoites cause invasion of the tissue through (i) colonization in the large intestine (ii) adherence of *Amoeba* to the colon and lysis of mucosal cells (iii) invasion of the intestinal mucosa and (iv) extra-intestinal invasion of tissue or serum (Parija, 2004).

### ***Cyclospora* and Cyclosporiasis**

*Cyclospora cayentanensis* is an obligate and sporulating coccidian parasite measuring 8-10µm in diameter. It was 1<sup>st</sup> reported from the Papua New Guinea (Ashford, 1973). It is an emerging parasitic pathogen of human being. It has been recognized as both food and water borne pathogen endemic in many developing countries (Singh *et al.*, 2009). Unsporulated cyst or oocysts are released in the environment along with the human feces. In the environment, sporulation occurs; sporant divides into two sporocyst and each contain two sporozoites. Infection to human occurs by ingesting sporulated oocyst. It infects the upper small intestinal tract and invade the epithelial cells of the intestine. The symptoms of the disease are low grade fever, fatigue, headache, loss of appetite and loss of weight (Chatterjee, 2009).

### ***Cryptosporidium* and cryptosporidiosis**

*Cryptosporidium* is a coccidian protozoan parasite, discovered by Current and Upton in 1985. It is an important causative agent of human and animal gastro-intestinal illness globally (Tandukar *et al.*, 2015). It causes the disease cryptosporidiosis which has high incidence in AIDS cases. The parasite measures 4-5 µm in diameter and exists in only one form: oocyst. They may contain 1-6 large dark granules and numerous small granules. Mature post-sporulation oocyst contain 2-4 sausage shaped sporozoites. Oocyst contain one thick and one thin wall. The principal mode of transmission of cryptosporidium is through the ingestion of oocyst contaminated water which spread mainly through the faeco-oral route (Karanis *et al.*, 2007). In immune-competent host, cryptosporidiosis is usually mild, self limiting and recovers within a week but the infection may have a severe, chronic and even fatal clinical course in immune-compromised individual (Ghimire *et al.*, 2004).

### **1.2.2 Intestinal helminthes parasites:**

The helminth parasites are multicellular, bilaterally symmetrical, elongated flat or round animal (Arora and Arora, 2012). Helminths have one or more intermediate host. They are classified into two phylums : Platyhelminthes and Nematelminthes. Platyhelminthes is further divided into classes Cestoda and Trematoda while Nematelminthes has only one class. Some common helminth parasites are *Enterobious vermicularis*, *Trichuris trichiura*, *Ascaris lumbricoids*, *Schistosoma* sp. and other.

#### ***Ascaris* and Ascariasis**

*Ascaris lumbricoides*, also known as round worm, is known to be existed since 1500 BC (Rai and Rai, 1999). It is the largest and common human intestinal nematode, coined by Linneaus in 1758. It has worldwide distribution being especially prevalent in developing countries with tropical and sub-tropical climate implicating as one of the cause of morbidity and mortality (Rai and Rai, 1999). These are large, stout and tapering at both ends. It has separate sex, females are longer and larger (200-400 × 3.6mm) than males (150-300× 2-4 mm). The worm does not require any intermediate host to complete its life cycle. Infection with the *Ascaris lumbricoides* is acquired by the ingestion of the embryonated eggs containing 2<sup>nd</sup> stage infective larva through the contaminated food, drinks and vegetables. In highly endemic areas, air borne transmission is also possible. Great majority (85%) of the infection are symptomless (Oli, 2016). The clinical manifestations are produced by migrating larva and adult worms. The main features of the disease are diarrhea, fever, cough dyspnoea, bloody sputum etc. The adult worm causes edema of face, fever, rashes, conjunctivitis, hemorrhagic pancreatitis, meningitis and other (Chatterjee, 2009).

#### ***Ancylostoma* and Ancylostomiasis**

In 1843, Dubini discovered the *Ancylostoma duodenale* in Italy. It is commonly known as Hook worm, and the disease is called Ancylostomiasis, which is highly prevalent in the tropics and sub tropics. The adults are small and grayish-white in color. The male is smaller (8-11mm× 0.45mm) than female (10-13mm×0.66mm). Single female can lays 15,000-20,000 eggs per day (Chakraborty, 2004). The male bears the copulatory bursa in an umbrella like fashion at the posterior end where as tail region of female is tapering. The hook worm species can be distinguish by the presence of buccal capsule lined with hard substance provided with six teeth, four hooks on the ventral surface and two knobs like on the dorsal surface. There is presence of 5 glands which are associated with the digestive system that secrete the anticoagulant substance. The adult worm reside in the human intestine, particularly in the jejunum, less often in duodenum and rarely in the ileum about 3-4 years (Cheng, 1999).The third stage filariform larva penetrate the skin and suck the blood, lymph, bites of mucous membrane and tissue fluid from the lining of the intestinal wall which give characteristic symptoms of gastro-intestinal disturbances, anemia and nervous disorders (Chatterjee, 2009).

#### ***Trichuris* and Trichuriasis**

This parasite was reported by the Lineaus in 1771, which is commonly called Whip worm. The disease caused is called trichuriasis or whipworm infection which occurs worldwide.

The worm is sexually dimorphic in which male measures 30-45mm in length with coiled posterior end where as female measures 35-50mm in length with comma shaped posterior end (Chakraborty, 2004). No intermediate host is required for the completion of the life cycle but change of host is required for the continuance of the species (Chatterjee, 2009). Human are exposed to infection by ingesting embryonated egg containing larva through contaminated food and water. The egg shell is dissolved by the digestive juice and larvae are liberated through mucous plug and migrate to the caecum (Ichpujani and Bhatia, 2002). Mild trichuriasis is often asymptomatic. The worm inhabiting vermiform appendix may give rise to acute appendicitis (Regmi, 2012). The main features of the heavy infections are abdominal pain, malnutrition, bloody diarrhea, anemia and weight loss.

### ***Hymenolepis* and *Hymenolepiasis***

It is the smallest tapeworm infecting human and was 1<sup>st</sup> identified by Bilharz in 1875 (Arora and Arora, 2012). It is commonly known as the dwarf tapeworm which causes a disease called Hymenolepiasis. It is cosmopolitan in distribution and its incidence is higher in the children in temperate areas. The adult worm is lodge in the upper two-third of the ileum with scolex embedded in the mucosa. The entire worm is small measuring only 4-5 cm in length and 1mm in diameter. The eggs are oval or spherical, and consist of thin and smooth outer membrane and an inner membrane containing embryophore. Some eggs hatch out in the lumen of the small intestine and released the embryos which directly invade the intestinal villi. The infection to man occurs by ingesting the food and water contaminated with the eggs. The infection are more common in children and symptoms are produced in immune-deficient and malnourished patient. The clinical features of the disease are headache, dizziness, anemia, pruritus of nose and anus, abdominal pain, diarrhea, restlessness, epileptiform convulsion and eosinophilia in excess of 5% (Arora and Arora, 2012).

### ***Strongyloides* and *Strongyloidiasis***

It was 1<sup>st</sup> observed in the faces of the French soldier in the Indo-china by the Normand in 1876. The disease caused by this nematode is called strongyloidiasis. It is worldwide in distribution, however, flourishes commonly in tropics and sub-tropics. It has two forms, parasitic and free living. The parasitic females are found in the mucous membrane of the small intestine. It reaches up to 2-3mm in length and 30-50µm in width. It is ova-viviparous and visible to the naked eyes. There is a debate over many years for the existence of parasitic male. It is believed that parasitic male exist, they are boarder and shorter than females and have no penetration power, therefore, they do not invade the intestinal wall (Arora and Arora, 2012). The larvae are of two types: Rhabditiform and filariform. The filariform larva is longer and slender than the rhabditiform and is highly infectious to the host. The disease is transmitted through the entry of the infectious filariform larva from the soft skin of the man, generally through the foot. The infection is usually asymptomatic in immune-competent host, but due to the autoinfection, the infection often progress to the fatal hyper-infected state under immune compromised condition (Kobayashi *et al.*, 1996).The patient may develop the superficial ulceration in the intestine, mild esinophilic infiltration, catarrhal and other.



## **Taenia and Taeniasis**

Taeniasis is referred as the human infection with *Taenia solium* and *Taenia saginata*. The description of the *Taenian saginata* was given by the Hippocrates and Goeze differentiated it from the *Taenia solium*. They are cosmopolitan in distribution. It is estimated that as many as 100 million people are infected with *Taenian saginata* and *Taenia solium* (Arora and Arora, 2012).

The scolex of the *Taenia saginata* is pear-shaped with the diameter of 1-2mm, has 4 suckers, and embedded in the mucosa of the wall of the ileum. The adult worm is white, semi-transparent which generally measures 5-12 meters but sometimes exceed 20 meters. Spherical eggs are not infective to man. In the intestine, eggs are ruptured; onchosphere liberated and get entered into the portal vein by penetrating the gut wall through their hooks. Human get infected after ingesting undercooked beef containing cysticercous larva which later develop into adult worm inside human body (Chatterjee, 2009).

*Taenia solium* was identified by the Linnaeus in 1758. The entire worm measures 2-3 meters in length which lives in the small intestine of the man. The scolex is about pin-head size and head is provided with the rostellum armed with the double row of large and small hooklet. The larval stage known as cysticercus cellulose occurs in pig and also in man. Eggs are morphologically similar to that of *Taenia saginata*. Man acquires infection either by eating the inadequately cooked pork containing cysticercus cellulosa or by ingesting the eggs of *Taenia solium* in contaminated food and water. Taeniasis is asymptomatic in cases of man. Occasionally mild diarrheas, abdominal pain, loss of appetite are the main symptoms due to cysticercus cellulosa (Chatterjee, 2009).

### **1.3 Objectives**

#### **1.3.1 General objective**

- To study the prevalence of intestinal parasites in Meche community of Jalthal VDC, Jhapa, Nepal.

#### **1.3.2 Specific objectives**

- To assess the age and sex wise prevalence of intestinal parasite.
- To determine the concurrency of the intestinal parasites.
- To know the relationship of socio-economic status and intestinal parasites of Meche people.
- To assess the knowledge, attitude and practices (KAP) regarding intestinal parasitic infection of the study area.

### **1.4 Significance of the study**

Intestinal parasites are widely prevalent in developing countries probably due to the poor sanitation and inadequate personal hygiene (Ramadas *et al.*, 2010). Among intestinal parasites, protozoan parasite alone is responsible for different kinds of mortality and morbidity (Rai *et al.*, 2011). The morbidity caused by helminthes consist of nutritional deficiency, intestinal obstruction, prostrating anemia, chronic dysentery, rectal prolapsed,

respiratory complications, poor weight gain, retarded growth and mental retardation (Regmi, 2012).

