PREVALENCE AND MORPHOLOGICAL STUDIES OF GASTROINTESTINAL HELMINTHS OF BACKYARD CHICKEN IN BANGLADESH

S. M. ABDULLAH

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR, DHAKA-1207

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BY

S. M. ABDULLAH

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APPROVED BY

Dr. Uday Kumar Mohanta
Supervisor
Chairman & Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Dr. Mohammad Saiful Islam (Rasel)
Co-Supervisor
Chairman & Associate Professor
Department of Anatomy, Histology and Physiology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Dr. Uday Kumar Mohanta
Chairman of Examination Committee
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Department of Microbiology and Parasitology

Sher-e-Bangla Agricultural University

Sher-e-Bangla Nagar, Dhaka-1207

MEMO NO: SAU/

CERTIFICATE

THIS IS TO CERTIFY THAT THE THESIS ENTITLED "PREVALENCE AND MORPHOLOGICAL STUDIES OF GASTROINTESTINAL HELMINTHS OF BACKYARD CHICKEN IN BANGLADESH" SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY, SHER-E-BANGLA AGRICULTURAL UNIVERSITY, DHAKA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PARASITOLOGY, EMBODIES THE RESULT OF A PIECE OF BONA FIDE RESEARCH WORK CARRIED OUT BY S. M. ABDULLAH, REGISTRATION NO. 12-04875 UNDER MY SUPERVISION AND GUIDANCE. NO PART OF THE THESIS HAS BEEN SUBMITTED FOR ANY OTHER DEGREE OR DIPLOMA.

I FURTHER CERTIFY THAT ANY HELP OR SOURCE OF INFORMATION, RECEIVED DURING THE COURSE OF THIS INVESTIGATION HAS BEEN DULY ACKNOWLEDGED.

Date: May, 2019

Place: Dhaka, Bangladesh

Dr. Uday Kumar Mohanta
Supervisor
Chairman & Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

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The Author

LIST OF CONTENTS

LIST OF CONTENTS (CONT'D)

LIST OF FIGURES

LIST OF FIGURES (CONT'D)

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

ABBREVIATION		FULL MEANING
et al.	=	And others/Associates
HCl	=	Hydrochloric acid
M.S.	=	Master of Science
>	=	More than

PREVALENCE AND MORPHOLOGICAL STUDIES OF GASTROINTESTINAL HELMINTHS OF BACKYARD CHICKEN IN BANGLADESH

ABSTRACT

Poultry is a promising sector in Bangladesh which is expanding day by day. It is also the most appropriate income generating sector for rural women as well as landless and marginal farmers. But the indigenous chickens are affected by various parasites. Therefore, the present study was aimed to study the prevalence and morphological identification of gastrointestinal helminths of backyard chicken in Dhaka, Bandarban, Mymensingh and Pabna. A total of 63 intestines were examined for helminth parasites from September 2017 to November 2017. This experiment was performed at the Microbiology and Parasitology Laboratory under the Department of Microbiology and Parasitology, Sher-e- Bangla Agricultural University, Dhaka-1207. The collected helminths were identified according to the keys and description given by Soulsby (1982) and Yamaguti (1958). A high rate of helminth infection (100%) was observed in backyard chicken in Bangladesh. One cestode, *Raillietina tetragona* (73.01%); two nematodes, *Ascaridia galli* (47.61%) and *Heterakis gallinarum* (38.09%); and two trematodes, *Catatropis verrucosa* (23.80%) and *Echinostoma revolutum* (7.93%) were encountered during the study. *Ascaridia galli* was mostly found in small intestine whereas *Heterakis gallinarum* was found in the caecum. *Echinostoma revolutum* and *Raillietina tetragona* were collected from both small and large intestines. *Catatropis verrucosa* were recovered only from caecum. Out of 63 samples, 25.39% were infected with single infection while the rest 74.61% were mixed infections. This work strongly suggests that helminthosis is a very serious problem in backyard chicken and therefore, appropriate control strategies are needed to design for better production.

