PREVALENCE OF INTESTINAL HELMINTH PARASITES OF CHICKEN (*Gallus gallus domesticus* Linnaeus,1758) IN LALITPUR DISTRICT, NEPAL

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In partial fulfillment of the requirement for the award of the degree of Master of Science in Zoology with special paper in Parasitology.

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DECLARATION

I, hereby, declare that the work presented in the thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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RECOMMENDATIONS

This is to recommend that the thesis entitled "**Prevalence of Intestinal Helminth Parasites of Chicken (*Gallus gallus domesticus* Linnaeus, 1758) in Lalitpur District, Nepal**" has been carried out by Tasneem Mujahid for the partial fulfillment of Master's degree of Science in Zoology with special paper in Parasitology. This is his 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 institution.

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On the recommendation of supervisor Mr. Janak Raj Subedi, this thesis submitted by Tasneem Mujahid entitled "**Prevalence of Intestinal Helminth Parasites of Chicken (*Gallus gallus domesticus* Linnaeus, 1758) in Lalitpur District, Nepal**" is approved for the examination and submitted to Tribhuvan University in partial fulfillment of the requirement for Master's Degree of Science in Zoology with special paper in Parasitology.

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This thesis work submitted by Tasneem Mujahid entitled "**Prevalence of Intestinal Helminth Parasites of Chicken (*Gallus gallus domesticus* Linnaeus, 1758) in Lalitpur District, Nepal**" has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper in Parasitology.

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LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
OECD	Organization for Economic Cooperation and Development
AFA	Alcohol Formalin Acetic Acid
DPX	Dibutylphthalate Xylene
ADPCD	Animal Disease and Parasite Control Division
CDZ	Central Department of Zoology
gm	Gram
m	Meter
ml	Milliliter
et al.	And his associates
i.e.	That is
km^2	Kilometer square
P value	Probability value
sp.	Species

ABSTRACT

Gallus gallus domesticus is the most common domestic fowl that harbours many intestinal helminth parasites.A study was conducted to investigate the prevalence of gastro intestinal helminth parasites of local chicken, *Gallus gallus domesticus* in Lalitpur district of Nepal. A total of 93 intestine and 32 stool samples of local chicken were collected from different places of Lalitpur district. The collected samples were examined thoroughly for the presence of helminth parasites in the form of adult or egg. The present study showed that only 40% of all the poultry examined were infected. Five species of nematodes , one species of cestode and four unidentified species were recorded. The highest prevalence rate was found with *Heterakis gallinarum* (22.4%) followed by *Capillaria* species (16%), *Ascaridia galli* (10.4%) and *Raillietina tetragona(*4%). The prevalence of unidentified species was (4.8%). Statistically there was a significant difference in the prevalence of helminth species ($\chi^2 = 33.83, p < 0.05, \alpha = 1$) with high prevalence of nematode (*Heterakis gallinarum*). The infection was more in free range chicken (70%) as they were reared in unhygienic environment with higher risk of parasitic infection due to their feeding habit. The intestine, collected from slaughter house were with least infection as they were bought from poultry farm where chicken were reared in hygienic environment with medication. Thus statistically there was significant difference in the prevalence rate of helminth parasites in free range chicken and poultry chicken ($\chi^2 = 22.055, p < 0.05, \alpha=1$).The present study showed that the free range chicken were mostly infected by one or more helminth parasites.

1. INTRODUCTION

1.1 Background

A parasite is an organism that lives in or on and takes its nourishment from another organism called host. The parasite derives benefits and the host gets nothing in return but always suffers some injury. The host at the same time, offers some resistance in the injury done by the parasite and there may be some adaptation (tolerance) between the parasite and the host. Intestinal parasites are those that are found or live in intestinal tract. Intestinal parasites includes both helminths and protozoans. Helminths is a polyphyletic group of morphologically similar organisms consisting of members of the following taxa: monogeneans, cestodes(tapeworms), nematodes(roundworms) and trematodes(flukes). Parasitism is the association in which the parasite is metabolically dependent to a greater or lesser extent to the host. Helminthology is the study of parasitic worms and their effect on hosts. Gastro- intestinal parasites are, however , the most prevalent and most devastating parasites affecting chicken productivity. According to Muchadeyi *et al.,* (2004) and Mwale and Masika (2009) village chicken are raised mainly under the free range (scavenging) product system, with partial or no housing and this predisposes the chicken to disease and parasites especially helminths (Swaton *et al* ., 2003).

A domestic fowl or chicken *(Gallus gallus domesticus*), belonging to the family Phasianidae, is a sub species of Red Jungle Fowl. It is one of the most common and domesticated birds than any other species in the world. Human keep chickens primarily as a source of food, consuming both their meat and their eggs. A zoo of parasitic worms can be found in chicken flocks. Worms find cozy places to stay in the crop, gizzard, intestine, caecum, windpipe and even the eyelids (Gauthier and Ludlow 2013). On the basis of their site of location helminths are of different types. The worm which are found in caecum of large intestine are called caecal worms .e.g. *Heterakis* while which are found in eye are called eye worm e.g .(*Oxyspirura mansoni*). Gape worms are found in trachea (*Syngamus trachea*). These worms are also called “red- worm” or “forked-worm” and birds infected with gape worm show “open mouth breathing characteristics”. Round worm (*Ascaridia*) and tape worms (*Raillietina*) are found in intestine while thread worm

(*Capillaria*) is found in crop or oesophagus (Janquera 2017). The eggs and immature stages of many parasitic worms can live outside of the chicken host for a long time, possibly several years. Some parasitic worms spend part of their life cycle in other creatures such as earthworms, insects, slugs or snails. Chicken pick up worms by eating dirt or litter contaminated with worms eggs or by eating small creatures carrying immature stages of worms.

Parasitic infection in chicken is the major problem of developing country like Nepal which leads to economic loss of the country. Domestic fowls are more often infected due to unhygienic management practices, malnutrition, lack of veterinary supervision and also the complicated life cycle of the parasites. Chicken infected with parasites show retarded growth, decreased egg production, reduced weight gain, significant haemoglobin depression (Nair and Nadakal, 1981), villous atropy, catarrhal enteritis, granuloma formation in duodenum, desquamation of villi and submucosal glands congestion, inflammatory reaction and vacuolation of epithelial cells (Kurkure and Ganorkar 1998). Actually the parasites can't be totally eradicated but their number can be controlled. So to prevent such infection, first of all, detail study of those parasites should be done including their life cycle and medication should be followed.

According to the report by the Organization for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization of the United Nation (FAO) indigenous chicken meat production was 95.8 million and broiler meat production was 108.7 million tonnes in 2014. Indigenous chicken meat production in Nepal was 40.3 thousand tonnes in 2012 . According to FAO, it was seen that in 2014 there was increase in poultry meat production by 1.7 percent and was expected that outcome will expand by more than two percent as it might approach to 98 million tonnes in 2015 (GPT, 2014).

1.2 Objectives of the study

Considerable work has been done on the intestinal parasites of chicken in various parts of country in the past. Identification of any new helminth species in this research was the success of all parasitologists who have worked on the same topic in Nepal. The present research work was also helpful to carry out future research programmes on helminth in Nepal. So keeping in

view the importance of these parasites in chickens, the present study was designed with the following aims:

1.2.1 General Objective

To study the prevalence of intestinal helminth parasites in chicken of Lalitpur district.

1.2.2 Specific Objectives

i. To identify the gastrointestinal helminth of poultry based on morphology.
ii. To determine the prevalence of gastrointestinal helminth parasite infections in local chicken.
iii. To compare the significant difference in the prevalence rate of poultry farm chicken and free range chicken.

1.3. Rational of the study

Gastrointestinal helminths infection interfere with host metabolism, resulting in poor feed utilization , reduced growth rate and size and death in severe cases. As a result there is a huge loss in economy of an individual or even of a country. So this study may help to reduce the infection in chicken by two ways. One of the ways is identification of parasites and medication and the other way is bringing change in management of chicken by providing the hygienic environment, healthy feed and veterinary supervision. The identification of new helminth species in Nepal will assist the future research work on helminth parasites.

2. LITERATURE REVIEW

2.1 Nematodes

Nematodes have been identified globally and at national level in chicken. While going through research paper published on prevalence of helminthes parasites of chicken globally and nationally, I found the following outcome. In Nepal there is no enough research work on the very topic but worldwide numerous works have been done to layout the helminth parasites of chicken.