No work has been done on the intestinal parasitic infection in Meche people, so there is necessity to study about the infection of the parasites in this ethnic group. The situation about the parasites and its infection remains almost unknown in this community. So, the present study plays an important role to find out the infection in this community due to intestinal parasites. Poor economic status, lack of health education, unhygienic habit etc. has contributed to the high prevalence of parasitic infection in the country as well as in the Meche community. Feeding habit, community sanitation, education level, availability of health services etc. are observe to be very poor in this community. These determine the poor economic status. So, this study will also be beneficial to find out the relationship between socio-economic status and prevalence of intestinal parasites of Meche people of Jalthal VDC of Jhapa, Nepal. This survey will help to create the awareness related to intestinal parasitic infection which will help in minimizing different intestinal parasitic disease and to improve sanitation behavior of the community. The survey will be fruitful for developing knowledge about intestinal parasites to the Meche people of Jalthal VDC. Moreover, the present study might help the future researcher to enhance the knowledge and carry out various investigations in rural communities about intestinal parasites.

## 2. LITERATURE REVIEW

### 2.1 History of Parasitology

The knowledge of parasitology was limited to recognition of the existence of a few common external parasites such as lice, fleas and few internal parasites like tapeworms, *Ascaris*, pinworms and guinea worms up to the middle of 17<sup>th</sup> century (Chandler and Read, 1961). These parasites were thought to be the natural product of human body. This idea was also supported by the Rudolphi and Bremser (Chandler and Read, 1961). In the Linnaeus time, it was believed that internal parasites were originated from the accidentally swallowed free living organism (Chandler and Read, 1961).

In the latter half of the 17<sup>th</sup> century, Francesco Redi, “Grandfather of Parasitology” discovered that maggots developed from the eggs of flies and even *Ascaris* had male and female. He also search for and found parasites not only in the human bowel but in other human organs, in the air sacs of birds and in the swim bladder of the fish (Chandler and Read, 1961). At the same time Leewenhoek discovered the microscope which helped him to describe various kinds of animalcule such as protozoa in the rain water, saliva, feces etc. In 1771, Linnaeus discovered the *Trichuris trichiura* (Arora and Arora, 2012) and latter life cycle was described by Grassi and Fulleborn. In 1800, Zeder recognized 5 classes of worms which Rudolphi Named Nematodea, Acanthocephala, Nematoda, Cestoda, and Cystica. In 1773 Muller discovered cercaria larvae (Chandler and Read, 1961).

Hookworm was discovered by Dubini in 1782. The penetration of the human skin by the hookworm larvae was discovered by Leoss in 1898. In 1849 Gros found the 1<sup>st</sup> human amoeba *Entamoeba gingivalis*. Lambl discovered *Entamoeba histolytica* in 1859. Kuchemeister and Leukart studied the life history of the *Taenia solium* in 1855 and 1856 respectively. In 1865 Leukart workout the life cycle of *Enterobius vermicularis* and latter in 1875 Losch proved its pathogenic nature. *Strongyloides stercoralis* was observed by Normand in 1876 (Arora and Arora, 2012). In 1903, Schoudinn differentiated pathogenic and non-pathogenic types of amoebae (Oli, 2016). Stewart experimentally proved tissue migration of *Ascaris* in 1961.

Many researches have been carried out on the intestinal parasites of the human by different workers. Some of the studies on human parasites and their infection are as follows:

### 2.2 Global context

Parasitic diseases have high prevalence and it is estimated that more than 3 billion people are infected by the intestinal parasitic infection in the world today (Rostami *et al.*, 2016). Globally, the infection rate due to the gastrointestinal parasites is on remarkable scale, particularly in undeveloped or developing countries due to the poor sanitation and inadequate hygiene. Both the protozoa and helminthes are responsible for the intestinal infections leading to high mortality and morbidity, particularly in developing countries (WHO, 2000). It is estimated that as much as 60% of the world population is infected with intestinal parasites (Ragunathan *et al.*, 2010).

Twenty-five percent of the world's population is estimated to be infected by one or more species of these parasites. Majority of the children of the developing countries are infected

by the *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm, *Entamoeba histolytica* and *Hymenolepis nana* (Opara *et al.*, 2007; Golia *et al.*, 2014; Wordemann *et al.*, 2006; Abahussain, 2005; Alamir *et al.*, 2013; Akingbade *et al.*, 2013). The second most common helminthic infection in human is the hookworm infection (Arora and Arora, 2012) and globally 1298 million people are ill due to hookworm infection (Crompton, 1999). It has been reported from most literature that IPI is common among the females (Akingbade *et al.*, 2013; Khanum *et al.*, 2015; Marothi and Singh, 2011). Rural students are more frequently encountered with parasitic infections than the urban students (Mohammad *et al.*, 2010). Socio-demographic and poor personal hygienic habits are main indicators for these infections. It has been reported that the commonest intestinal parasitic infection factors among pregnant woman and children are Ascariasis, Hookworm, Trichuriasis and *Entamoeba histolytica* which leads to increased anemia along with the high mortality and morbidity (Chatterji *et al.*, 2015).

Several researches have been carried out in the Asian continents about intestinal parasites. Asian continent is the home of the most of the developing countries. Poverty, illiteracy, ignorance etc. are common among the people of developing countries. Many researches have been done in the different countries of the Asia. It was claimed that overall prevalence of IPI has been found very high (75.18%) in India (Wani *et al.*, 2010; Wani *et al.*, 2009) while IPI in the Malaysia was found to be 64.1% (Lim, *et al.*, 2009). Similarly study by Alyousefi *et al.* (2011) revealed comparatively less prevalence (30.9%) in the Yemen whereas 28.5% of prevalence was revealed in Jordan (Ammoura, 2010). Research done in Indonesia showed variety of results regarding IPI. It was reported that *Ascaris lumbricoides* was the major helminth parasite infecting the people of the Muslim community (Hasegawa *et al.*, 1992) while some argued *Ancylostoma duodenale* as the predominant intestinal parasite (Bangs *et al.*, 1996). Some study revealed *Trichuris trichiura* as most frequently encountered parasite in the Muslim population of Indonesia (Hadju *et al.*, 1995; Toma *et al.*, 1999; Uga *et al.* 2004). Diverse species of parasites have been recorded from the different countries of the Asia. *Ascaris lumbricoides* has been ranked as most prevalent helminth parasite in India (Wani *et al.*, 2010) i.e.68.30%. However *Trichuris trichiura* has been found as major helminth in Malaysia (Lim *et al.*, 2009) and Korea (Kyong-Rock., 2010). *Blastocystis hominis* and *Giardia lamblia* have been reported as most common intestinal parasites (Arani *et al.*, 2008) while in national scale *Giardia lamblia* alone was ranked as most encountered intestinal parasites in Islamic Republic of Iran (Sayyari *et al.*, 2005). In Pakistan, *Ascaris lumbricoides* was reported as most frequent intestinal parasites in Muslim population contributing over 60% of total intestinal parasitic infection (Hiroshi *et al.*, 2002). Regarding the sex-wise distribution of the IPI, male are more victimized than females but statistically not explained in United Arab Emirates (Dash *et al.*, 2010).

Out of 224 stool samples of Bhil tribal individuals of Udaipur district, microscopic examination showed 51.78% of the overall prevalence of parasites and most common parasites revealed were *Entamoeba histolytica* (14.73%) followed by *Entamoeba coli* (8.92%), *Taenia solium* (5.35%), *Ascaris lumbricoides* (4.46%), *Hymenolepis nana* (2.23%), Hookworm (0.89%), *Strongyloides stercoralis* (0.89%), *Trichuris trichiura*

(0.44%) and *Hymenolepis diminuta* with 0.44% (Jaroli *et al.*, 2012). Similar study was carried out in the Bharia tribe of the India which showed that 30% of the children suffered from the severe anemia and 50% of the children had intestinal parasite with major parasites as *Ancylostoma duodenale* (16.3%) and *Ascaris lumbricoides* with 18.53% (Chakma *et al.*, 2000). Study performed by Rayapu *et al.* (2012) revealed 15.55% of overall prevalence of helminthes among children aged 5-14 years in rural area of Kupam, Andhra Pradesh and the most common helminth recorded were Hookworm, *Ascaris* and *Hymenolepis nana*. Another study by Chatterjee *et al.* (2015) in Paschim Medinipur district, West Bengal among pregnant women and children showed that *Ascaris lumbricoides*, Hook worm, *Trichuris trichiura* and *Entamoeba histolytica* were the leading cause of the prevalence of anemia along with the high infant mortality and morbidity. Similarly study carried out in Iran among students who took part in practical parasitological courses in Lorestan University of Medical sciences revealed overall prevalence of 11.93% (Rostami *et al.*, 2016).

Like Asian continent several researches have also been performed in the African continent. A study carried out in semi-urban village of Osun state, Nigeria revealed 50% prevalence of one or more helminth in the pre-school children, more prevalent being *Ascaris lumbricoides* i.e.47.6% (Kirwan *et al.*, 2009). Among the protozoan parasite, *Entamoeba histolytica* has been found as the most prevalent parasite in hospitalized AIDS patients in Congo (Wumba *et al.*, 2010). Similarly *Entamoeba histolytica* (36.7%), *Cryptosporidium* sp. (30.5%) and *Giardia lamblia* (16%) were most common intestinal parasites in the children presenting with diarrhea in outpatient and inpatient setting in an informal settlement of Nairobi, Kenya (Mbae *et al.*, 2013). In the district of Vhembe of South Africa, it was recorded that *Entamoeba histolytica*, *Entamoeba dispar* and *Cryptosporidium* sp. were the commonest parasite and *Giardia lamblia* was the cause of the diarrhea among the school children (Samie *et al.*, 2009). Finger sucking children were more susceptible to intestinal parasitic infection than non-finger sucking children and toilet facility of children also affected the infection in the finger sucking who used pit latrines regarding higher prevalence in Nigeria (Idowu *et al.*, 2011). There was high prevalence of *Ascaris lumbricoides* (20%) followed by *Enterobius vermicularis* (17.8%), *Trichuris trichiura* (12.9%) and *Ancylostoma duodenale* (6.5%) in Nigeria (Akinbo *et al.*, 2010). Similarly the overall prevalence of IPI was found to be very high (77.9%) among the school children in Dagi primary school, Ethiopia (Alamir *et al.*, 2013) where *Ascaris lumbricoides* (8.3%) was most prevalent. Similarly the prevalence of protozoa and helminth were reported to be 98% and 16.7% respectively with the overall prevalence of 84% in the children of six indigenous communities inhabiting Coli, Colombia (Florez *et al.*, 2012).