Keywords: Helminths, Backyard chicken, Morphological identification, Prevalence, Bangladesh

CHAPTER 1

INTRODUCTION

Poultry, specially chicken is one of the most intensively reared domesticated species, and is the most developed and profitable animal production businesses (FAO, 1987). Its importance in developing countries and its role in improving the nutritional status and income of many small farmers have been recognized by various scholars and rural development agencies in the last two decades (Eyinnaya, 1992). Animal production in general and chickens in particular play important socioeconomic roles in developing countries (Alders, 2004). The purposes of backyard chicken production are for income, egg hatching for replacement, consumption, for cultural and/or religious ceremonies and egg production (Moges *et al*., 2010). Poultry productivity is enhanced by application of sound principles of health protection and management (Shane, 2005). The economic contribution of the sector is not still proportional to the large chicken numbers, attributed to the presence of many productions, reproduction and infrastructural constraints (Aberra, 2000). Free-range chickens from rural areas supply all of the chicken meat and egg requirements for rural people, and about 12–13% of urban requirements (Melewas, 1989).

Like other country, poultry production is a promising sector in Bangladesh which is increasing day by day. Poultry meat and eggs contribute approximately 37% of total animal protein in Bangladesh (Ahmed and Islam, 1990). In Bangladesh, Poultry rearing is the most appropriate income generating activities for rural women, especially for landless and marginal farmers. The production of backyard poultry under semi scavenging system is found suitable to the villagers as additional source of income and nutrient supplement (Latif, 2001).

Poultry diseases is a major hindrance which interfering with poultry productivity, by decreasing economic returns, and may therefore, negatively affect the development of the industry (Abebe *et al*., 1997). Among poultry diseases, helminthosis is considered to be the most important problem of local chickens, and major causes of ill-health and loss of productivity in different parts of the world (Yimer *et al*., 2001). Parasitic diseases are problem wherever poultry are raised, whether in large commercial operations or in small backyard flocks and economic losses caused by parasites on poultry can be significant (Fatihu *et al*., 1991).

In fact, the indigenous chickens of Bangladesh are parasitized by various parasites (Sarkar, 1976). The domestic chicken has a wide range of feeding habits from grains, fruits and insects which may carry stages of parasites, thus predisposing them to parasitic infections (Adang, 1999; Oniye, 2000). The chickens pick up the parasite eggs directly by ingesting contaminated feed, water, or litter or by eating snails, earthworms, or other insects. Clinical signs of parasitism are unthriftyness, poor growth and feed conversion, decreased egg production and even death in severe infections. Furthermore, parasites can make the flock less resistant to other diseases and exacerbate existing disease conditions. Parasitic infection or their concurrent infections result in immunosuppression, especially in response to vaccines against some poultry diseases. Of all the intestinal worms, large roundworms (*Ascaridia galli*) probably inflict the most damage in young birds being more severely affected. A mild infection is often unnoticed but large numbers of worms, however, interfere with feed absorption causing poor growth and production. In severe infections, there can be actual intestinal blockage by the worms, causing death (Yousuf *et al*., 2009).

However, large numbers can have a devastating effect on growth, egg production, and over-all health. Parasitism has been attributed to cause reduced growth, low egg production, emaciation, anaemia as well as mortality (Belonwu, 1993; Hassouini and Belghyti, 2006; Heyradin *et al*., 2012). These authors reported that mortality due to parasitic diseases was higher than those attributed to Newcastle disease and mortality causing viral infection of poultry. The concentration of parasite eggs in the poultry environment is an important factor which plays a major role in determining the severity

of the infection (Pinkney *et al*., 2008). Viral, bacterial and protozoan diseases may appear to be more economically important to the farmer because they cause obvious losses in the form of deaths of many birds at a time. However, the less obvious, but ubiquitous, losses due to reduced productivity caused by helminthosis are economically very important to the poultry industry (Ssenyonga, 1982).