2.1.1 *Ascaridia galli*

Ascaridia is a nematode parasite of intestine that causes ascariasis in chickens, guinea fowl, turkeys, geese and other wild birds worldwide. It lives in the small intestine. Adult worms are semi-transparent; males measure 50-76 mm, while female worms are 72- 16 mm long. Their oral opening has 3 large lips and the esophagus has no posterior bulb. Rai (1988) and Shrestha (1990) reported *Ascaridia galli* in domestic fowl in Nepal. Mikail and Adamu (1998) studied gastro-intestinal parasites of local chicken in Sokoto Metropolis, Nigeria and reported the prevalence of *Ascaridia galli* as (8.66%). Anand *et al.*, (2008) reported *Ascaridia galli* (91.4%) in Bangalore India. Dawet *et al.*, (2012) found highest prevalence of *Ascaridia galli* (19.21%) in Jos, Plateau State, Nigeria. Adang *et al.*, (2014) found less prevalence of *Ascaridia galli* (0.7%) in Gombe Main Market, Gombe State Nigeria. Wongrak *et al.*, (2014) reported high prevalence of *Ascaridia galli* (96.2%) in free range chicken in Lower Saxony, Germany.

2.1.2 *Heterakis gallinae*

Heterakis is a caecal worm found in caecum of large intestine of chickens, guinea fowl, turkeys, ducks, and geese. These are small white worms with 3 lips in the mouth and the eosophageal bulb with a valvular apparatus. Its clinical effects are minimal, but heavy infections do cause thickening of caecal mucosa, petechial haemorrhages, and hepatic granulomas. Shrestha (1990) and Rai (1988) reported *Heterakis gallinae* in Nepal. Mukaratirwa and Khumalo (2010) studied the helminth parasites in South Africa and found *Heterakis gallinae* (80-94.4%) as the most

prevalent nematode. Mikail and Adamu (1998) also found *Heterakis gallinae* (28.66%) the most prevalent nematode. Anand *et al.,* (2008) studied the gastro-intestinal parasites in poultry in and around Bangalore, India and found *Heterakis gallinae* (4.3%). Dawet *et al.*, (2012) recorded least prevalence of *Heterakis gallinae* (0.56%) in Plateau State, Nigeria. Wongrak *et.al.*, (2014) found *Heterakis gallinae* (98.5%) the most prevalent nematode in the intestine of free range chicken in Lower Saxony, Germany.

2.1.3 *Capillaria* species

Capillaria is a thread worm found in crop and oesophagus of hen. These are small, hair like worms that include: *Capillaria annulata, C. contorta, C. caudinflata, C. bursata, C. obsignata,* and *C. anatis. C. annulata* and *C. contorta* are found in the crop and esophagus. *Capillaria caudinflata, C. obsignata, C. bursata* and *C. anatis* which are found in the intestine. The *Capillaria* species are cosmopolitan. In Nepal *Capillaria* spp was identified in intestine of chicken by Animal Disease and Parasite Control Division(ADPCD) in 1982. This parasite was also reported from the intestine of other animals like buffalo, resus monkey and cat. However Mukaratirwa and Khumalo (2010) reported three species of *Capillaria* in the gastro-intestinal tract of hen. They were *Capillaria obsignata, Capillaria annuletus* and *Capillaria contortus*. Wongrak *et al.*, (2014) also reported three species of *Capillaria*: *Capillaria obsignata, Capillaria bursata* and *Capillaria caudinflata*.

2.1.4 Other Nematodes : Anand *et al.*, (2008) studied the gastro-intestinal parasites of poultry in and around Bangalore, India and reported *Subulura* species (4.3%). Mukaratirwa and Khumalo (2010) reported *Subulura suctoria* and *Gongylonoma ingluvicola* (43.86%) as gastro-intestinal tract parasite in free range chicken in Kwazulu Natal province of South Africa.

2.2. Cestodes

2.2.1 *Raillietina*

Raillietina is a tapeworm found in intestine. Rai (1998) identified *Raillietina cesticellus*, *Raillietina tetragona* and *Raillietina echinobothrida* in the intestine of hen in Nepal. Shrestha (1990) identified only two species of *Raillietina*, they were *Raillietina echinobothrida* and *Raillietina tetragona* in hen of Nepal . Bhure *et al.*, (2013) reported high prevalence of cestode compared to nematode parasites and found the prevalence of *Raillietina* species (73.95%) in Latur District, India. Tasawar *et al.*,.(1999) reported high prevalence of *Raillietina tetragona* (51.66%) in the domestic fowl in Pakistan. Anand studied gastro- intestinal parasites of poultry in and around Banglore, India. He found the prevalence of two cestodes *Raillietina tetragona* (80%) and *Raillietina echinobothrida* (20%). Adang *et al.*, (2014) reported four species of *Raillietina* in the Gombe State , Nigeria. They were *Raillietina tetragona* (34.7%), *Raillietina cesticellus* (21.3%), *Raillietina echinobothrida* (25.3%) and *Raillietina magninumida*(3.3%). *Raillietina tetragona* is usually buried in the intestinal mucosa and is associated with weight loss and decreased egg production. *R. echinobothrida* is associated with catarrhal enteritis, nodular formation, and granulomas, and causes nodular disease. *R. cesticillus* causes emaciation and degeneration of intestinal villi.

2.2.2 *Amoebotaenia cuneata* :

Shrestha (1990) and Rai (1988) both reported the cestode in *Gallus gallus domesticus* in Nepal. Adang *et al.*, (2014) reported the prevalence of *Amoebotaenia cuneata* (4%) in domestic chicken slaughtered at Gombe Main Market, Gombe State, Nigeria.

2.2.3 *Cotugnia digonopora*

Shrestha (1990) identified *Cotugnia digonopora* in the intestine of *Gallus gallus domesticus* in Nepal. Bhure *et al.*, (2013) reported high prevalence of cestodes compared to nematode parasites and found 72.9% prevalence of *Catugnia* species.

2.2.4 *Fernandezia kantipuri* :

Shrestha (1990) and Rai (1988) identified this cestode in the intestine of hen in Nepal.

2.2.5 *Other Cestodes*

Adang *et al.*, (2014) reported *Hymenolepis carioca* (12.0%) in the gastro-intestinal tract of domestic chicken slaughtered at Gombe Main Market, Gombe State, Nigeria. Bhure *et al.*, (2013) reported the prevalence of *Davainea* species (72.39%) in chicken in India.

2.3 Tremetode

2.3.1 *Catatropis verrucosa*

Catatropis is an intestinal fluke of the family Notocotylidae. In Nepal this species was identified by Rai (1988) and Shrestha (1990) in the intestine of hen. Suhardono and Gatot (2002) also reported *Catatropis* species in poultry which were reared in rice growing field in West Java.

2.3.2 *Echinostoma revolutum*

Echinostoma revolutum is a fluke of family Echinostomidae found in intestine of hen and also can be a parasite in human. In Nepal this fluke was identified by Shrestha (1990) and Rai (1988) in the intestine of hen. This species was also identified by Suhardono and Gatot (2002) in poultry reared in rice growing environment in West Java.

2.3.3 *Prosthogonimus ovatus*

It is actually an oviduct fluke of hen but also reported in caecal area. Rai (1988) reported the very fluke in hen. Suhardono and Gatot (2002) also reported *Prosthogonimus* species infecting hen reared in rice growing environment in two district of Sukabuni and Serang, West Java.

2.3.4 *Other Trematode*

Suhardono and Gatot (2002) reported few more species of trematode like *Cotylurus* species, *Hypoderaeum* species, *Philopthalmus* species, *Psilochasmus* species, *Paramonostomum* species and *Apatemon* species in poultry reared in rice growing environment in two districts of Sukubuni and Serang, West Java.

3. MATERIALS AND METHODS

3.1. The Study Area

The study was carried out in Lalitpur Sub Metropolitan City which is one of the major cities of Nepal located in the south- central part of Kathmandu valley. It is best known for its rich cultural heritage particularly its tradition of arts and crafts. At the time of 2011 Nepal Census it had a population of 226728 in 54748 individual households. The city has an area of 15.43 sq. km and is divided into 22 municipal wards. It is situated at 27° 40'N 85° 19'E/27.66°N 85.317°E. Climate is characterized by relatively high temperature and evenly distributed precipitation throughout the year. Many people in this area are involved in poultry farming in small as well as large scale. The district experiences a sub- tropical type of climate. Summers are very hot and winters are very cold. The district experiences four distinct seasons. Summers last from March to mid- June. Monsoon season last from mid- June to September Winter last from December to February. .