Even though being highly developed continent, Europe also contributes to the some forms of the parasitic infection in the world. People residing in different parts of the Europe are suffering from varieties of the intestinal parasites. When fecal samples from Greek population and refugees were examined, it was revealed that 18.02% were found to be infected with one or more species of parasites where the most common parasites detected were *Blastocystis hominis*, *Cryptosporidium parvum*, *Entamoeba coli* and *Giardia lamblia*

among the protozoan whereas among helminthes *Enterobius vermicularis*, *Taenia* sp., *Ascaris lumbricoides* and *Trichuris trichiura* were common (Frydas *et al.*, 2004). Intestinal parasitic infection such as enterobiasis, giardiasis and ascariasis were detected most frequently in Romania but their importance was definitely surpassed by trichinelosis, echinococcosis and toxoplasmosis (Neghina, 2011). In Turkey, *Blastocystis hominis* was found as the most prevalent parasite followed by *Enterobius vermicularis* (7.31%), *Hymenolepis nana* (6.2%), *Giardia intestinalis* (5.6%), *Entamoeba coli* (2.27%), *Entamoeba histolytica* (1.13%), *Entamoeba harmonic* (1.13%) and *Taenia* sp. (0.56%)(Alver *et al.*, 2011).

Similarly different researches in the American continent regarding intestinal parasitic infection have been conducted. In Brazil, prevalence of the *Strongyloides stercoralis* was surveyed and infection was confirmed in 11.3% of the total cases (Hasegawa *et al.*, 1996). Similar study was conducted in the same country and prevalence rate was found to be 13.0% out of total sample (Machado and Costa, 1998; Siddique and Berk, 2001). A retrospective study was carried out in Parasitological unit of medical laboratory services of Princess Margaret Hospital, Dominica and revealed 10.47% of the positive cases. The commonly encountered parasites were *Entamoeba coli* (14%), *Giardia lamblia* (1.4%), *Strongyloides stercoralis* (1.0%), *Ascaris lumbricoides* (0.8%) and *Trichuris trichiura* with 0.9% (Adedayo *et al.*, 2004). A study carried out in the rural village of Lucia among the children showed very high (61.6%) overall prevalence of IPI (Kunup and Hunjan, 2010), in which common parasite detected was *Ascaris lumbricoides* (15.7%) followed by Hook worm (11.9%), *Strongyloides stercoralis* (9.9%) and *Trichuris trichiura* (4.7%).

Like other continent, Australia is also untouched from infection caused by intestinal parasites. In Sydney, Business district, study showed high prevalence of different parasites (Fletcher, 2012) in which recorded parasites were *Blastocystis* sp. (57%), *Giardia intestinalis* (27%) and *Dientamoeba histolytica* (12%). Similarly the study performed by Shield *et al.*, (2015) among 314 participants of Aboriginal community, Australia revealed overall prevalence of 89% and the parasites detected were *Trichuris trichiura* (86%) followed by hookworm (36%), *Entamoeba* sp. (29%), *Strongyloides stercoralis* (19%), *Hymenolepis nana* (16%) and *Giardia intestinalis* (10%).

It seems that there is no any part of the world which is free from the IPI and, is mostly distributed in Asian and African continent. Even highly developed countries harbor the varieties of the parasites.

### **2.3 National context**

In context of Nepal, many researches have been carried out by different researcher regarding IPI and reported the different species of helminth and protozoan parasites. *Ascaris* has remained as leading human parasites among helminth where as *Entamoeba* sp. has been found higher among different species of the protozoan parasites (Rai and Rai, 1999).

Most of the research on the basis of IPI was carried out from different parts of the Nepal and intestinal parasitic infection has been reported by different researcher such as (Rai and Gurung, 1986; Shrestha and Maharjan, 2013; Shrestha *et al.*, 2012, Malla *et al.*, 2004;

Tandukar *et al.*, 2015; Pandey *et al.*, 2015) where *Ascaris Lumbricoides* was most dominant parasite. Some author carried out study in children and adult showed *Entamoeba histolytica* as the major intestinal parasites (Sherchand *et al.*, 2010; Agrawal *et al.*, 2012; Shakya *et al.*, 2012; Shrestha *et al.*, 2012; Singh *et al.*, 2014). In Nepal, giardiasis, ascariasis, amoebiasis and taeniasis are common IPI (Acharya, 1997). Intestinal protozoan infection and helminthic infection rank third and fourth respectively in Nepal (DHS, 2004).

*Ascaris lumbricoides* (roundworm), hookworm (*Ancylostoma duodenale* and *Necator americanus*) and *Trichuris trichiura* (whipworm) are the most common soil-transmitted helminthes (STH) which infect man (Rai *et al.*, 1994). It was observed that non vegetarian was highly infected with parasitic infection than the vegetarians (Pandey *et al.* 2015). Other contributing factors for IPI are unawareness (Pandey *et al.*, 2015), lack of toilet (Karunaithas *et al.*, 2011) and farming practices (Tandukar *et al.*,2013). Low socio-economic status, poor hygienic condition (Khanal *et al.*, 2011; Rosino *et al.*, 2009), lack of proper sanitary disposal and lack of health education are considered to be the root cause of the parasitic infection (Rashid *et al.*, 2011).

Several authors reported the high prevalence of the IPI among the school aged children. The prime victim of the intestinal parasites is the school children that affect the physical development, school attendance and ability to learn (Mohammed *et al.*, 2010; Jasti *et al.*, 2007). It was revealed by Bhandari *et al.* (2011) that 40% prevalence of the intestinal parasitic infection among the school going children while Shrestha *et al.* (2012) reported 21.05% prevalence in school aged children. IPI is also found associated with malnutrition among the children. A study from the Sarlahi, Nepal showed out of 35.11 and 36.88 percentages of the severely and moderately malnourished children, 41.77% were infected with some kind of the parasitosis (Malla *et al.*, 2004). Similarly in Bara district, the prevalence of IPI among the school children was found to be 31.7% (Regmi, 2012), 40% among children of Kathmandu valley (Bhandari *et al.*, 2011), 23.3% in children of Chitwan district (Sherchand *et al.*, 2015) and 16.7% in the children of Lalitpur district (Tandukar *et al.*, 2013).

Several studies showed the very high prevalence of IPI in different parts of the country. The overall prevalence of 76.4% was reported from the Achham district (Rai *et al.*, 2001) while 81.94% was reported among the children of age between 7-12 years old in Lalitpur district (Shrestha, 2001). Terai people of low socio-economic status revealed 84.2% of the sample positive (Rosino *et al.*, 2009). Similarly children of the Sukumbasi Basti of Kathmandu below 16 years of age were found to be more susceptible to IPI and study conducted in that community showed 43.3% of prevalence with no significant difference in boys and girls (Rai *et al.*, 2011) where most common parasite detected was *Giardia lamblia* followed by *Entamoeba histolytica*, *Trichuris trichiura* and others. Another study carried out in Squatter community of Dharan municipality, Sunsari also showed high prevalence (41.4%) with male children more infected (45.8%) than females (37.5%) where positive rate was higher in Dalits (59.7%) and least in Tibeto-Burman (27.1%) ethnic group (Chongbang *et al.*, 2016). IPI in relation among the HIV and AIDS patient was

carried out in the Nepalese patient which showed the 42% of the prevalence (Sapkota *et al.*, 2004).

However, comparatively low prevalence of parasites has also been reported from some parts of the countries. Among 300 stool samples, only 5% was recorded as positive in Northern Kathmandu in which commonly found parasites were *Entamoeba histolytica* (1.67%) followed by *Giardia lamblia*, *Ascaris lumbricoides*, *Hymenolepis nana* and *Cyclospora* (Pandey *et al.*, 2015). Similarly in a public high school of Kathmandu, reported only 17.6% of prevalence of IPI among the children aged 6-16 years (Khanal *et al.*, 2011). The school children of Kathmandu valley revealed 16.5% of prevalence (Tandukar *et al.*, 2015). The prevalence of the IPI was found to be 21.4% in a study carried out among the patient of Deukhuri hospital, Dang (Khanal *et al.*, 2011) where most prevalent parasite were *Entamoeba histolytica*, *Ascaris lumbricoides*, Hookworm, *Trichuris trichiura*, and *Taenia* sp.

Among protozoan parasite, *Giardia lamblia* has been found as most prevalent (Chandrasekhar, 2005; Shakya *et al.*, 2012; Tandukar *et al.*, 2013). Similarly *Giardia lamblia* (12.0%) was found as major parasite followed by *Entamoeba histolytica* (9.6%), in Kanti children hospital, Maharajgunj, Kathmandu (Rai *et al.*, 2011). Similar study conducted in Sukumbasi tole of Chorsangu-7 Pokhara also revealed *Giardia lamblia* as most prevalent protozoan parasite (Tiwari, 2012). However some authors reported *Entamoeba histolytica* as most prevalent among protozoan (Pandey *et al.*, 2015; Rai *et al.*, 2008; Singh *et al.*, 2014). The prevalence of *Entamoeba histolytica* was found to be very high (61.0%) followed by *Trichuris trichiura* (22.0%), Hook worm and *Ascaris* in the children of Bharatpokhari, VDC, Kaski, Nepal (Jaiswal *et al.*, 2014). Similarly study carried out among the subject undergoing cataract surgery at the eye camp in rural hilly areas of Nepal also claimed *Entamoeba histolytica* as most prevalent protozoan (Rai *et al.*, 2008).

Among helminth, *Ascaris* has been recorded as most common (Rai *et al.*, 2001; Rai *et al.*, 2002; Chongbang *et al.*, 2016; Maharjan and Shrestha, 2013; Khanal *et al.*, 2011). Some investigations confirmed Hook worm as most prevalent (Singh *et al.*, 2007; Gyawali, 2012; Rai *et al.*, 2008). A retrospective study carried out in tertiary care hospital showed Hook worm as most prevalent (10.50%) followed by *Ascaris* (5.72%), *Strongyloides stercoralis* (4.77%), *Hymenolepis nana* (3.34%), *Trichuris trichiura* (0.95%) and *Enterobius vermicularis* with 0.23% (Singh *et al.*, 2007). Apart from *Ascaris* and Hook worm, *Trichuris trichiura* has also been recorded as common parasite (Rai *et al.*, 2008; Rai *et al.*, 2011; Shakya *et al.*, 2012). The soil transmitted helminth in Kathmandu was reported by Shrestha *et al.* (2014) where contamination rate of soil with parasite eggs and larvae was found to be 28.5%. The prevalence of soil transmitted helminth was ranged from 3.3 to 51.5% in different parts of Nepal (Parajuli *et al.*, 2014).