Gastrointestinal parasites which invade the host possess morphological and physiological features such as small thread like cylindrical body, hooks and hard body cuticle enhance their adaptation to long living and existence in their hosts. These parasites constitute a major factor limiting productivity of the poultry industry (Soulsby, 1982).

Of the helminth parasites of poultry birds, nematodes constitute the most important group of helminth parasites of poultry both in number of species and the extent of damage they cause: the main genera include *Ascaridia*, *Heterakis* and *Capillaria*. There are a few species of cestodes in poultry. Like them *Choanotaenia infundibulum*, *Davainea proglottina*, *Raillietina cesticillus* and *Raillietina tetragona* are also found in the intestine of poultry (Matur, 2010).

Therefore, the aim of this study was:

1. To carry out the detailed morphological identification of different species of gastrointestinal helminths affecting backyard poultry.
2. To determine the prevalence of those endoparasites in different districts of Bangladesh.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Morphological studies on *Ascaridia galli*

Lalchhandama (2010) conducted a detailed morphological study of *Ascaridia galli* by scanning electron microscopy and revealed that the extreme anterior cephalic region was a triangular mouth consisting of three prominent lips. Each of the lip was lined with fine teeth on the internal rim and studded with eye-like sensory papillae. The body cuticle constituted series of striations called annulations. Annuli were transverse concentric rings and were divided into parallel subannuli. Female had a simple straight tail with a ventrally located anal opening. The male posterior was curved and pointed, and relatively elaborate having a precloacal sucker in addition to the anus. These posterior openings were surrounded on both sides by a row of minute protrusions called caudal papillae and the lateral caudal alae. The precloacal sucker was surrounded by a sclerotized ring. Light microscopy showed that the cuticle was multilayered and continuous with the hypodermis, which in turn was supported with a thick musculature composed of fibrillar contractile and granular noncontractile protoplasmic portions. The body space, pseudocoel, contained digestive tract and reproductive organs such as testis, vas deferens and seminal vesicle in male, and ovaries, oviducts and uteri in female. The seminal vesicle housed spermatozoa, and the uteri, fertilized eggs. The eggs were elliptical, covered with chitinous shell that enclosed the embryo.

Katakam (2010) analysed *A. galli* larval recovery from the chicken intestine. The number of larvae recovered from the intestinal wall of chickens infected with 1000 embryonated *A. galli* eggs and killed 15 days post infection (p.i.) by three methods (ethylenediamine tetraacetic acid [EDTA], pepsin digestion and scraping) were compared. The EDTA and pepsin digestion were found to be the most efficient methods with no significant difference ($P > 0.05$) in the number of recovered larvae between the

two. Subsequently, three different *A. galli* cohorts were established using the polymerase chain reaction-linked restriction fragment length polymorphism (PCR-RFLP) technique. A 533-bp long region of the cytochrome c oxidase subunit 1 gene of the mitochondrial DNA was targeted and 22 *A. galli* females were allocated to three different haplotypes. The four females with the highest embryonation rate from each haplotype group (total 12 females) were selected and used to inoculate each of 12 chickens with a dose of 1000 embryonated eggs. The chickens were killed 15 days p.i. and *A. galli* larvae were recovered from the small intestinal wall by the EDTA method and by sieving the lumen content on a 90 µm sieve. DNA of 40 larvae from each of the three different haplotypes was extracted using a worm lysis buffer, and PCR-RFLP analysis of these larvae revealed the same haplotype as that of their maternal parent. The identification of distinguishable cohorts may be a powerful tool in population studies of parasite turnover within the animal host.