Figure 1: Map of Nepal showing Lalitpur district

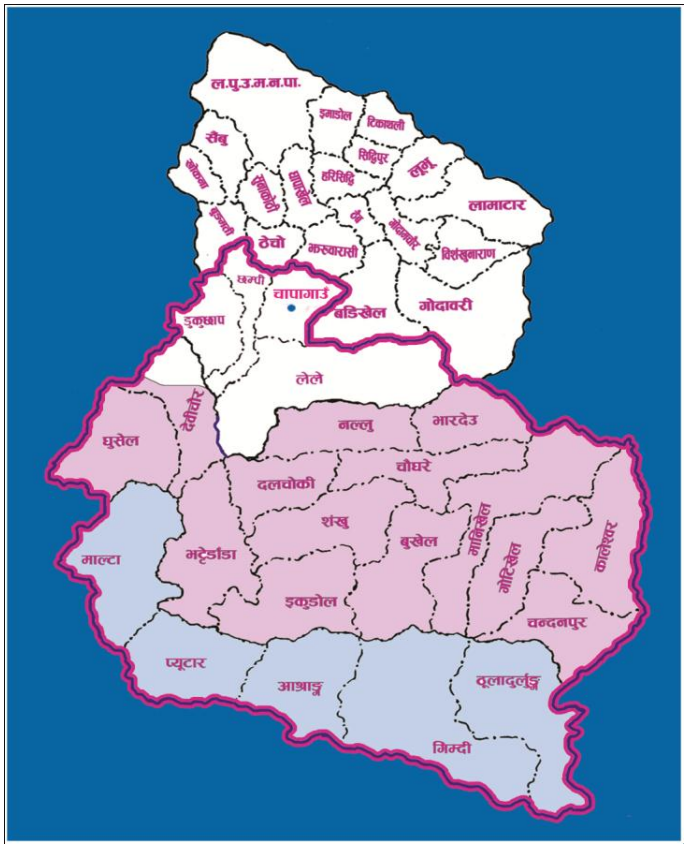

Figure 2: Map of Lalitpur district (Study Area)

3.2. Materials Required

i. Compound Microscope ii. Collecting Vials

iii.Wooden Spoon iv. Tooth Pick

v. Glass Slides vi. Cover Slips

vii. Cotton viii. Gloves

ix. Foreceps x. Stickers

xi. Dustbin xii. Polythene Bags

3.3. Chemicals Required

i. Potassium dichromate (5%) ii. Normal saline

iii. Lugol's Iodine Solution iv. 70% Alcohol

iv. AFA Solution (Alcohol Formalin Acetic Acid)

v. Acid Alcohol

vi. DPX (Dibutyl Phthalate Xylene)

viii. Benzene

ix. Gower's Solution

3.4. Study Design

3.4.1 Extensive survey of literature review: Several articles, research papers and books related to intestinal helminth parasites of chicken *(Gallus gallus domesticus*) of national and international level were gone through.

3.4.2 Sample Size: A total of 125 samples (93 alimentary canals of freshly killed chicken and 32 stool samples from ground and fowl runs) were collected from Lalitpur District. Out of 93 alimentary canals 17 were collected from free range chicken and 76 were from slaughtered house i.e. from poultry farm chicken. These samples were collected from different places of Lalitput districts from February 2014 to March 2015 A. D.. The samples collected from different places of Lalitpur district were brought to the laboratory (at home) for examination of helminth parasites.

3.4.3. Sample Collection and Preservation: The faecal sample were collected in the sterile vials containing 2.5% potassium dichromate with the help of wooden stick. Potassium dichromate was used as preservative which helped to maintain the morphology of eggs and also prevented further development of helminth eggs. The alimentary canal were collected in polythene bag and were immediately brought to the laboratory for the examination. (Gurung 2016)

3.4.5 Sample Examination: Depending upon the convience, following methods were used for the identification of helminth parasites from collected samples.

a. Post Mortem Examination Method
b. Direct Smear Fecal Exam
c. Differential Floatation Method

a. Post Mortem Examination Method:

The collected parasites were examined in freshly killed condition because many parasites deteriorate after the death of the host. The alimentary canal was cut longitudinally from oesophagus to rectum including both caecal tubes. All worms visible to necked eye were removed using thumb forceps and brush (Fowler 1990). Cestodes were whole mounted for identification while nematode were fixed in glycerol jelly and observed under the microscope. The freshly collected helminths parasites were kept in normal saline before fixation. Permanent slides (whole mount slides) of parasites were prepared for their identification according to the method described by Cable (1975).

Trematode and Cestode whole mount procedure

Fixation: Flatworms were killed in flattened position . The best general fixative for this purpose was AFA (Alcohol Formalin Acetic Acid) solution. The fluke was kept under the coverslip or glass slides depending upon their size so that it could be pressed. Thread or rubber band was used to tie around the slide so as to keep the slides immovable .

Preparation of permanent slides of endoparasites

The fluke or cestodes were placed in between two slides. Then rubber band or thread was tied around the two slides so that the parasites may not be displaced . The large and muscular fluke were tied more by rubber . Such slides were kept in AFA for 20-24 hours. The time necessary for fixation depends upon the size of the parasites. Then the specimen were washed in running water for the removal of fixative. Then they were dipped into the prepared Gower's solution for 20-24 hours. After staining , the worms were washed carefully in water. Then they were dehydrated in graded alcohol i.e. 30%, 50%, 70%, 90% and 100% alcohol. After 70% alcohol the parasites were dipped in acid alcohol for removing extra staining. After acid alcohol , parasites were dipped in 70% alcohol to remove the effect of acid alcohol further and continue the process upto

absolute alcohol. After 100% alcohol methyl salicylate was used as a clearing agent so the materials were dipped for about 10-20 minutes , then materials were transferred to benzene for 10 minutes. Then the parasites were mounted on DPX (Rai 1998)

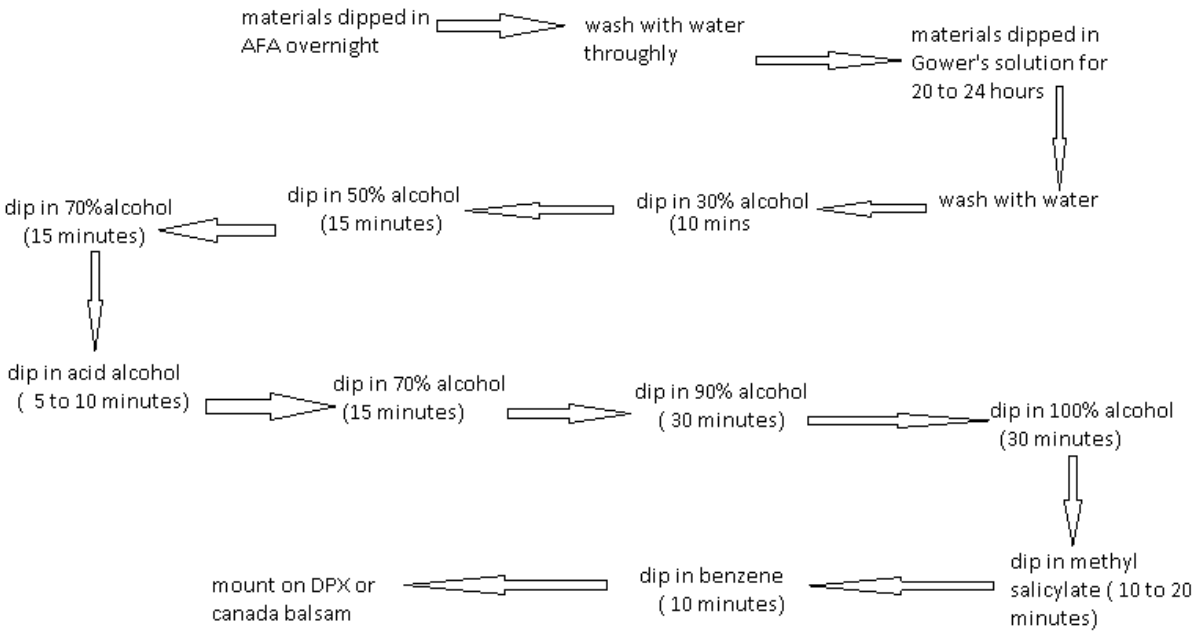

Figure 1: Flowchart showing steps of preparation of permanent slides of cestodes and trematodes

b. Direct Smear Fecal Exam

This method of examination was used for the observation of eggs, cysts and larva from the faecal matters. In this method small amount of feces was placed on a slide . A drop of normal saline or Lugol's solution was added to the feces and mixed thoroughly. Since we were looking for the helminth eggs , larva and cysts Lugol's solution or normal saline was used . Then the fecal materials was covered with cover slip. The cover slip was moved around until it laid flat. The

smear film was made thin so that the light from the microscope was able to pass through the sample in order for us to examine it. The slide was examined at 10X objective and again at 40X objective (Chatterji 2009 and Soulsby 1982)