Regarding ethnicity, Tibeto-Burman ethnic group showed highest prevalence (70.1%) compared with Indo-aryan (64.7%) and Dalits (57.7%) among the patients of cataract in hilly areas (Rai *et al.*, 2008). Positive rate in Tibeto-Burman was highest (23.3%) followed by Indo-Aryan (22.1%) and Dalits (29.6%) among the school children of Chitwan district (Bhattachan *et al.*, 2015). Newar ethnic group was found to be more susceptible (36.84%)



to IPI followed by Chhetri (32.97%) among the children of Kathmandu valley (Bhandari *et al.*, 2011). Similarly prevalence rate of Protozoan parasite was found to be higher in Dalits (20.3%) than in Indo-Aryan (19.6%) of school aged children of Kathmandu valley (Mukhiya *et al.*, 2012). Out of 422 stool sample of Mushar community of Saptari district, fecal examination revealed overall prevalence of 27.48 % with *Ascaris lumbricoides* being most predominant parasites showing prevalence of 10.45% (Yadav, 2014) and other detected parasites were *Entamoeba histolytica*, *Giardia lamblia*, *Taenia solium* and *Ancylostoma duodenale*.

A significant association has been found in prevalence of IPI between rural and urban area in which rural area was more susceptible (Chandrasekhar, 2005). Amongst the infected children of public school of rural village of Kathmandu, single infection was found in 93.5% of cases and mixed in 6.5% (Shakya *et al.*, 2012). The percentage of single infection was higher (89.4%) than multiple parasitosis (Regmi, 2012) among the children of Kalaiya, Bara, Nepal. The rate of double and multiple infections were found 18.98% and 2.19% among the children of Bhaktapur district (Maharjan and Shrestha, 2013). Research conducted in public and private school of Thimi area showed overall prevalence of 35.6%, where positive rate was slightly higher (40.3%) in public school than private (26.0%) (Rai, *et al.*, 2001).

The IPI was correlated with food habit, sanitary condition and drinking water quality. Subjects undergoing cataract surgery without toilet and those with vegetarian food habit had marginally high positive rate as compared to those having toilets and non-vegetarians (Rai *et al.*, 2008). Children drinking untreated water, living in bigger family and those without toilet had high positive rate (Shrestha *et al.*, 2009). Those belonging to family of agricultural workers were most commonly affected by the IPI, and hand washing practice, type of drinking water also showed the significant difference (Tandukar *et al.*, 2013). Similarly occurrence of IPI was found associated with unhygienic way of life, poverty, ignorance and poor sanitation among the children of Bhaktapur district (Maharjan and Shrestha, 2013). Consumption of untreated drinking water and raw vegetables among infected children showed correlation between low income and prevalence of IPI (Bhandari *et al.*, 2015). From previous studies, it shows that many people of different parts of Nepal are suffering from different helminth as well as protozoan parasitic infection due to illiteracy, poor hygiene, poor sanitary condition and low socio-economic condition.

### 3. MATERIALS AND METHODS

#### 3.1 Study area

The study was conducted in Jalthal VDC which lies in Jhapa district. Jhapa district is situated in terai region of eastern belt of Nepal. Jhapa district is divided into 37 VDCs and 8 municipalities. Among 37 VDCs Jalthal is located in the southern part of the Jhapa district extending from Mahespur VDC in the east to Rajgadh VDC to the west with the location 26° 7' 01.24" N and 87° 56' 27.62" E to 26° 31' 33.14" N and 88° 02' 57.37" E. The study population comprised of Meche people residing in the Jalthal VDC of Jhapa district which is least developed rural area.

In Nepal, 125 ethnic groups are present speaking 123 languages (CBS, 2011). The total population of Meche in national context is 4,380, of which 4,076 reside in Jhapa district (CBS, 2011). Jalthal VDC includes 9 wards in which 3, 4, 5,6,7,8 and 9 wards are inhabited by Meche people. Out of 13,363 population of Jalthal VDC, 1,011 are Meche people (NPHC, 2011). Meche ethnic group is one of the communities of Nepal with least population, so, categorized as rare indigenous caste of Nepal. Meche are Mongolian people residing in Mechi River (eastern boarder of the country) of Jhapa district. They are mainly called Bodo, who mainly inhabit in Jalthal and Dhaijan VDCs (Rai and Dhungana, 2002). The main occupation of the Meche people is agriculture and alcohol making by traditional method.

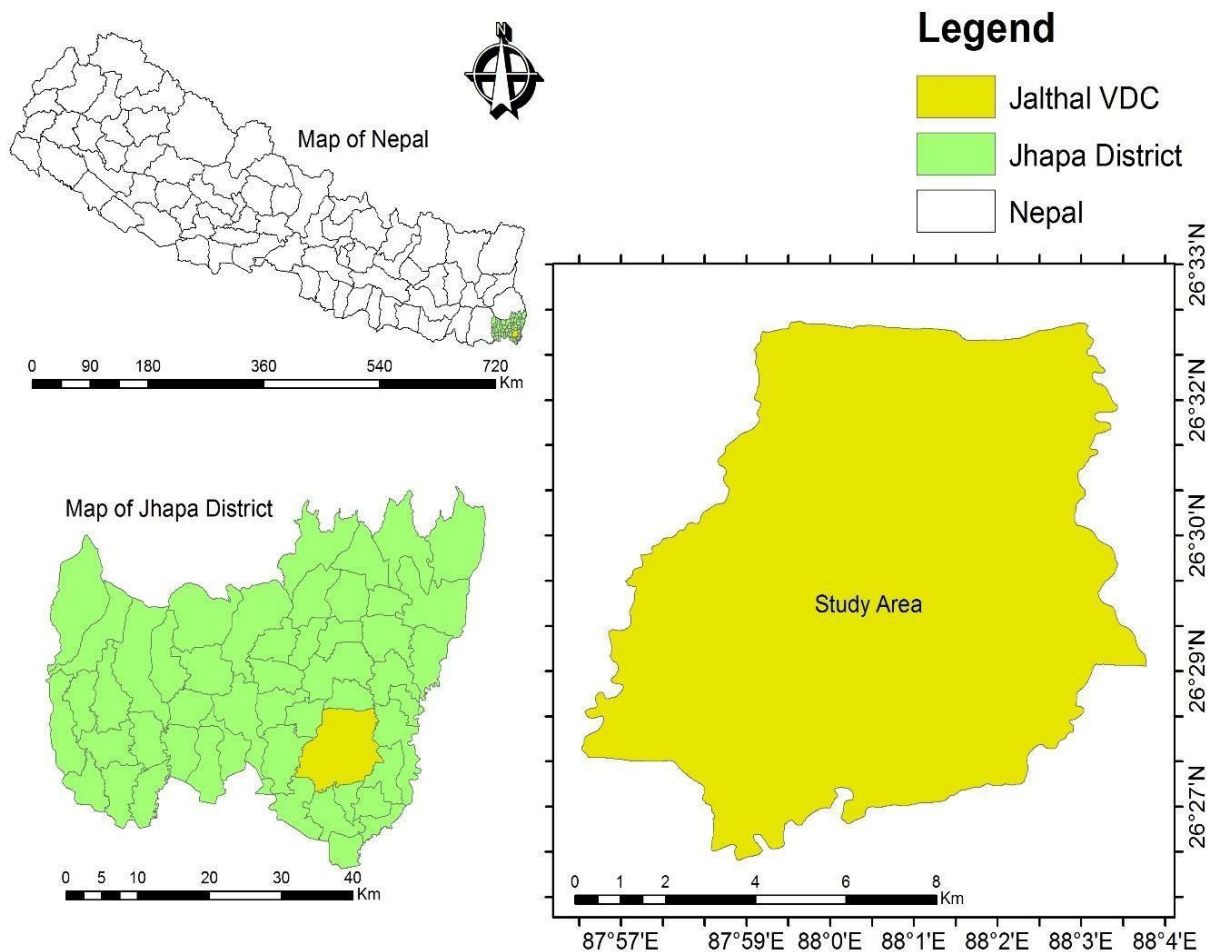


Figure 1: Map of study area

### 3.2 Study duration

Random sampling method was applied for collecting information and stool sample in the study area with prepared questionnaire during June to July, 2017.

### 3.3 Study design

The study was designed to assess the intestinal parasitic infection in the Meche community of Jalthal VDC, Jhapa. The study design includes:

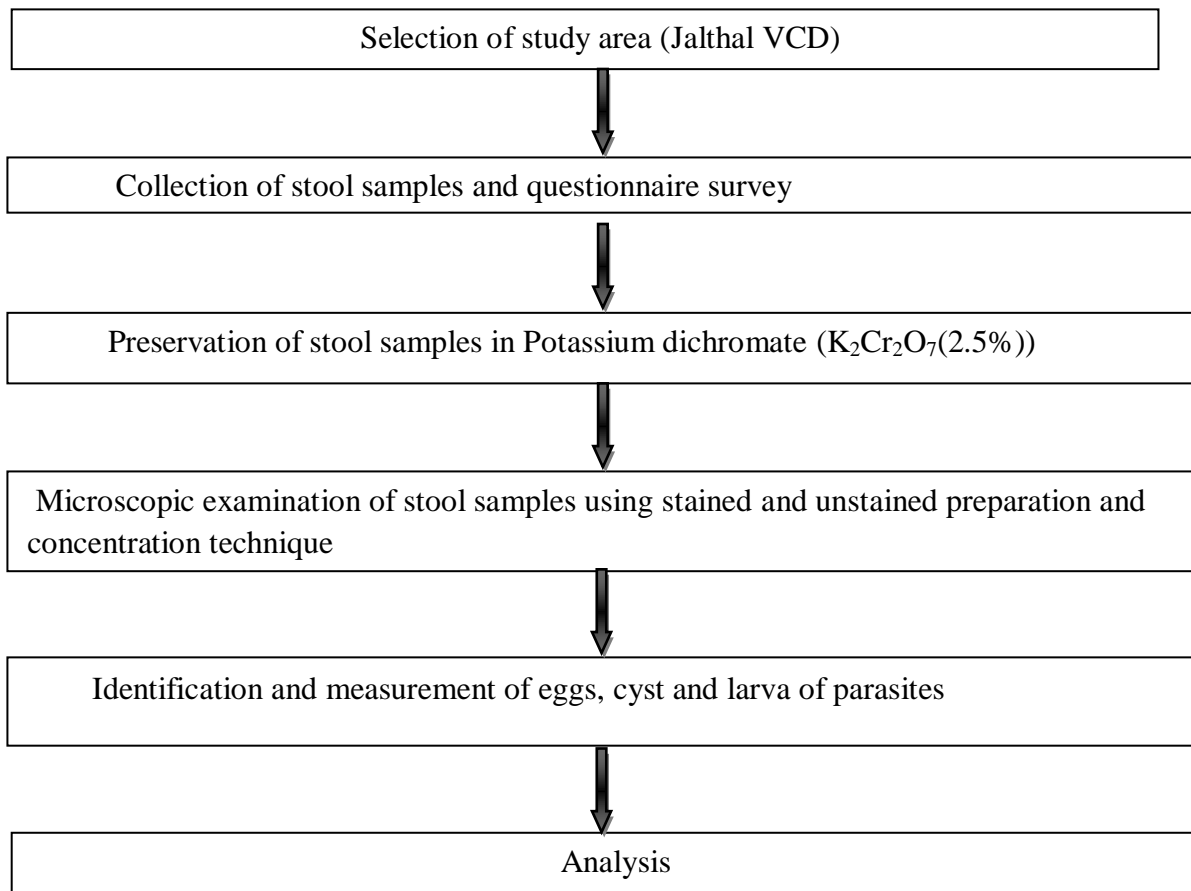


Figure 2: Flowchart of research outline

### 3.4 Sample size

Total sample size of the present study was 150 from different age and sex of Meche peoples. The study of population was divided into 3 age groups i.e. 5 to 20 years, 21-40 years and above 41 years of age and stool samples were collected.