Hafiz *et al*. (2015) performed a study to carry out the prevalence and severity of *A. galli* in White Leghorn layers (housing type: battery cage and deep litter, 50 each) and Fayoumi-Rhode wasland Red crossbred (male and female: 50 each) flock rearing at Government Poultry Farm, Dina, Punjab, Pakistan. Two hundred faecal samples were examined by using standard parasitological and McMaster egg counting technique. The overall prevalence was 24.5% at farm, 13% in White leghorn layer (battery cage=2%, deep litter=24%) and 36% in Fayoumi-Rhode wasland Red (male=34%, female=38%). It was also observed that White leghorn layer rearing in deep litter had more severe infection (EPG=1920) of *A. galli* compare with battery cages birds (EPG=500). Parasite prevalence was significantly related with sex ($P<0.05$) in Fayoumi-Rhode wasland Red and male birds had less number of average parasites (0.34±0.47) as compared to females (0.38±0.490). Additionally, female birds were under serious threat of infection (EPG=2270) compared with its counterpart (EPG=1250). Given the high infection rates, particular attention should be paid to management and provision of feed supplement to White leghorn layer housing in deep litter and female bird of Fayoumi-Rhode was land Red crossbred.

Zhao *et al*. (2016) performed a research to find out the detailed morphology of *A. galli* using light and scanning electron microscopy, based on specimens collected from the endangered green peafowl *Pavo muticus Linnaeus* (Galliformes: Phasianidae) in China. The results revealed some erroneous and previously unreported morphological features, including the lips lacking real denticles, the lateral alae beginning at some distance posterior to the base of the ventrolateral lips and the caudal papillae with 4 different morphotypes.

Akter *et al*. (2016) conducted a research to determine the incidence of ascariasis in Polashbari upazilla of Gobindhaganj district during July to November 2012. Out of 500 chickens examined for presence of *A. galli* infestation by faecal sample examination, 365 hens and 135 cocks. The 292 female (80%) and 119 male (88.15%) were found infected with *A. galli*. The highest infection rate 95.26% was found in 60 to 90 days of age group. Infected chickens were treated with pineapple leaves extract @ 1ml/kg body weight per OS for 7 consecutive days. The efficacy of anthelmintic treatment was evaluated by counting fecal egg per gram (EPG) compared with pretreatment values. Body weight and hematological changes of each chicken was recorded in pre and post treatment. In the untreated control chickens the average EPG increased from 300 ± 11.07 to 340 ± 13.96. The average EPG reduced from 300 ± 11.07 to 60 ± 7.40 within 28 days of pineapple treatment. The mean body weight gain in treated chicken was significantly ($p<0.01$) higher than the control. Pineapple leaves extract increased the TEC, Hb and PCV and decreased TLC and ESR values of chickens. But in control group TEC, Hb and PCV decreased and TLC and ESR values increased. It may be concluded that pineapple leaves extract treatment effectively reduced the ascariasis load in chicken and improved body weight.

2.2 Morphological studies on *Heterakis gallinarum*

Everett *et al*. (1974) reported that the reproductive potential of *Heterakis gallinarum* was substantially higher in the ring-necked pheasant than in any of the eight other species of galliform birds used on the 67 tests. Pheasants on four tests yielded an average 19.4 times as many eggs that embryonated as were used to infect the birds, while for those on tests

with a highly virulent strain of *Histomonas meleagridis* present the return was 21.1 eggs per egg used. Corresponding returns for chickens were 5.2 and 2.4; for guinea fowl, 9.7 and 1.3; and for turkeys, 1.9 and 0.17. Birds of the other five species gave even poorer returns. Previous studies had indicated that 10–30 times as many heterakid eggs must embryonate as survive to be ingested, under natural conditions. Inasmuch as the traditional host of *Heterakis gallinarum* must also have been that of the virulent strains of *Histomonas meleagridis* that have become man's contemporaries, they regard the ring-necked pheasant, or some very close relative, as being the most likely host of these parasites in the late Cenozoic and Recent Eras.