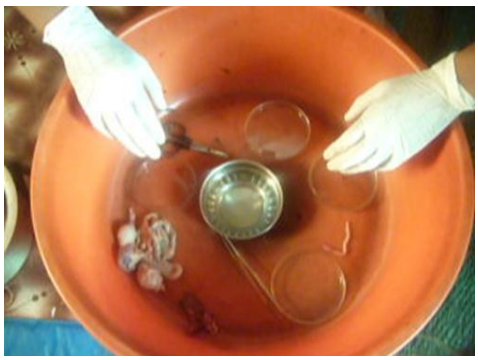

Photo 3: Intestine of hen ready to be dissected

Photo 4: Isolation of specimen recovered

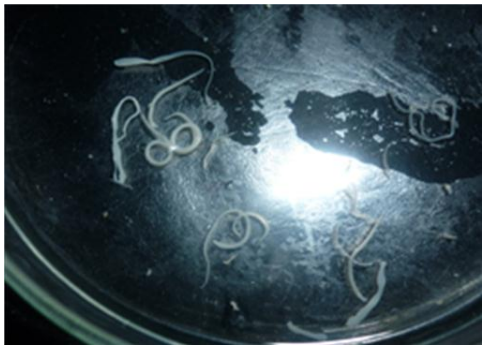

Photo 5: Collected specimen from the intestine

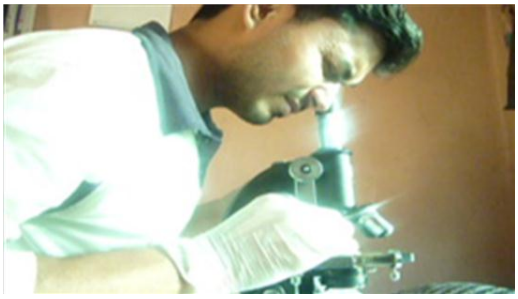

Photo 6: Examination of collected specimen

Photo 7: Specimen in AFA solution

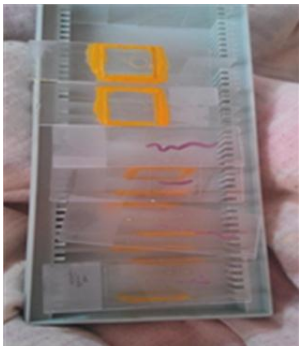

Photo 8: Permanent slides of collected specimen

c. Differential Floatation Method

Differential floatation method was used to separate the diagnostic products of endoparasitic organisms (egg, larva, oocysts and cysts) in the feces of animals by the use of suspension medium with a higher specific gravity than the parasite products (MAFF 1986). Parasite egg, cyst, and oocysts are concentrated on the surface of the medium because of their lighter densities .

The saturated salt solution of specific gravity 1.2 was prepared by allowing an excess of common salt to boil in a basin until a scum was formed on the surface. It was cooled and stored in a bottle leaving an excess of undissolved salt at the bottom. Four gram of fecal material was taken in a test tube and a few drops salt solution was added (Hansen and Perry 1994). It was then stirred with a glass rod or a small piece of stick so as to make an even emulsion. After that more salt solution (15 to 20 ml according to the capacity of the test tube used) was added till the test tube was nearly full, stirring was continued through the process . Any coarse matter, which float up, were removed without fear of removing any egg, as egg takes a long time(20 to 30 minutes) to come to the surface of the fluid. At this stage the test tube was placed on the level surface and the final filling of the test tube was done by means of a dropper until a convex meniscus was formed . A glass slide was carefully laid on the top of the test tube so that its center is in contact with the fluid. The preparation was allowed to stand for 20 to 30 minutes, after which the glass slide was quickly lifted, turned over smoothly so as to avoid spilling of the liquid and was examined under the microscope (Chatterji 2009 and Soulsby 1982).

3.4.6 Identification of Parasites

The parasites were identified by the study of their morphology under light microscopy using the identification keys described in annex (Soulsby 1892 and E.B.Cram 1936).

3.4.7 Data Analysis

Prevalence of infection of identified species in each area was calculated as the number of individual chicken infected by a specific helminth species at the time of study was divided by the total number of chicken examined multiplied by 100. Variation in the prevalence of gastrointestinal helminths in relation to helminth species and chicken type were analysed using

Chi- square statistics. In all cases $p < 0.05$ was considered indicative of statistically significant difference or association.

4. RESULTS

The present study revealed that 40% among all poultry examined were infected by one or more species of helminth parasites. 36 intestine of chicken out of 93 were infected with helminth parasites while 14 stool samples or droppings were found positive for helminth eggs.

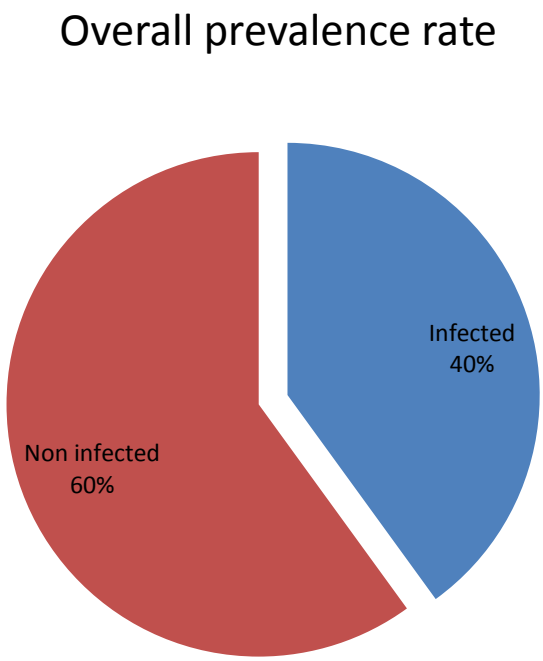

Figure 2: Showing overall prevalence of helminth parasites in Lalitpur district.

After the examination of 93 alimentary canals and 32 stool samples of local chicken in Lalitpur district, five species of nematode and one species of cestode were recovered. No any species of trematode were recovered during research work and four unidentified species were also recorded. Among nematodes *Ascaridia galli*, *Heterakis gallinae*, *Capillaria* species were identified while among cestodes *Raillietina tetragona* was identified on the basis of identification keys given by Soulsby, 1982.

Table 1: Showing helminth parasites recovered

Class of parasite	Name of parasite	Location
Nematoda	*Ascaridia galli*	Intestine
	Heterakis gallinarum	Caecum
	Capillaria annulata	Crop, Oesophagus and Intestine
	Capillaria obsignata	Crop, Oesophagus and Intestine
	Capillaria contortus	Crop, Oesophagus and Intestine
Cestoda	*Raillietina tetragona*	Intestine
Trematoda	Nil	---

4.1. Prevalence Rate on the basis of Species

The highest prevalence of *Heterakis gallinarum* (22.4%) was seen followed by *Capillaria* species (16%), *Ascaridia galli* (10.4%), and *Raillietina tetragona* (4%). Similarly the prevalence of unidentified species was found 4.8%. Statistically, there was a significant difference in the prevalence of helminth species (χ^2 = 33.832, $p < 0.05$) with the highest prevalence of *H. gallinarum.*

Table 2: Showing species- wise prevalence rate (%), n= 125

Name of parasite	No. of chicken infected by		t	Prevalence Rate
	Adult	Egg		
Ascaridia galli	9	4	13	10.40%
Heterakis gallinarum	23	5	28	22.40%
Capillaria species	17	3	20	16%
Raillietina tetragona	5	0	5	4%
Unidentified	4	2	6	4.80%

where, t = total no. of infected chicken , Prevalence Rate = (t/n)*100%, n= 125

4.2. Prevalence Rate on the basis of Class

Among different classes of helminth parasites, Nematoda was most prevalent (48.80%) followed by Cestoda (4%) while no any species from class trematoda was recovered. Statistically there was significant difference in the prevalence rate of different class of helminth ($\chi^2 = 138.10$, $p < 0.05$) with high prevalence rate of class Nematoda.

Table 3:Showing class- wise prevalence rate (%), n= 125

Name of class	No. of chicken infected(t)	Prevalence Rate
Nematoda	61	48.80%
Cestoda	5	4%
Trematoda	0	0.00%
Unidentified	6	4.80%

where, t = total no. of infected chicken , Prevalence Rate = (t/n)*100%, n= 125

4.3 Prevalence Rate on the basis of Chicken Type

Out of 40 samples examined from free range chicken, 28 were found to be infected with the prevalence rate of 70% while from 85 samples of poultry chicken (from slaughtered house) , 22 were infected with the prevalence rate of 25.88%. Statistically there was significant difference in the prevalence rate of helminth in free range chicken and poultry chicken ($\chi^2 = 22.055$, $p < 0.05, \alpha=1$).