### 3.5 Stool sampling

One hundred fifty peoples were randomly selected for the study purpose. After proper instructions, vials were given to the people regarding collection of the stool sample, they were given sterile labeled vials and application sticks. An instruction was provided to avoid the contamination of stool and urine. Each of the specimens was checked for its labeling .The collected stool samples were preserved in potassium dichromate (2.5%) and transported to the Laboratory of Central Department of Zoology for further investigation of eggs, adult of intestinal parasites.

### 3.6 Materials

#### 3.6.1 Equipments

- Compound microscope,
- Filter paper or Cotton
- Tray, Cover slip
- Gloves
- Needle and Sticks, Slides
- Vials for sample collection.

### **3.6.2 Chemicals**

- 2.5% Potassium dichromate solution
- Dettol soap
- 0.5% normal saline,
- Saturated NaCl solution
- Methylene blue
- Iodine solution

### **3.6.3 Preparation of Potassium Dichromate:**

2.5 gm of Potassium Dichromate was weighted accurately with the help of electric balance and dissolved in 100 ml of distilled water. This solution was used for the preservation of parasite found in the stool (Chatterjee, 2009).

### **3.6.4 Preparation of Normal Saline:**

Normal saline was used for observing the characteristics movement of parasites. This solution was prepared by dissolving 8.5 gm of Sodium chloride in 1000 ml of distilled water, which was used in unstained preparation (Chatterjee, 2009).

### **3.6.5 Preparation of Iodine Solution:**

Iodine solution was used for studying the internal characters of identification of the species of protozoan parasites as well as helminthes eggs, a stained preparation was required. Iodine solution was prepared by dissolving 10 gm of potassium iodine in 100 ml of distilled water and slowly adding 5 gm iodine crystals in it. The solution was filtered and then kept in bottle (Chatterjee, 2009).

### **3.7 Laboratory work**

All the laboratory works were done in Central Department of Zoology, TU, Kirtipur, Kathmandu under the supervision of supervisor.

### **3.8 Methods of stool examination**

#### **3.8.1 Unstained preparation of stool smear:**

A minute portion of stool was taken with the help of small stick and emulsified with normal saline (0.5) and a drop of it was taken on a clean glass slide. Then a cover slip was placed gently over it so as to spread out the emulsion into a thin, fairly uniform and transparent layer and excess of fluid was removed with the help of filter paper (Arora and Arora, 2012). The slide was fixed in microscope and examined under low power 10X objective. Observation was starting from one end of the slide to another. When the parasites, eggs were seen then objects were centered and focused under the high power for detailed diagnosis

#### **3.8.2 Stained preparation of stool smear:**

Stained preparation was necessary for the identification and the study of the nuclear membrane and the iodine stained preparation was used for this purpose which was diluted in the ration of 1:5 with distilled water (Arora and Arora, 2012). The method was applied for observing the eggs, cyst and larva of parasites.

### **3.8.3 Concentration methods**

#### **3.8.3.1 Differential flotation technique**

This technique was used widely for detecting eggs of nematodes and cestodes. As their eggs are lighter and small, they can float by this method. Approximately 3gm of stool sample was taken in a beaker and added 15ml of saturated sodium chloride solution then stirred properly and solution was filtered by tea strainer. The filtrate was poured into a centrifuge tube of 15ml and tube was filled with more sodium chloride solution and centrifuge at 1000 rpm for 15 minutes. After centrifuge more saturated sodium chloride was added to develop the convex surface at the top of the tube and one drop of methylene blue was added then a cover slip was placed for 5 minutes. Then the cover slip was removed and placed on a slide and examined. The photographs of the eggs, cysts, and larva of parasite was taken and identified on the basis of shape, color and size (Chatterjee, 2009).

#### **3.8.3.2 Sedimentation technique**

This technique is very sensitive and has minimum chances of error which is used for detecting trematode eggs. It provides good results as trematode eggs are a bit heavier than other. Saturated salt solution was removed gently from the test tube after examining the flotation portion and poured the sediment content into the watch glass and stirred the content gently to mix it. One from the mixture was taken to prepare a second slide. The specimen was stained with iodine wet mount solution. The slides were examined under objectives of 10x X 10x and 10x X 40x objectives of microscope to detect the eggs of intestinal parasites (Chatterjee, 2009).

### **3.9 Eggs, Cyst and larva identification**

Identification was done on the basis of shape and size of published books (Arora and Arora, 2012), (Chatterjee, 2009) and other published and unpublished articles and internet sources.

### **3.10 Questionnaires**

The questionnaires were done to know about the knowledge, attitude and practices of Meche people which include population of different age groups and sex. Short questionnaires were designed which included (a) socio-demographic: address, age, gender and socio-economic status. (b) behavioral data: hand washing habits, types of drinking water, treatment method for infection etc. (c) Participant's present medical history: any complaints of abdominal pain/discomfort, nausea and vomiting. The entire questionnaires were checked for accuracy and completeness. The questionnaires are shown in the annex 2.

### **3.11 Data analysis and Interpretation**

All data as well as laboratory finding were analyzed according to their age, sex, feeding habit, and infection rate. Thus, analyzed data was interpreted by representing with table and pie-chart. Association of intestinal parasites with age, sex, occupation, drinking water etc. were assessed by using "R", version 3.3.1 software packages. In all cases 95% confidence interval (CI) and  $p < 0.05$  was considered for statistically significant difference.

## 4. RESULTS

The study was carried out among the Meche people of the Jalthal VDC of Jhapa district. A total of 150 stool sample of different age and sex group were collected and examined from June to July, 2017. The result of present study was categorized in two ways:

1. Results of stool examination
2. Results of questionnaires survey

### 4.1 Results of stool examination

#### 4.1.1 General Prevalence of Intestinal Parasites:

Out of 150 samples, the general prevalence of the intestinal parasites of Jalthal VDC were found to be 41 (27.33%).

#### 4.1.2 Prevalence individual intestinal parasites:

Out of 150 stool samples examined, five intestinal parasites were identified with list of *Ascaris lumbricoides* 29 (19.33%), *Entamoeba coli* 4 (2.67%), *Taenia solium* 4 (2.67%), Hookworm 4 (2.67%) and *Trichuris trichiura* 3 (2%).

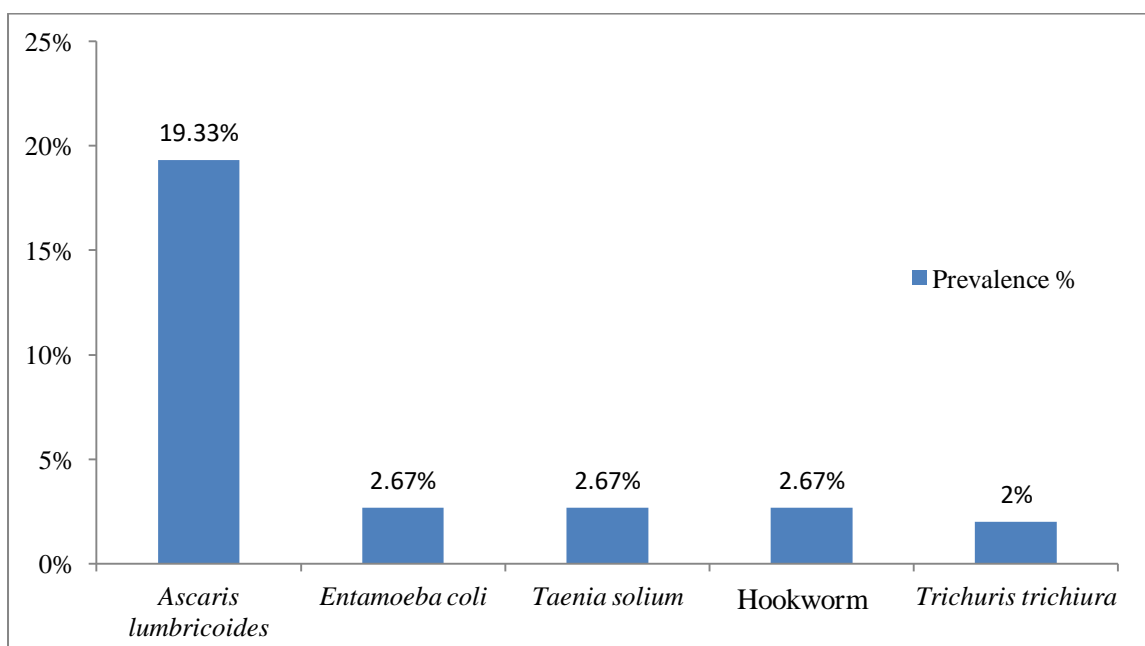


Figure 3: Individual prevalence of intestinal parasites

#### 4.1.3 Distribution of Protozoan parasite:

Among total positive samples (41), prevalence of protozoan parasite was observed in 4 (9.75%) cases which is *Entamoeba coli*.

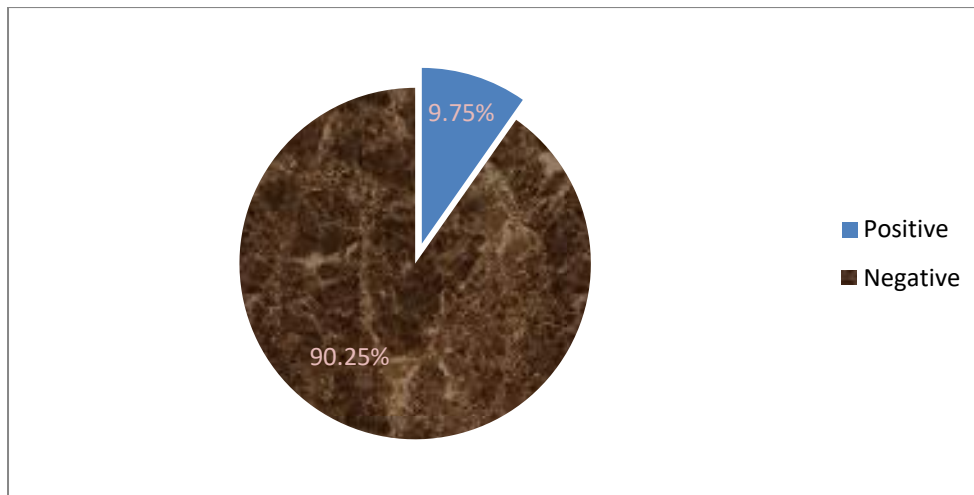


Figure 4: Distribution of Protozoan parasite

#### 4.1.4 Distribution of helminth parasite:

Out of total positive cases, prevalence of helminth parasites was observed in 40 (97.56%) of the samples. The detected helminths were *Ascaris lumbricoides*, *Taenia solium*, Hookworm and *Trichuris trichiura*.