Chalvet-Monfray *et al*. (2004) developed a mathematical model to describe the population dynamics of *Heterakis gallinarum* in a turkey flock to study its kinetics in a number of hosts. The model includes quantitative (parasite burden) and qualitative (number of hosts without mature parasite) descriptions of these dynamics. To understand the role of *Heterakis* as a transport host, the various elements that delay the beginning of development of the parasite population (e.g., necessary delay of larval stage, the probability of having a male and female in the same host) were taken into account. From published data, the negative binomial distribution parameter k = 0.24, which described the aggregated distribution of the *Heterakis* among the hosts, was calculated. The sensibility study showed that when the k parameter decreased (i.e., when the population was more aggregated), infestation increased quantitatively (mean parasite burden increased) but not qualitatively (the number of host without mature parasite increased). The model demonstrated that the population dynamics of *Heterakis* takes time; for instance, with an aggregated population of *Heterakis* at day 90, the host was mainly free of adult parasite. These results may be used in the future to test the role of *Heterakis* in the spread of *Histomonas*.

Papini & Cacciuttolo (2008) performed a study to conduct *Heterakis gallinarum* infection in a flock of Rhode was land Red laying hens. These hens were entirely kept in houses on a farm for commercial egg production, where a deep litter production system was adopted. Faecal samples from 120 hens selected at random were examined by common flotation technique and modified McMaster’s technique. *H. gallinarum* eggs were detected in 50%

of the examined samples with very low faecal egg counts. There was no evidence of clinical signs, gross pathological lesions, and consequences on production level linkable to heterakiasis. *H. gallinarum* was transmitted by direct ingestion of infective eggs from the soil and was one of the most important intestinal helminths of poultry due to the role it plays as vector of histomoniasis. In accordance with European legislation on the welfare of laying hens, a progressively increasing number of farmers can adopt breeding programs on soil.

Schwarz *et al*. (2011) performed two experiments. In two experiments 3-week-old chickens were inoculated with embryonated *H. gallinarum* eggs, which were positive for *H. meleagridis*. While birds of the first experiment were left untreated, those of the second experiment were treated with dimetridazol to prevent *H. meleagridis* co-infection. Mild to moderate histological lesions and local immune reactions with a significant increase in CD4(+), CD8α(+), TCRαβ(+) and TCRδγ(+) cells in the lamina propria and induction of the T-helper type 2 (Th2) cytokine interleukin-13 dominated the *H. gallinarum* immune response at 2 weeks post infection. Co-infection with *H. gallinarum* and *H. meleagridis* induced an increase in mRNA expression of the T-helper type 1 (Th1) cytokine interferon-γ, a decrease in splenic CD4(+) cells and severe destruction of the caecal mucosa in association with strong T-cell infiltration in the caecal lamina propria. There was no obvious effect on the chloride secretion of the caecal epithelium, which was investigated once the mucosa had almost recovered from the infection, in either experiment.

Das *et al*. (2014) investigated egg production dynamics and fecundity of *H. gallinarum* residing in different caecal environments induced through different types of dietary fibre. Growing layers were fed a standard (CON) or an insoluble- (I-) or soluble- (S-) non-starch polysaccharides-(NSP) supplemented diet for the first 11 weeks (wk) of life in a twice-replicated experiment. At 3wk of age, the birds were infected with 200 embryonated eggs of *H. gallinarum*. Starting from 3wk post-infection (p.i.), individual daily total excreta were collected. The number of eggs per gram of faeces (EPG) was determined (N=2240), and the number of eggs per day (EPD) were estimated. The birds were necropsied 8wk p.i. and the worm burdens were quantified. The nematode began to lay eggs as early as 23 d.p.i. and thereafter laid on average 436 eggs/d. I-NSP- and S-SNP-supplemented diets

expedited the onset of patency by approximately 5 days, and increased total egg excretion by 110% and 185%, respectively, due to higher worm counts. The latter diet (S-SNP) additionally increased total egg excretion by 94% due to enhanced fecundity.