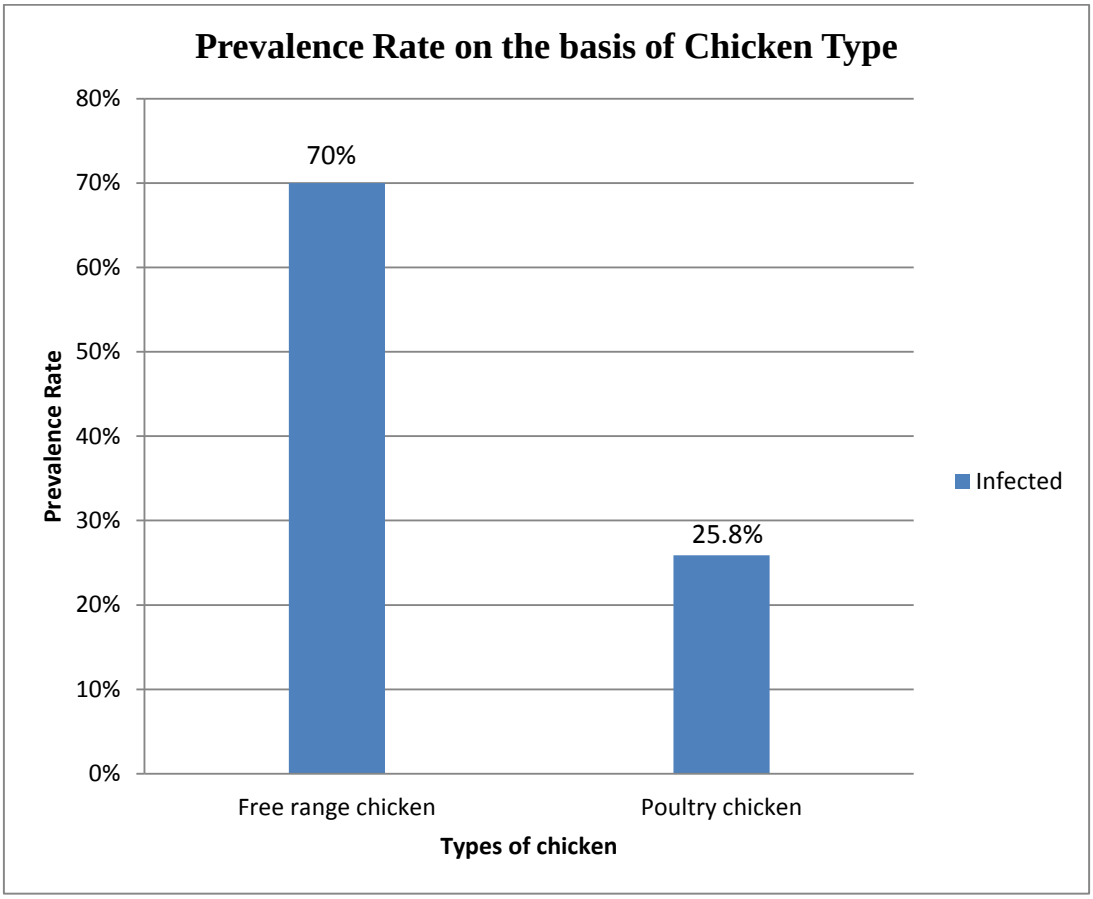

Fig 3: Prevalence rate on the basis of chicken type

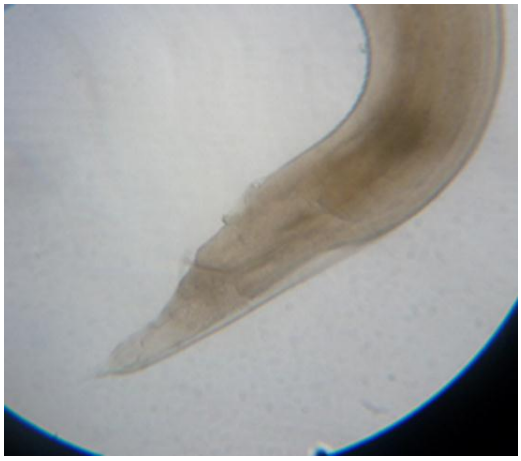

Photo 9 : Showing tail of male *A. galli*(10X10)

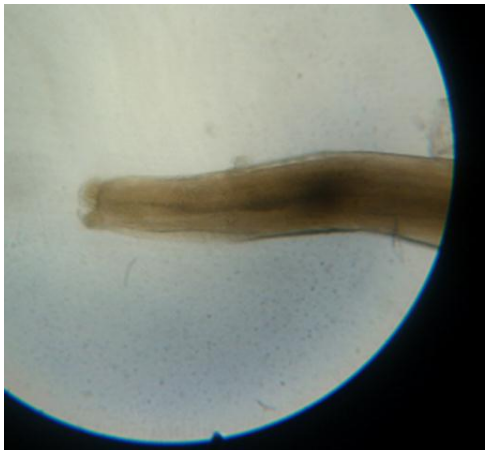

Photo 10 :Showing head of male *A. galli* (10X10)

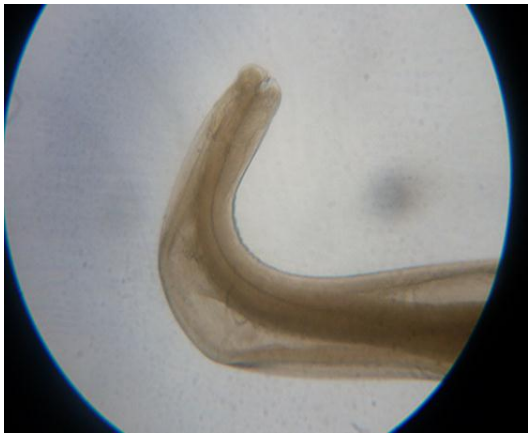

Photo 11 : Showing oesophagus without posterior bulb of male *A. galli*(10X10)

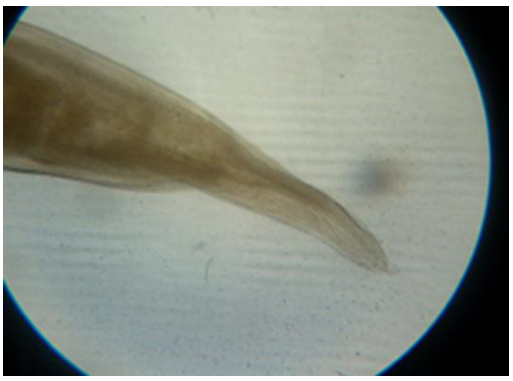

Photo 12 : Showing tail of female *A. galli* (10X10)

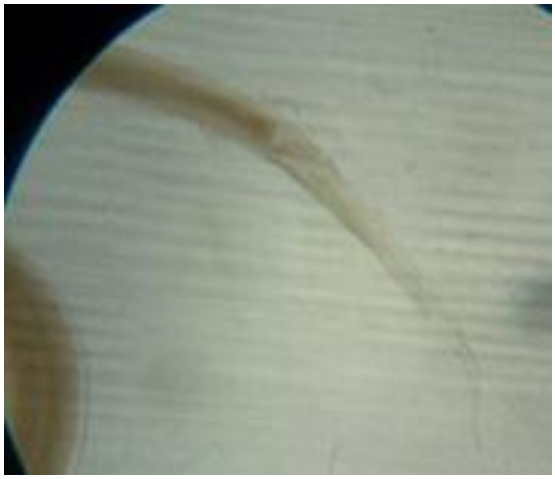

Photo 13: Tail of female *H. gallinarum* (10X10)

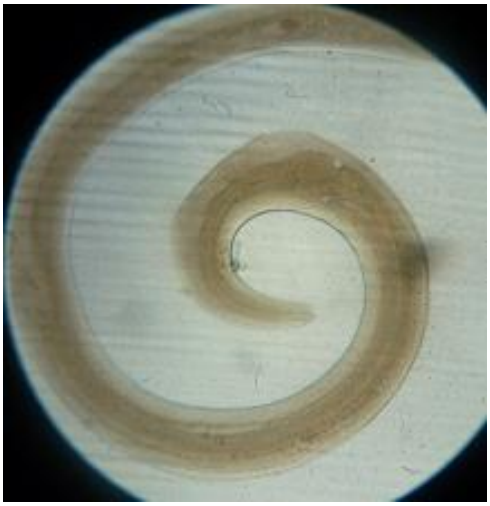

Photo 14 : Head of female *H.gallinarum*(10X10)

Photo 15: Head of male *H. gallinarum* (10X10)

Photo 16 : Tail of male *H. gallinarum* (10X10)