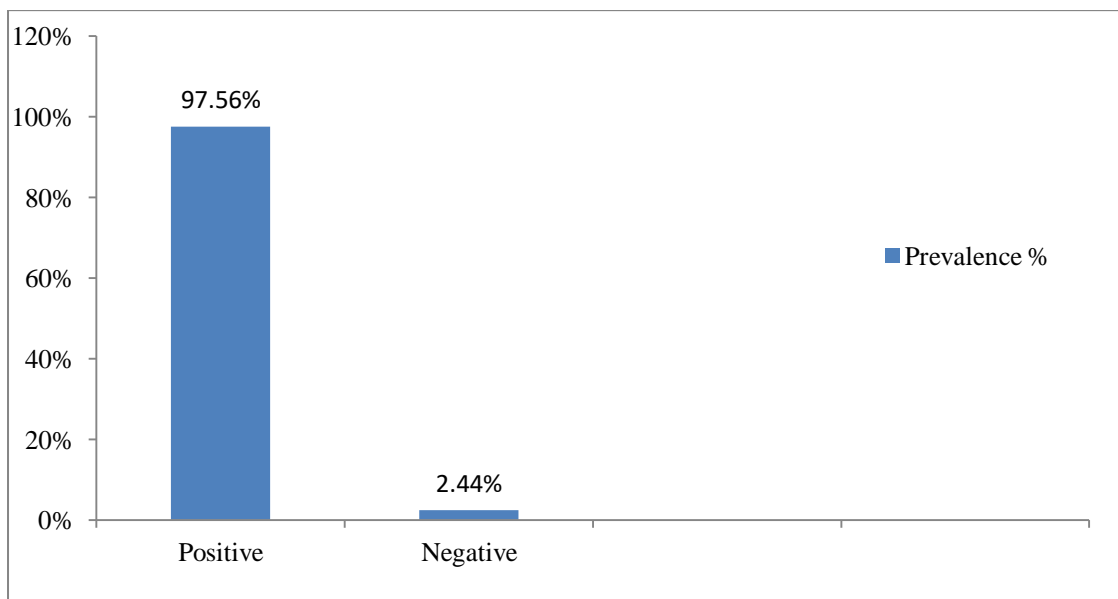


Figure 5: Distribution of Helminth parasites

#### 4.1.5 Sex-wise Prevalence of intestinal Prevalence

In total of 150 stool samples, 73 were of males and 77 were of females. Out of 73 samples examined from male, 16 (21.91%) were found to be positive. Similarly out of 77 samples examined from female, 25 (32.47%) were found to be positive. Hence, infection rate was found higher in female than in male. There was not significant difference in the distribution of parasites between male and female ( $\chi^2=1.6022$ ,  $df=1$ ,  $p\text{-value}= 0.2056$ ).



Table 1: Sex-wise Prevalence of Intestinal Parasites

S.N.	Sex	Total Examined sample	Positive Cases	Positive %
1	Male	73	16	21.91
2	Female	77	25	32.47
3	Total	150	41	27.33

#### 4.1.6 Age group-wise prevalence of Intestinal parasites

The study population was categorized into three age groups which were 1-20 years, 21-40 years and above 41 years old. The parasites were recorded maximum 17 (47.22%) in age groups 21-40 and minimum 8 (20.51%) above 41 years old age group. Parasitic infection among age group was found to be statistically significant ( $\chi^2=5.999$ ,  $df = 2$ ,  $p\text{-value}=0.0498$ ).

Table 2: Age group-wise Prevalence of Intestinal Parasites

S.N.	Age	Total no. of samples	Positive cases	Positive %
1.	1-20	45	16	35.56
2.	21-40	36	17	47.22
3.	Above 41	39	8	20.51
	Total	150	41	27.33

#### 4.1.7 Concurrency of intestinal parasites:

Out of 41 (27.33%) positive samples, 38 (92.68%) samples were found to have single infection whereas only 3 (7.32%) samples were detected to have double infection.

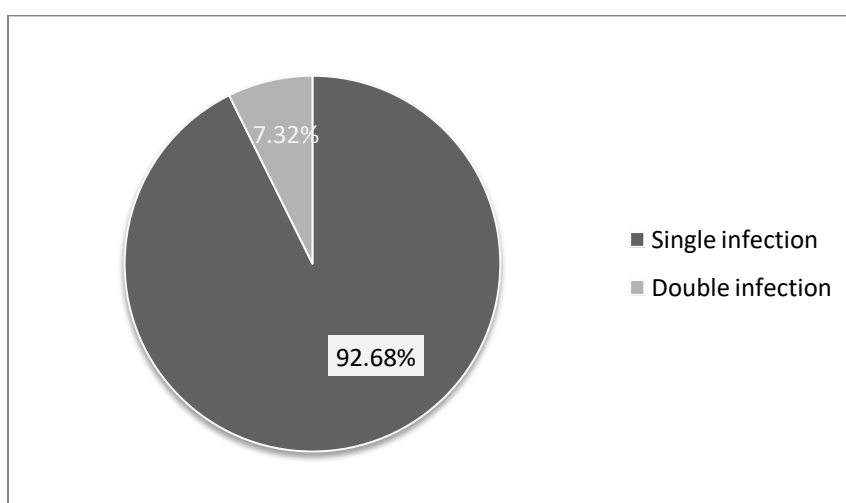


Figure 6: Overall concurrency of intestinal parasites

## 4.2 Results of Questionnaire Survey Analysis

The survey was carried out in the Meche community and a set of questionnaire were asked to those people whose stool sample were collected to examine for the information of knowledge, attitude and practice of the people towards the parasitic infection

### 4.2.1 Knowledge of the interviewed people

Meche people were interviewed on the basis of the parasitic infection, knowledge of the transmission, control and prevention of the parasites. Maximum number 105 (70%) of the people were unaware and 45 (30%) people were aware about the infection of the parasites. The infection was found higher (30.48%) among unaware people and lower (20%) among aware people. Statistically, distribution of parasites between aware and unaware people was not found significant ( $\chi^2=1.253$ ,  $df=1$ ,  $p\text{-value}=0.263$ ).

Table 3: Knowledge towards intestinal parasites among Meche people

S.N.	Sex	No. of respondent	Aware			Unaware		
			No.	Infected	%	No.	Infected	%
1	Male	73	22	2	9.1	51	14	27.45
2	Female	77	23	7	30.4	54	18	33.33
3	Total	150	45	9	20	105	32	30.48

### 4.2.2 Occupation wise Prevalence of Intestinal Parasites

The prevalence of the intestinal parasitic infection was found to be maximum 14 (32.59%) among students and minimum among the drivers 1(20%) whereas business man and teacher had not any infection. The distribution of intestinal parasites was not found significant according to different occupation statistically ( $\chi^2=4.2039$ ,  $df=5$ ,  $p\text{-value}=0.519$ ).

Table 4: Occupation wise prevalence of intestinal parasites

S.N.	Occupation	No. of respondent	Positive samples	%
1	Business man	8	0	0.00
2	Driver	5	1	20.00
3	Farmer	66	19	28.79
4	Housewife	27	7	25.93
5	Student	43	14	32.59
6	Teacher	1	0	0.00
7	Total	150	41	27.33

### 4.2.3 Domestic Animals Ownership-wise Prevalence of Intestinal Parasites

Most of the interviewed respondent 117 (78%) out of 150, had domestic animals mainly pigs, cows, dogs, cats and hens whereas 36 (24%) didn't have livestock. The prevalence was found higher 36 (30.77%) among the people who had livestock and domestic animals while 5 (15.15%) is found in peoples who didn't have livestock and domestic animals. There was no significant difference found in the prevalence of intestinal parasites of the people on the basis of livestock and domestic animal ownership ( $\chi^2=2.4235, df=1, p\text{-value}=0.12$ ).

Table 5: Livestock and Domestic animals ownership-wise prevalence of intestinal parasites

S.N.	Animal husbandry	Observation	Positive Cases	Positive %
1	Having domestic animals	117	36	30.77
2	Not having domestic animals	33	5	15.15
3	Total	150	41	27.33

### 4.2.4 Treatment Method-wise Prevalence of Intestinal Parasites

Out of 150 stool samples, it was found that most of the people who believed in traditional method (Dhami, Jhakri) were found to be highly infected 11(42.31%) and those consulting with doctors showed minimum infection 3(12%). There was no significant difference in the prevalence of intestinal parasites on the basis of treatment method ( $\chi^2=5.895, df=2, p\text{-value}=0.0525$ ).

Table 6: Treatment method-wise prevalence of intestinal parasites

S.N.	Methods	No. of respondents	positive cases	Positive %
1	Direct taking medicine	99	27	27.27
2	Traditional method	26	11	42.31
3	Consulting doctor	25	3	12.00
4	Total	150	41	27.33

### 4.2.5 Prevalence of Intestinal Parasites on the basis of Food habit

Out of total sample examined, 134 were non-vegetarian and only 16 were vegetarian. Among 134 non-vegetarian, 38 (28.36%) were infected whereas 3(18.75%) were infected among vegetarian, which showed the high risk of infection among non-vegetarian. The distribution of the parasite between vegetarian and non-vegetarian was not significant ( $\chi^2=0.269, df=1, p\text{-value}=0.604$ ).

Table 7: Prevalence of Intestinal Parasites on the basis of Food habit

S.N.	Sex	No. of respondent	Vegetarian			Non-vegetarian		
			No.	Infected	%	No.	Infected	%
1	Male	73	12	3	25.0	61	13	21.31
2	Female	77	4	0	0	73	25	34.25
3	Total	150	16	3	18.75	134	38	28.36

#### 4.2.6 Hand washing wise Prevalence of Intestinal parasites

Among 150 samples, the prevalence of parasitic infection was found to be maximum 12 (42.86%) in those who used soil and water as cleaning agent to clean hands and minimum 23 (21.50%) in those people who used soap and water as cleaning agent. There seems significant difference in the prevalence of the intestinal parasites according to the different agent for cleaning hands ( $\chi^2=6.445$ ,  $df=2$ ,  $p\text{-value}=0.0399$ ).