Stehr *et al*. (2018) quantified the extent and duration of worm expulsion by chickens experimentally infected with both *Ascaridia galli* and *Heterakis gallinarum*, and investigated the accompanying humoral and cell-mediated host immune responses in association with population dynamics of the worms. Results demonstrated the strong co-expulsion of the two ascarid species in three phases. The expulsion patterns were characterized by non-linear alterations separated by species-specific time thresholds. *Ascaridia galli* burden decreased at a daily expulsion rate (e) of 4.3 worms up to a threshold of 30.5 days p.i., followed by a much lower second expulsion rate ($e = 0.46$), which resulted in almost, but not entirely, complete expulsion. *Heterakis gallinarum* was able to induce reinfection within the experimental period (9 weeks). First generation *H. gallinarum* worms were expelled at a daily rate of $e = 0.8$ worms until 36.4 days p.i., and thereafter almost no expulsion occurred. Data on both humoral and tissue-specific cellular immune responses collectively indicated that antibody production in chickens with multispecies ascarid infections was triggered by Th2 polarisation. Local Th2 immune responses and mucin-regulating genes were associated with the regulation of worm expulsion.

2.3 Morphological studies on *Raillietina tetragona*

Elowni *et al*. (1989) examined the effect of niclosamide on *Raillietina tetragona* as it has poor or variable activity against some cestodes. Since the niclosamide had clearly not removed the worms from the birds at either 7 days of age or 17 days of age, it can only have caused destrobilation, leaving intact scoleces in the gut. These small scoleces were so deeply embedded and firmly attached in the intestinal mucosa that very few were found at necropsy 24 h after treatment. Presumably they began to grow again when treatment ceased, so that the worms from birds exposed to niclosamide treatment were consistently smaller than worms of the same age from the untreated controls. Niclosamide was poorly absorbed from the gastrointestinal tract and so may not affect deeply embedded scoleces.

The destrobilation may also have been a manifestation of a drug-induced impairment of the parasite carbohydrate metabolism. The anticestodal activity of niclosamide has been attributed to inhibition of glucose absorption by the tapeworm and uncoupling of the oxidative phosphorylation process in mitochondria and destrobilation of cestodes was known to occur as a result of reduced carbohydrate in the host's diet.

Mu *et al*. (2009) compared the morphology and development of two species of *Raillietina* from chicken. The body of the two species consists of scolex, neck and strobilae. Each mature proglottid showed a set of male and female reproductive system and genital openings on one side. Testes located on both sides of the ovary and behind vitellarium. A complete worm of *R. echinobothrida* was shorter than *R. tetragona*, with a round scolex and suckers and short neck. The ovary looked like leaf and vitellarium was in kidney-shape. There were many acid particles and calcareous corpuscles in gravid proglottids. Egg capsule showed no clear boundary and contained only one egg. However, the scolex and suckers of R. tetragonal were oval in shape, and the neck was long and thin. The ovary was flower-like. Each egg capsule contained 4-12 eggs and many calcareous corpuscles, each of which was surrounded by a membrane. The male reproductive system matures first in both species. As the two reproductive systems matured, the proglottids became gravid after fertilization. The formation of egg capsule in the two species was similar.

Salam *et al*. (2010) undertook a research into *Raillietina cesticillus* infection in scavenging indigenous chicken in the Kashmir valley from January 2005 to December 2006. A total of 478 birds of different age groups and both sexes were randomly selected from 10 villages and screened through clinical, parasitological and pathoanatomical examinations. The study indicated that 23.22% (111/478) of the chicken were infected with *R. cesticillus* either singly or in association with other parasites - *Amoebotaenia sphenoides, Raillietina tetragona* and *Choanotaenia infundibulum*. Annual occurrence of the infection was found to be 24.03% (56/233) and 22.44% (55/245). There was a marked seasonal difference in load and mean intensity of infection. A histologically variable degree of degenerative changes was observed with more severe changes in heavy infestation. The inflammatory reaction was characterized by predominant infiltration of heterophils and lymphocytes.