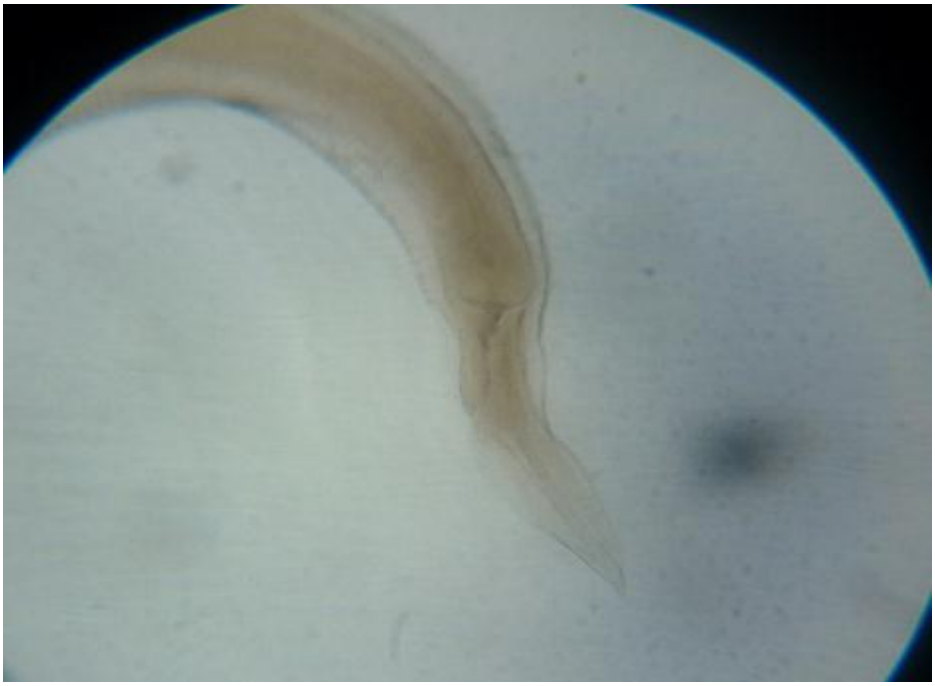

Photo 17 : Showing head with bulbous swelling of cuticle of male *Capillaria annulata*(10X10)

Photo 18: Showing tail with spicule sheath beset with spines of male *Capillaria annulata* (10X10)

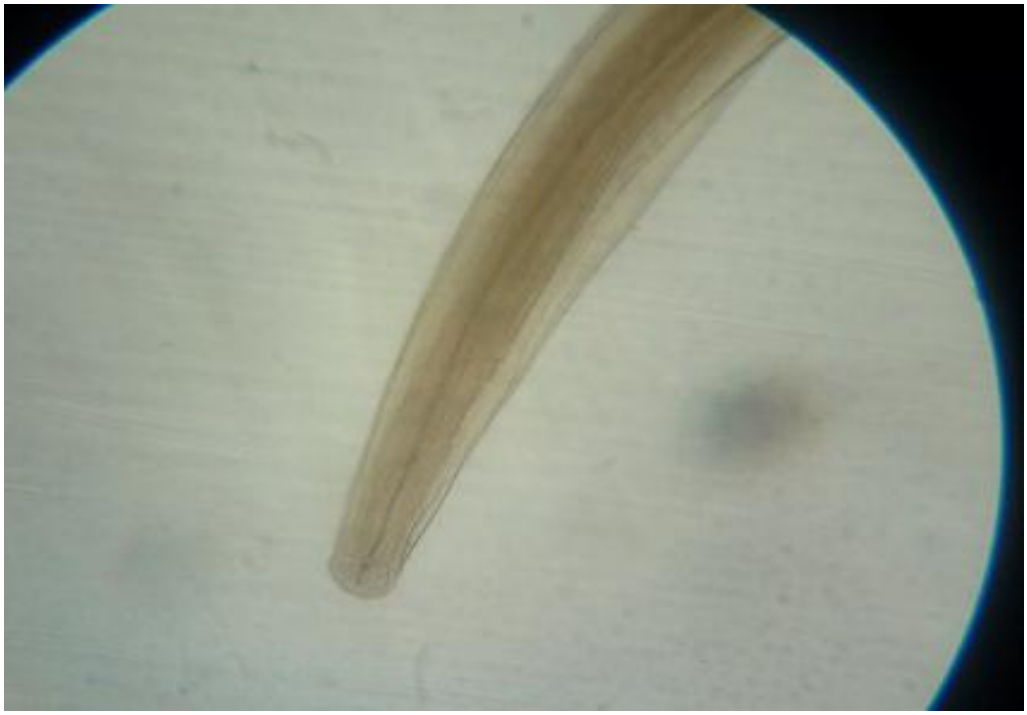

Photo 19: Showing head of male *Capillaria contortus*(10X10)

Photo 20 : Showing obliquely truncated tail of male *Capillaria contortus* with spine of long spicule sheath (10X10)

Photo 21 : Showing scolex and neck of *Raillietina tetragona*(10X10)

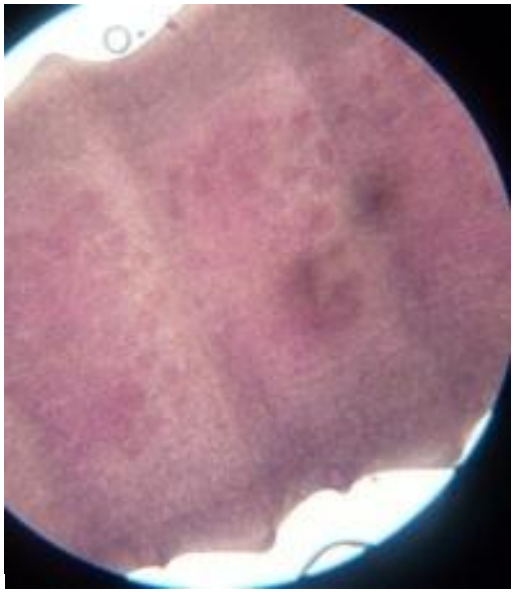

Photo22 : Showing gravid proglottid of *Raillietina tetragona* (10X10)

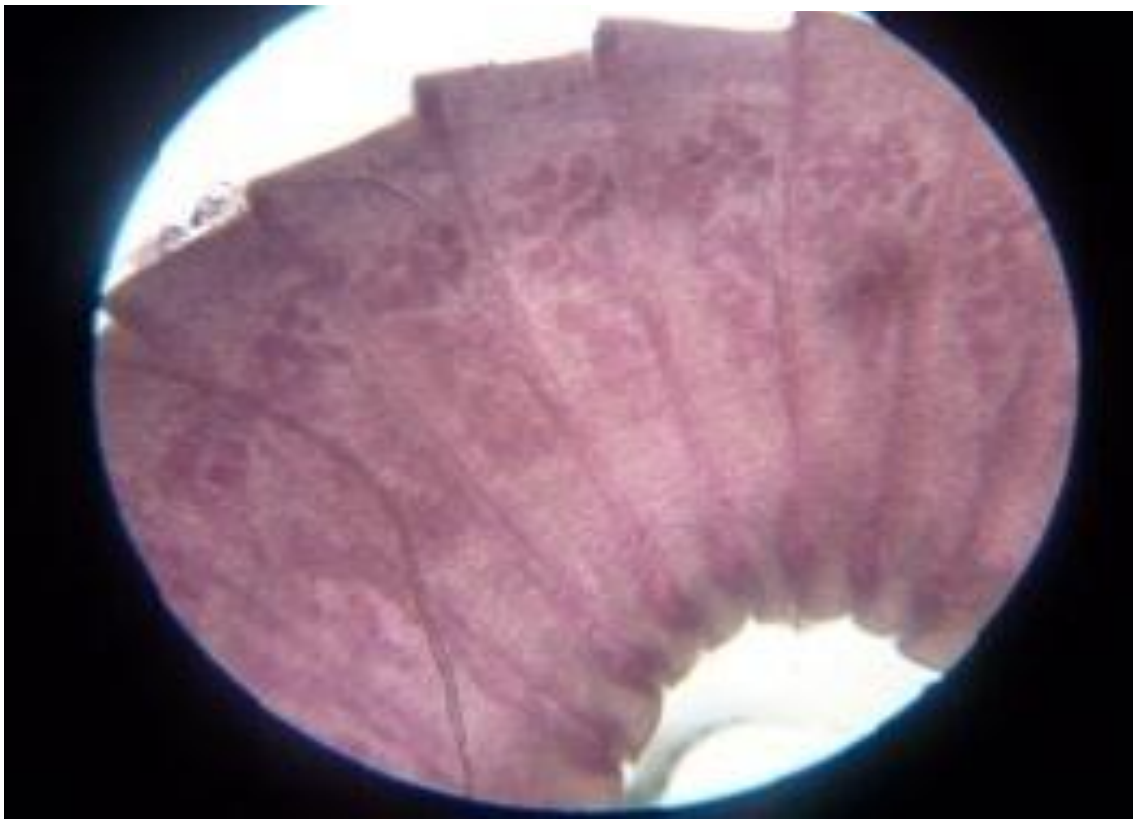

Photo 23 : Showing mature proglottid with unilateral genital pore of *Raillietina tetragona* (10X10)

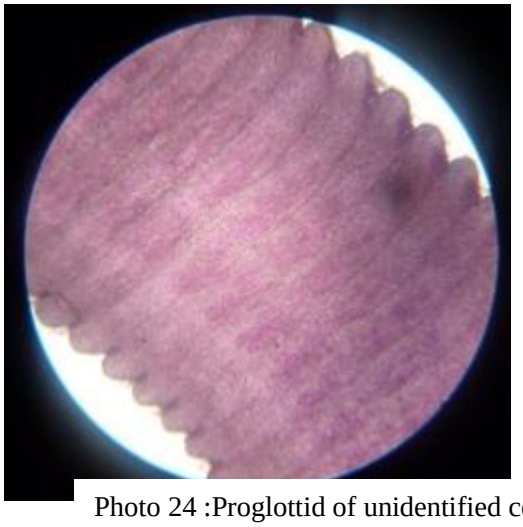

Photo 24 :Proglottid of unidentified cestode .