Table 8: Hand washing wise Prevalence of Intestinal parasites

S.N	Agent	Observation no.	Positive cases	positive %
1	Only with water	15	6	40.00
2	With soil and water	28	12	42.86
3	With soap and water	107	23	21.50
4	Total	150	41	27.33

#### 4.2.7 Prevalence of Intestinal Parasites on the basis of water-treatment method

Out of total stool sample examined, the prevalence rate was found maximum 35 (85.37%) among those people who directly drink water without treatment and lowest 6(14%) in those who drink water after filtration while respondent drinking boiled water didn't have any infections. There was significant difference in the prevalence of intestinal parasites on the basis of water treatment method ( $\chi^2=14.392$ ,  $df=2$ ,  $p\text{-value}=0.00075$ ).

Table 9: Prevalence of Intestinal Parasites on the basis of water-treatment method

S.N.	Water treatment method	Total respondent	Positive cases	Positive %
1	Boiling	10	0	0.00
2	Filter	48	6	14.63
3	No treatment	92	35	38.04
	Total	150	41	27.33

## 5. DISCUSSION

Intestinal parasites are present throughout the world in various degree of prevalence and are the major health problems in areas where there is overcrowding, poor environmental sanitation and personal hygiene practice especially in developing countries (WHO, 2010). Soil transmitted helminth infections caused by *Ascaris*, *Trichuris* and hookworms are most prevalent and more than two billion people are estimated to be affected with these parasites (WHO, 2012). Intestinal parasites are responsible for much morbidity among rural population of rural communities (Sinniah *et al.*, 2012).

The overall prevalence of intestinal parasites in the present study was found to be 27.33% which is slightly similar to the result of Golia *et al.* (2014), Mohammed *et al.* (2010), Mbae *et al.* (2013), Oli (2016), Yadav (2014), Ammoura (2010), Alousefi *et al.* (2011), Tiwari *et al.* (2013) and Shrestha and Maharjan (2013) who reported 26.74%, 27.2%, 25.6%, 27.48%, 28.5%, 30.9%, 31.13% and 27.67% from Banglore, Al-asha, Saudi Arabia, Kenya, Dang district, Nepal, Saptari district, Nepal, Jordan, Yemen, Dadeldura district, Nepal and Bhaktapur, Nepal respectively who have also recorded the same prevalence with minor differences. In contrast study done by Rai *et al.* (2001), Shrestha (2001), Rosino *et al.* (2009), Florez *et al.* (2012), Alamir *et al.* (2013), Kirwan *et al.* (2009), Jaroli *et al.* (2012), Kunup and Hunjan (2010), Al-Mekhlafi *et al.* (2016) Rai *et al.* (2011), Bhandari *et al.* (2011) who reported 76.4%, 81.94%, 84.2%, 84%, 77.9%, 50%, 51.78%, 61.6%, 54.8%, 43.3% and 40% from Achham district, Lalitpur district, Terai, Nepal, Coli, Colombia, Ethiopia, Nigeria, Udayapur district, India, Lucia, Yemen, Sukumbasi Basti, Kathmandu, Kathmandu valley respectively have recorded high prevalence than present study. These differences might be due to the place and time differences of the study, health, awareness, education and living standard of people.

The present study recorded higher prevalence as compared with the study of different authors such as Tandukar *et al.* (2013), Bhattachan *et al.* (2015), Bhandari *et al.* (2015), Tandukar *et al.* (2015), Jaiswal *et al.* (2014), Pandey *et al.* (2015), Khanal *et al.* (2011), Adedayo *et al.* (2004), Frydas *et al.* (2004), Rayapu *et al.* (2014), Chakman *et al.* (2000) who reported 16.7%, 23.3%, 22.68%, 16.5%, 11%, 17.6%, 21.4%, 10.47%, 18.02%, 15.55% and 18.53% respectively from Lalitpur, Nepal, Chitwan district, Kathmandu valley, Bharatpokhari, VDC, Kaski, Northern-Kathmandu, Katmandu valley, Dang, Deukhuri, Dominica, Greek, Andhra Pradesh, India and Bharia tribe of India respectively. The high prevalence of the intestinal parasites may be attributed to the lack of health education (Rasshid *et al.*, 2011), hand washing behavior (Karunaithas *et al.*, 2011; Sah *et al.*, 2016), farming profession (Tandukar *et al.*, 2013) and non-vegetarian food habit (Pandey *et al.*, 2015). Most of the vegetable farmer use human excreta as manure which might be the potential source of the infection since farmers go to the farm to tender vegetables.

Among the Meche people the most prevalent intestinal parasite recorded was *Ascaris lumbricoides* (19.33%) which is similar to the work of Rai *et al.* (2001), Chongbang *et al.* (2016), Maharjan and Shrestha (2013), Khanal *et al.* (2011), Yadav (2014), Kupun and Hunjan (2010), Abera and Niber (2014), Salam and Azam (2017), Malla *et al.* (2004), Rai *et al.* (1986), Agrawal *et al.* (2012), Khadka *et al.* (2013), Tandukar *et al.* (2015), Estevez

*et al.* (1983), Singh *et al.* (2009) who also reported *Ascaris lumbricoides* as most predominant parasites respectively from hilly area in Western Nepal, Sunsari, Kathmandu, Kathmandu valley, Saptari district, Lucia, Northwest, Ethiopia, India, Sarlahi, Nepal, Birgunj city, Teaching Hospital, Kathmandu, Pokhara, Kathmandu, Remote village of Nepal and Biratnagar. The present study confirmed that *Ascaris lumbricoides* is the most common helminth parasites among the Meche community of Jalthal, VDC, Jhapa which may be due to the water contamination with helminth eggs in the locality, inadequate agent for washing hand after defecation and low economy of poor farmers who usually work bare foot in farm.

The present study revealed more prevalence of the helminth than protozoan parasites. The findings of this study matched with results of the Malla *et al.* (2004), Chongbang *et al.* (2016), Golia *et al.* (2014), Abossie and Mohammed (2014) who also reported helminth parasites are more prevalent than protozoan parasites respectively from Sarlahi, Nepal, Sunsari, Bangalore and Southern Ethiopia. Other helminthes recorded in present study are Hookworm (2.67%), *Taenia solium* (2.67%) and *Trichuris trichiura* (2%). These parasites were also reported by the Nduka *et al.* (2006), Bhattachan *et al.* (2015) and Frydas *et al.* (2004) from Abia state, Chitwan district, South Ethiopia and Greek respectively. This study presents that single infection was found to be maximum (92.68%) than double (7.32%) parasitic infection. Similar finding was reported by Shrestha *et al.* (2012), Khanal *et al.* (2011) and Abera *et al.* (2014). This might be indication of the condition of insanitary environment, poor living condition, lack of health education associated with poverty, lack of clean water and personal hygiene.

*Entamoeba coli* was the protozoan parasites recorded in this study which contribute to 2.67% of total infection, is generally non-pathogenic but sometimes it causes diarrhea and its prevalence could be due to faecal contamination of drinking water which in turn shows poor sanitary practices. Present study is in agreement to the work of Mukhiya *et al.* (2012), Dhanabal *et al.* (2014), Mordi and Ngwodo (2007) and Saksirisampant *et al.* (2006) who also reported *Entamoeba coli* as most common protozoan in their study respectively from school children of Kathmandu, South Chennai, India, Edo state and Central Region of Thailand.

Gender-wise parasitic infection rate was found slightly more common in females (32.47%) than in males (21.91%) which agrees with the findings of Sinniah *et al.* (2012), Sherchand *et al.* (2010), Khadka *et al.* (2013), Bhandari *et al.* (2011), Bhattachan *et al.* (2015), Uga *et al.* (2004), Ntulume *et al.* (2017), Gyang *et al.* (2017), Amer *et al.* (2015) who reported slightly higher prevalence of parasites in female than in males respectively from Perak, Malaysia, school going children of Kathmandu, children in Pokhara, elsewhere in Nepal, Chitwan, Nepal, Kanti children Hospital Kathmandu, Bhusenyi district, Western, Uganda, African urban slum in Nigeria and North-west, Saudi Arabia. The risk factors for high prevalence of parasites among females include lower educational status, their involvement in child care as well as other household works (Shrestha *et al.* 2012) and consuming raw vegetables.

Based on the age of the people included in study, the infection was found higher in the middle age group 21-40 (47.22%) and lowest among the elder people (20.51%) which is

slightly similar to the report of Nduka *et al.* (2006). The people showing higher prevalence are those involved in farming and house hold activities. On the other hand people of (1-20) years showed high prevalence (35.56%) compared to elderly people. This age group include school children who are generally highly infected as a result of age and lack acquired immunity (Mbanugo and Onyebuchi, 2002; Adeyeba and Akinlabi, 2002). The present study is different from the reports of studies done by Ojuronbe *et al.* (2014), Pandey *et al.* (2015) and Rayapu *et al.* (2014) who showed high prevalence among the school going children under 15years of age. However high prevalence was recorded among elderly people in the study of Oli (2016) which is not in the agreement of the finding of the present study.

The finding of the present study showed the higher prevalence (28.36%) among the non-vegetarian and lower among vegetarian (18.75%) which is supported by the study of Pandey *et al.* (2015), Ara (2014), Sah (2012) , Oli (2016) who reported higher prevalence of parasites among non-vegetarian respectively from Northen-Kathmandu, Chorni VDC, Parsa, Rajhena, VDC, Banke and Pawanagar VDC, Dang. This might be due to consuming infected raw and improperly cooked meat (Oli, 2016). However the study done by Rai *et al.* (2008) showed high prevalence among the vegetarian patient of cataract surgery at the eye camps in the rural hilly areas of Nepal.

In the present study, the infection was found higher (42.86%) among those people who use soil and water as hand cleaning agent which seems similar to the result of Yadav (2014), Regmi (2012), Yadav and Pakash (2016) who reported higher prevalence among those using soil/ mud and water as hand cleaning agent. This study reflects that the highest percentage (38.04%) of positive cases of parasitic infection was found in those who used direct tube well water without any treatment which is supported by the finding of the Yadav and Praksh (2016). These might be due to the lack of health awareness and health education. This study highlights that prevalence was found maximum (30.77%) in the people having live stock and domestic mainly pig, duck, cow, goat, hens, which is supported by the results of Oli (2016), Yadav (2014) who also reported high prevalence in people having live stock and domestic animals. This might be due to the insufficient sanitary conditions, lack of personal hygiene and having nearby live stock and domestic animals.