Waghmare *et al*. (2014) Described on *Raillietina echinobothrida* (Pasquale, 1890) (Cestoda: Davaineidae) and Studied Conserved Domain across Divergent Phylogenetic Lineages of Class Cestoda. *Raillietina* (Fuhrmannetaa) echinobothrida, (Magnin, 1881) cestode parasite of *Gallus gallus domesticus* was redescribed on the basis of type material from Aurangabad, Marathawada, Maharashtra, India. The worms resembled with *R. echinobothrida*, (Magnin, 1881) in having all essential morphological characters. having scolex oval, rostellum elongated/rounded, presence of four suckers, short neck, mature proglottids were broader than long, testes rounded and excretory canal long tube. But the same differed due to number of testes.Butboonchoo *et al*. (2016) performed a research work on *Raillietina* species in domestic chickens (*Gallus gallus domesticus*) in Phayao province, northern Thailand. The identification of *Raillietina* has been based on morphology and molecular analysis. In that study, morphological observations using light (LM) and scanning electron microscopies (SEM) coupled with molecular analysis of the internal transcribed spacer 2 (ITS2) region and the nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1) gene were employed for precise identification and phylogenetic relationship studies of *Raillietina* spp. Four *Raillietina* species, including *R. echinobothrida, R. tetragona, R. cesticillus*, and *Raillietina* sp., were recovered in domestic chickens from 4 districts in Phayao province, Thailand. LM and SEM observations revealed differences in the morphology of the scolex, position of the genital pore, number of eggs per egg capsule, and rostellar opening surface structures in all 4 species. Phylogenetic relationships were found among the phylogenetic trees obtained by the maximum likelihood and distance-based neighbor-joining methods. ITS2 and ND1 sequence data recorded from Raillietina sp. appeared to be monophyletic. The query sequences of *R. echinobothrida, R. tetragona, R. cesticillus,* and *Raillietina* sp. were separated according to the different morphological characters. This study confirmed that morphological studies combined with molecular analyses can differentiate related species within the genus *Raillietina* in Thailand.

Simões *et al*. (2017) conducted a detail the morphology and morphometry of *R. celebenis* specimens collected in the municipality of São Gonçalo, Rio de Janeiro state, Brazil. They examined by light and confocal scanning laser microscopy and also report the results of molecular phylogenetic analyses to determine its relationships within the family

Davaineidae. Analysis of the number and size of testes, number and shape of rostellar hooks; cirrus sac length; capsules and eggs per capsule and morphology of the mature proglote allowed concluding that the present specimens constitute a new record of *R. celebensis* in South America. Our genetic and phylogenetic analyses, based on the partial small subunit (SSU) 18S rRNA gene, revealed *R. celebensis* to be in the Davaineidae family within the *Raillietina* genus, in agreement with the morphological taxonomy. Phylogenetic trees obtained by neighbor-joining and maximum likelihood methods demonstrated *R. celebensis* as a unique taxonomic unit, but also some taxonomic inconsistences. The incorporation of Brazilian *R. celebensis* sequences derived from mammals in the phylogeny of davaineids was consistent with the assertion that neither *Raillietina* nor *Fuhrmannetta* can be supported as a distinct genus.

2.4 Morphological studies on *Echinostoma revolutum*

Kanev (1994) completed an experiment with infected snails collected at the type-locality, near Erlangen, Germany. Based on the specimens obtained, each stage of the life-cycle had been redescribed. Important taxonomic features were discussed and hitherto unknown characteristics were described. Based on extensive experimental life-cycle studies beginning with infected snails from type-localities, it was shown that the first intermediate host was a lymnaeid snail; the second intermediate hosts were various pulmonate and prosobranch snails, mussels, frogs and freshwater turtles; the final hosts were birds. *E. revolutum* adults had 37 collar spines and specific characteristics were expressed only in the larvae and the host-parasite relationships. The adults of *E. revolutum* could not be identified using morphological criteria and it was proposed that worms with 37 collar spines belonging to the genus *Echinostoma* and occurring in naturally infected birds in Europe and Asia be referred to an *E. revolutum* group.