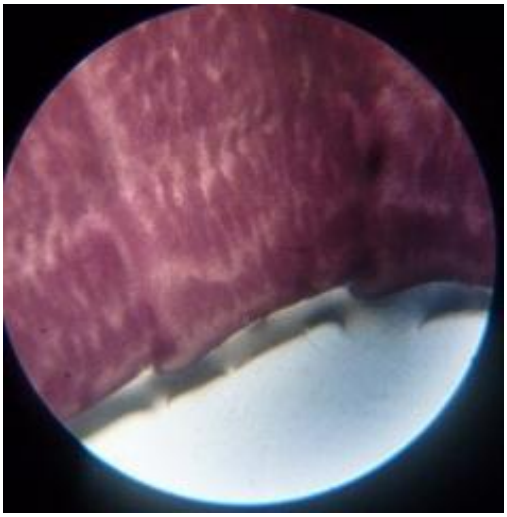

Photo25 :Proglottid of unidentified cestode

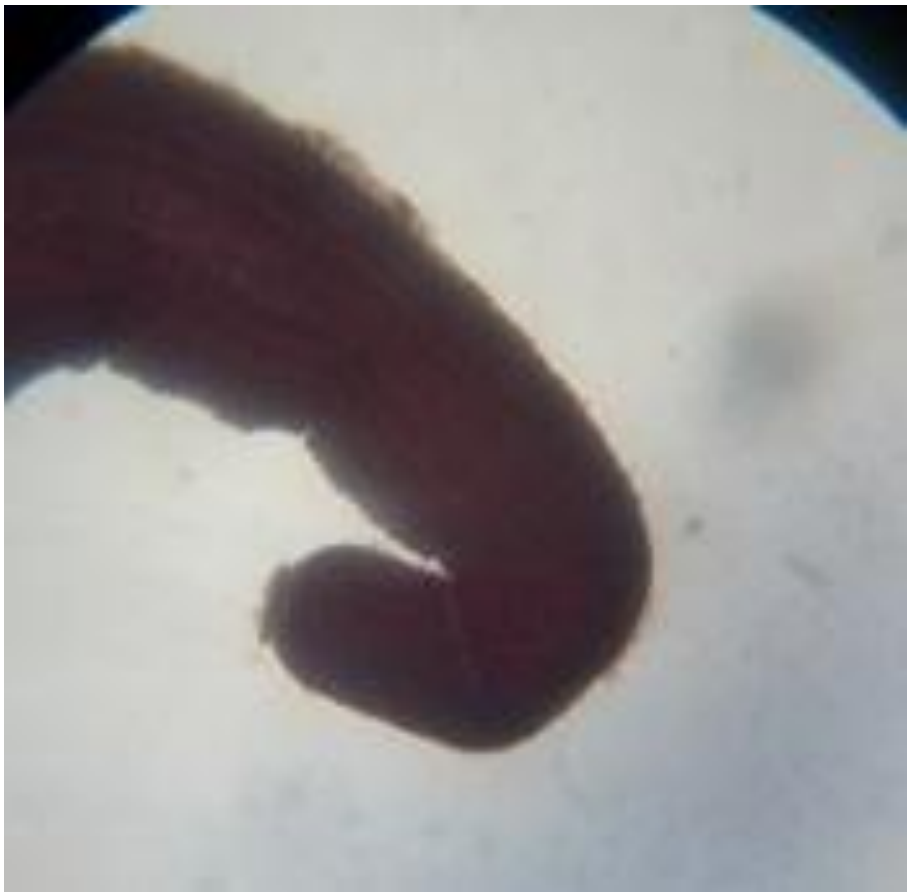

Photo 26 :Scolex and neck region of unidentified cestode at 10X10.

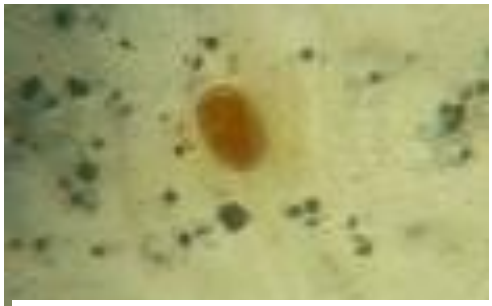

Photo 27 : Egg of *Heterakis gallinarum* (10X10)

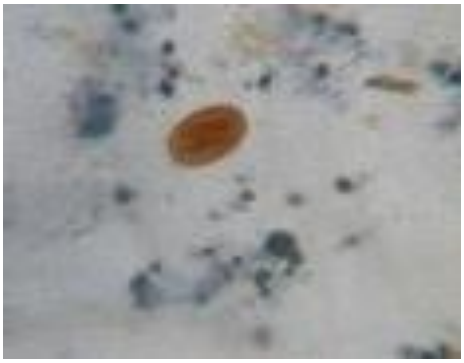

Photo 28 : Egg of *Ascaridia galli* (10X10)

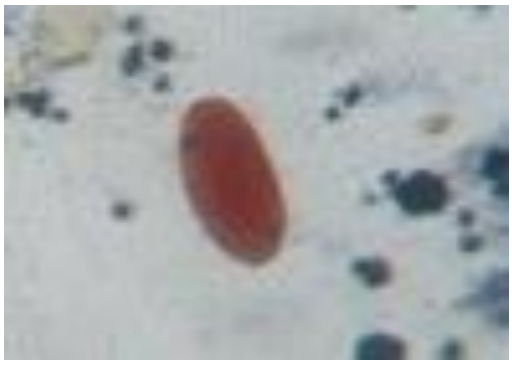

Photo 29: Unidentified egg at 10X10

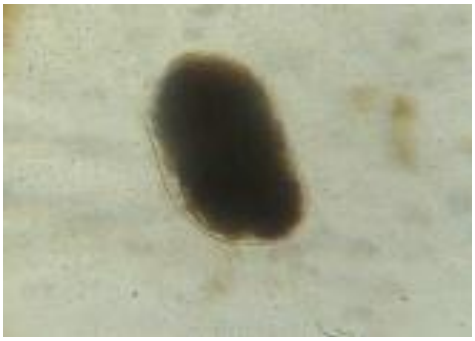

Photo 30 : Unidentified egg at 10X10

5. DISCUSSION

The domestic fowl or chicken harbours many intestinal parasites due to its feeding habit .According to Muchadeyi *et al*., (2004) and Mwale and Masike (2009) village chicken which are raised mainly under the free range (scavenging) product system, with partial or no housing have higher rate of infection of disease and parasites especially helminthes (Swaton *et al* ., 2003).

The present study was mainly conducted to find the prevalence rate of parasitic infection in chicken in Lalitpur district. Thus after dissecting 93 alimentary canals and observing 32 stool samples of chicken in Lalitpur district, four intestinal helminth parasites were recovered. The study showed 40% among all poultry examined were infected by one or more species of helminth parasites which agrees with the work of Sudhir (2013) who found 51.67% infection in free range chicken in India. The present study is also more or less similar to the report of other worker who reported the prevalence rate of 41.4% (Tesfaheywat *et al.,* 2010) ,53.00% (Matur *et al*., 2010) and 37.9% (Dawet *et al*., 2012).

In the present study, six species of helminth were identified comprising five nematodes and one cestode compared to seven species of helminth identified by Adang *et al*., (2014) comprising of six cestodes and one nematode, five species by Kose *et al*., (2009) comprising four nematodes and one cestodes , five species by Rayyan *et al*., (2010) comprising of three nematodes and two cestodes and three species by Matur (2002) comprising two cestodes and one nematode.

The species identifed in this work were *Heterakis gallinarum, Capillaria* species , *Ascaridia galli* and *Raillietina* species which is in accordance with the findings of Matur *et al*., 2010. In the present study the highest prevalence rate was seen in nematode(48.80%) followed by cestode(4%). Similarly the prevalence of unidentified species was found 4.8%. Statistically there was a significant difference in the prevalence of different class of helminth ($p < 0.05$) with high prevalence rate of class nematode. Hamad (2013) also found significant difference ($p < 0.001$) in the prevalence rate of nematode and cestode infection.