Most of the respondent (70%) in this study are unaware about the intestinal parasitic infection among them 38.04% were infected by the intestinal parasitic infection where as of 30% aware people, 20% were infected. Most of the people of the Meche community do not have any knowledge of means and modes of the parasitic disease transmission. This finding matched with the result by Pandey *et al.* (2015), Sah (2012) and Yadav (2014). The finding of the present study shows that knowledge of the parasitic infection is very poor in this community due to lack of public health awareness and health education. The present study revealed that most of the people who believe in traditional method such as Dhama, Jhakri, were highly infected (42.31%) and people consulting doctors show lower prevalence (12%) of the parasitic infection which is supported by the results of Sah (2012) and Oli (2016). It is due to the lack of knowledge, attitude, cultural and behavioral variations.

## 6. CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

The present study was carried out to observe the prevalence of intestinal parasites in Meche community of Jalthal VDC of Jhapa district in relation to their socio-economic status. Out of 150 stool samples investigated, 41 (27.33%) were positive in which positive rate was higher in females (32.47%) than that of males (21.91%). Age wise prevalence of parasites was found highest among the adult age group 21-40 (47.22%) followed by age group 1-20 (35.56%) and elderly people above 41(20.51%) years of age respectively. Altogether 5 species of the intestinal parasites were detected, the most prevalent was *Ascaris lumbricoides* (19.3%) followed by Hookworm (2.67%), *Taenia solium* (2.67%), *Entamoeba coli* (2.67%), which was only protozoan recorded, and *Trichuris trichiura* (2%). The present study demonstrated that overall prevalence of helminthes (97.56%) was higher than that of protozoan (9.75%). The study revealed that single infection (92.68%) was common than double infection (7.32%) among Meche people of Jalthal VDC.

Mostly the unaware respondents are more infected (30.48%) than aware (20%). The people who used soil and water as hand cleaning agent, people who drink direct tube well water and who believed in traditional method of treatment showed high infections of 42.86%, 38.04% and 42.31% respectively. This main reason behind all these activities is low socio-economic status and unawareness of the people towards parasitic infections. In the present study the prevalence of intestinal parasites was slightly higher (32.59%) in student. This is due to the negligence of school aged students towards their personal hygiene, less immunity and lack of health education. The present study has revealed high prevalence of intestinal parasites among Meche people of Jalthal VDC. The infection is attributable to the individual hygiene behavior and agriculture related risk factors. Lack of health education program, poverty, lack of awareness, poor environmental sanitation, habit of consuming raw and uncooked food, drinking unsafe water are some of the predisposing factors reflected by this study as cause of parasitic infections. Therefore the public health interventions and control program including treatment of infected individuals, education on personal and environmental hygiene, school-based awareness program, development of the health care facilities and other are required to minimize the risk of intestinal parasitic infections in such communities.

### 6.2 Recommendations

Based on conclusion, it was recommended that:

- Public health education in the Meche community should be made compulsory for awareness of the people.
- People should be encouraged for sanitary improvements including personal hygiene and environmental sanitation.
- Pure and safe drinking water facility should be made easily accessible for the tribal communities.



- Basic health education program should be conducted time to time in communities and school for raising awareness towards parasitic infection, prevention and control.
- The research work on the prevalence of intestinal parasites in relation of socio-economic status and prevention should be encouraged.

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## PHOTOGRAHS



Photo 1: Examining under microscope



Photo 2: Slide preparation



Photo 3: With Meche people



Photo 4: Meche Basti

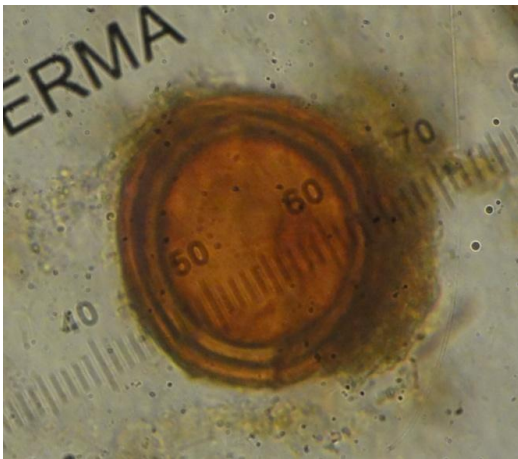


Photo 5: Egg of *Ascaris lumbricoides*(48 $\mu$ m)

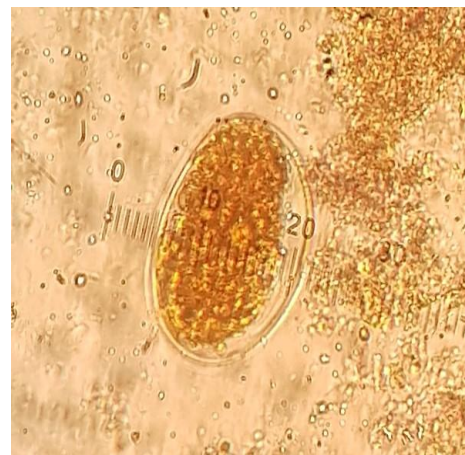


Photo 6: Egg of Hookworm(55 $\mu$ m)



Photo 7: Egg of *Trichuris trichiura* (50µm)

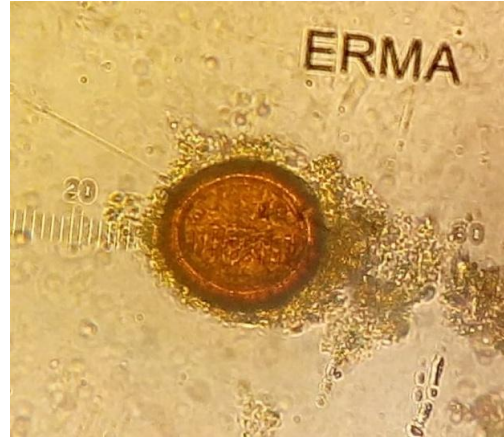


Photo 8: Egg of *Taenia solium* (35µm)

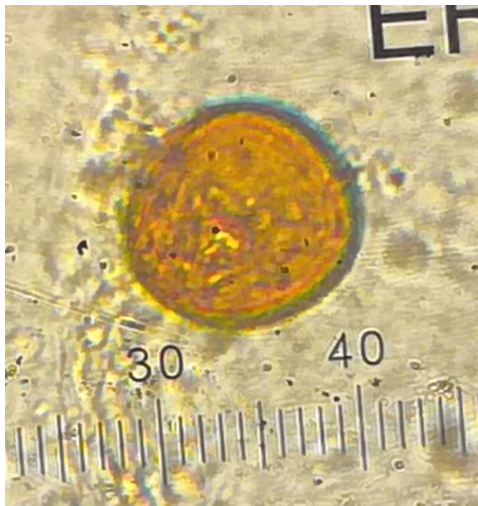


Photo 9 : Egg of *Entamoeba coli* (25µm)

## ANNEX- 1 QUESTIONNAIRES

1. S.N.

Name: \_\_\_\_\_ Age: \_\_\_\_\_ Sex: M/F \_\_\_\_\_ Education: \_\_\_\_\_

Address: \_\_\_\_\_ No. of family: \_\_\_\_\_

2. What is your main occupation?

Teacher \_\_\_\_\_ Farmer \_\_\_\_\_ Other.....

- What is your main source of income in your family?  
.....

- How many productive members in your family?  
.....

3. Drinking water

Tap \_\_\_\_\_ Tube well \_\_\_\_\_ River \_\_\_\_\_ Spring \_\_\_\_\_

4. How do you use water for family?

Direct \_\_\_\_\_ Boiling \_\_\_\_\_ Filtration \_\_\_\_\_

5. Where do you defecate?

Toilet \_\_\_\_\_ Open place \_\_\_\_\_ River bank \_\_\_\_\_

6. Do you wash your hand before meal?

Yes/No

If yes, what type?

With soap \_\_\_\_\_ Only water \_\_\_\_\_ With soil \_\_\_\_\_ Other.....

7. Do you cut your nail regularly?

Yes/No

If yes, when do you cut? Once a week \_\_\_\_\_ Twice a week \_\_\_\_\_ more.....

8. Do you have any domesticated animals?

Yes/No

If yes, what type?.....

9. What type of food habit you have?

Vegetarian \_\_\_\_\_ Non-vegetarian \_\_\_\_\_

If non-vegetarian, which meat frequently you take?

Pork \_\_\_\_\_ Chicken \_\_\_\_\_ Buffalo \_\_\_\_\_ Mutton \_\_\_\_\_ Fish \_\_\_\_\_ Beef \_\_\_\_\_

How do you prepare your meat to eat?

Boiled \_\_\_\_\_ Well cooked \_\_\_\_\_ Half cooked \_\_\_\_\_ Sekuwa masu \_\_\_\_\_

10. How do you clean your vegetables and fruits?

Rubbing on clothes \_\_\_\_\_ Tap/well water \_\_\_\_\_ Without washing \_\_\_\_\_

11. Have you suffered by diarrhea /dysentery worm?

If yes, when?

1 week before    1 month before    6 month before    Now    Don't know

12. When you taken deworming tablet?

Yes/No

If yes: 1 week before    2 week before    1 month before    6 month before

13. Do you know the cause of diarrhea?

Yes/No

If yes, what are they?.....

14. Do you know the causes of worm infection?

Yes/No

If yes, what are they?.....

15. How do you treat in case of infection?

Traditional methods                      By taking medicine                      By consulting  
doctors

16. Do you know the methods of prevention of worm infection?

Yes/No

If yes, what are they?.....

17. Do you use human excreta as manure?

Yes    No



## ANNEX- 2

Identification keys for eggs and cyst of parasites found:

Name of parasites	Measurement of eggs and cyst (in $\mu\text{m}$ )		Morphological characters	References values (Arora and Arora, 2012; Chatterji, 2009)
	Length	Breadth		
<i>Trichuris trichiura</i>	50	22	Double shelled brown in color, barrel shape with two mucous plugs.	50-55 $\mu\text{m}$ $\times$ 22-25 $\mu\text{m}$
<i>Ascaris lumbricoides</i>	-	48	Round or oval in shape, brownish in color, surrounded by thick shell.	60-75 $\mu\text{m}$ $\times$ 40-50 $\mu\text{m}$
<i>Taenia solium</i>	-	35	Spherical, brown in color, contain onchosphere.	31 $\mu\text{m}$ -43 $\mu\text{m}$
Hook worm	55	35	Oval in shape, surrounded by transparent cell membrane, contain segmented ovum.	55-65 $\mu\text{m}$ $\times$ 35-40 $\mu\text{m}$
<i>Entamoeba coli</i>	-	25	Cyst is colorless, round or oval shape, surrounded by smooth cyst wall.	15 $\mu\text{m}$ $\times$ 25 $\mu\text{m}$