There were no any trematode roported in this study . In accordance with this study, Anand *et al*.,(2008), Sudhir (2013) and Hamad (2013) did not report any trematode. The absence of these worms appeared to be linked with the complex life cycle requiring atleast an intermediate host

which is aquatic . This helps to break life cycle where water is not available and hence reducing the spread of worms.

The highest prevalence of *Heterakis gallinarum* (22.4%) was seen followed by *Capillaria* species (16%), *Ascaridia galli* (10.4%) and *Raillietina* species (4%). There was statistically significant difference in the prevalence of helminth species ($p < 0.05$) with high prevalence of *Heterakis gallinarum.* Mikail and Adamu (2008) reported similar findings with highest prevalence of *Hetakis gallinarum* (28.66%) . Similarly, Mukaratirwa and Khumalo (2010) and Hamad (2013) also reported the highest prevalence of *Heterakis gallinarum.*

Among cestode only *Raillietina* was the species reported with the prevalence rate of (4%) which agrees with the findings by Anand *et al*., (2008) who reported *Raillietina* as the gastrointestinal parasite in and around Banglore, India. *Raillietina tetragona* which is the only cestode parasite identified in this study is known to be cosmopolitan and contributes to nutrients depletion in birds as reported by Cheng (1973) and Soulsby (1982). Their intermediate hosts which are ants and beeltes are available more abundant and form an important part of diet of chicken. It is therefore safe to assume that the birds might have acquired helminth infection from their diets.

Out of 40 samples examined from free range chicken, 28 were found to be infected with the prevalence rate of 70% while 85 samples of poulty farm chicken (from slaughtered house) were found to have prevalence rate of 25.88% which agrees with the work of Hamad (2013) and Yoriyo *et al.,* (2005). Similarly, Mikail and Adamu (2008) reported high infection rate in free range chicken (92.66%) .

Statistically there was a significant difference in the prevalence rate of helminth in free range chicken and poultry chicken ($p < 0.05$). This may be because the free range chicken or backyard poultry are more susceptible to parasitic infection. The main food of backyard chicken consists of different types of seeds, kitchen wastage, insects, slugs, earthworm, etc. insects slugs worms act as intermediate host of many bioparasites (Soulsby, 1982). Besides, backyard poultry can easily ingest the infective stage of many parasites during taking food from the environment. Probably for the above mentioned causes backyard chicken is more susceptible to helminth infection. In case of chicken of poultry farm have less chance of gaining infection as they are reared in intensive system maintained with strict hygienic measure.

6. CONCUSION AND RECOMMENDATIONS

6.1 CONCLUSION

A study was carried out to find the prevalence of intestine helminth parasites of local chicken in Lalitpur district. A total of 93 intestine and 32 stool sample of local chicken, *Gallus gallus domesticus*, were collected from different places of Lalitpur district. The collected samples were examined thoroughly for the presence of helminth parasites in the form of adult or egg. A zoo of parasitic worm can be found in chicken flocks. Worms find cozy places to stay in the crop, gizzard, intestine , caecum, windpipe and even the eyelids. Chicken get infected because of their feeding habit as they pick up worms by eating dirt or litter contaminated with eggs or by eating small creatures carrying immature stages of worms.

Among 93 intestine of chicken only 36 was found to be infected by one or more helminth parasites while 14 stool sample showed positive for the presence of helminth eggs. The identification of helminth parasites was done by studying their morphological characteristics. In the present study five species of nematode , one species of cestode and four unidentified species were recorded.

The present study showed that only 40% of all the poultry examined were infected. The highest prevalence rate was found with *Heterakis gallinarum* (22.4%) followed by *Capillaria* species (16%), *Ascaridia galli* (10.4%) and *Raillietina tetragona (*4%). Similarly the prevalence of unidentified species was (4.8%).

The infection was more in free range chicken (70%) as they are reared in unhygienic environment and are more susceptible to parasitic infection due to their feeding habit. The intestine which were collected from slaughter house were with least infection (25.88%) as they were bought from poultry farm where chicken are reared in hygienic environment with medication.

6.2 RECOMMENDATIONS.

- Keep wild bird away from the flock as they may be infected and shed worm eggs in the dropping.
- Use integrated pest management (IPM) practice to control insect population which mainly act as vector of many helminth parasites.
- Further, extensive study needs to be carried out to explore parasitic fauna of chicken covering wide range of habitat.
- Improvement in the technique used for more scientific identification (molecular level) of helminth parasites.

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ANNEX 1

Identification keys of Nematode Family (Soulsby, 1982)

1. Worms with free- living adult generation , that is, males and females developing outside of body; in digestive tract, hermaphrodite females only……..**Strongyloididae**

Worms without a free-living generation, that is , incapable of producing males and females outside of body.....…**2**

2. Worms hair like or thread like ; oesophagus tubular and capillary, the tube embedded in or otherwise in relation to a single row of cells; in crop and small intestine……..……**Trichuridae**

Worms thick as with above , oesophagus well developed and muscular and with definite triangular lumen, not in relation to a single row of cells…….……..**3**

3. Cordons or other cephalic ornamentations present……**Acuriidae**

Cordons or other cephalic ornamentations absent ……..……..**4**

4. Preanal suckers present…….…….**5**

Preanal suckers absent ……..……**6**

5. Oesophagus with distinct posterior bulb containing a apparatus…….**Heterakidae**

Oesophagus without a distinct posterior bulb ……..**Ascarididae**

6.Bursa present …….……..…..**7**

Bursa absent…….……..**8**

7. Buccal capsule well developed and containing at least six teeth at base; oral opening hexangular…….……**Syngamidae**

Buccal capsule reduced and not containing not more than three teeth at base or none……**Trichostrongylidae**

8 .Pseudolabia absent……..……**Thelaziidae**

Pseudolabia present……..**Spiruridae**

ANNEX 2

Identification keys to species of *Capillaria* in the upper digestive tract of birds (E.B.Cram,1936)

1. Head with bulbous swelling and neck with cuticular thickenings……***C. annulata***

Head and neck simple, without above structures…….2

2. Female with large, bell-shaped, vaginal protuberance at vulva…….***C. cylindrica***

Female without such vaginal protuberance……. 3.

3.Posterior part of body much thicker than anterior part, especially in female …….. ***C. dispar***

No such marked change in width of body…….. 4.

4.. Spicule sheath unarmed; male tail simple, without lateral lobes…….. ***C. obtusiuscula***

Spicule sheath thickly covered with spines; male tail with 2 lateral lobes……..***C. triloba***

Spicules without above structure……. 5

5. Esophagus of male unusually short, measuring 410µ to 480µ in specimens 12.8 to 15.6 mm Long……***C. corvicula***

Oesophagus of male considerably longer than above…… 6

6. Tail end of male with 3 pairs of lobular projections, each with a pair of papillae, and the whole hidden by a bell-shaped bursal structure……***C. laricola***

Tail end of male simpler than above, having dorso- laterally 2 prominences or papillae…….7

7. Spicule apparently absent-…… ***C. lophortygis***

Spicule present, although sometimes difficult to see……8

8. Spicule very long, measuring more than one-third body length……..***C. perforans***

Spicule length much shorter than above; when deter minable, not more than one-tenth body length ***C. contorta***

ANNEX 3

Identification keys of families of cestode (Soulsby, 1982)

The species of cestodes parasitizing poultry belonging to different families can be differentiated by the following identification keys:

1. Head armed with numerous hammer shaped hooks……..**Davaineidae**

   Head armed with hooks not hammer shaped or unarmed …….2

2. Testes few, 1 to 4, rarely more……..…..……**Hymenolepididae**

   Testes numerous, more than 4…….....3

3. Head lacking rostellum; no paruterine organ in species occurring in poultry

Anoplocephalidae

Head with retractile rostellum, usually round , or rarely unarmed; rarely without rostellum; with or without paruterine organ……..…….**Didelpididae**

Identification keys of tapeworms of poultry (Soulsby, 1982)

1 .Rostellum absent ……..……2

Rostellum present……....……..3

2.Paruterine organ present……...……***Metroliasthes***

Paruterine organ absent ……..……***Aporina***

3. Mature worm small, usually not longer than 4 to 5 mm……..……4

Mature worms larger, longer than above ……..……..5

4. Strobila consisting of 2 to 9 segments ……..…….***Davainea***

Strobila consisting of numerous segments ……..…….***Amoebotaenia***

5. Testes three in number……..……6

Testes more than three in number ……..7

6. With a well developed pseudo- holdfast organ , in addition to a small ,true holdfast organ containing no genital premordia……..***Fimbriaria***

With only a true holdfast organ……..***Hymenolepis***

7. Rostellum armed with a single row of 16 to 20 hooks, each 20 to 30 µ long…..***Choanotaenia***

Rostellum armed with either a single row or double row of 100 or more hooks , each 6 to 15 µ long .……..……..….***Raillietina***