Chantima el al. (2013) investigated the occurrence of 37-collar spined echinostome metacercariae in freshwater snails in 6 districts of Chiang Mai Province, Thailand, from October 2011 to April 2012. A total of 2,914 snails that belong to 12 species were examined, and 7 snail species (*Clea helena, Eyriesia eyriesi, Bithynia funiculata, Bithynia siamensis siamensis, Filopaludina doliaris, Filopaludina sumatrensis polygramma,* and

Filopaludina martensi martensi) were found infected with echinostome metacercariae. The prevalence of metacercariae was the highest in Filopaludina spp. (38.5-58.7%) followed by *B. funiculata* (44.0%), *E. eyriesi* (12.5%), *B. siamensis siamensis* (8.2%), and *C. helena* (5.1%). Metacercariae were experimentally fed to hamsters and domestic chicks, and adult flukes were recovered from both hosts at days 15 and 20 post-infection. The adult flukes were identified based on morphological features, morphometrics, host-parasite relationships, and geographical distribution. They were compatible to *Echinostoma revolutum* or *Echinostoma jurini*, with only minor differences. As the adults were recovered from both hamsters and chicks, our specimens were more compatible to *E. revolutum* rather than *E. jurini* (reported only from mammals). This was the first report for metacercariae of *E. revolutum* in the snail host, *C. helena,* and also confirmed that *Filopaludina* spp., E. eryresi, and *Bithynia* spp. act as the second intermediate hosts of *E. revolutum* under natural conditions, which were indigenously distributed in Chiang Mai province.

Georgieva *et al*. (2014) conducted a study through an integration of morphological and molecular approaches in the investigation of a dataset with larger taxonomic and geographical coverage. More than 20,000 freshwater snails belonging to 16 species were collected during 1998 to 2012 from various localities in eight countries in Europe. Snail screening provided representative larval wasolates for five species of the revolutum group, identified by their morphology. Adult wasolates for four species recovered from natural and experimental infections were also identified. Partial fragments of the mitochondrial nad1 and 28S rRNA genes were amplified for 74 and 16 wasolates, respectively; these were analysed together with the sequences of *Echinostoma* spp. available on GenBank. Delineation of the European *Echinostoma* spp. was carried out based on molecular, morphological and ecological data. The large-scale screening revealed infections with five Echinostoma spp., including one new species: *E. revolutum* (sensu stricto), *E. miyagawai, E. paraulum, E. bolschewense* and *Echinostoma* n. sp. The newly-generated nad1 sequences from Europe fall into six distinct, well-supported, reciprocally monophyletic lineages corresponding to the species identifications based on morphology; this was corroborated by the 28S rDNA sequences. The analyses of the total nad1 dataset provided evidence for 12 monophyletic groups and five singletons, which

represent seven described/named species and ten cryptic species-level lineages of *Echinostoma*. They concluded that nad1 should be the first choice for large-scale barcode-based identification of the species of the revolutum group.

Mohanta *et al*. (2018) Precised a discrimination of *Echinostoma* species within the 'revolutum' group was quite difficult because of their morphological similarities. The study was to precisely characterize the echinostomes of ducks from Bangladesh based on both morphological and molecular characteristics. Two *Echinostoma* species were identified: *E. revolutum* and *E. robustum*. In the phylogenetic trees (ITS2 and nad1), *E. revolutum* and *E. robustum* belonged to their respective Eurasian clade, which was distinct from the American clade. These results suggested that both species have two distinct and geographically separated lineages, Eurasian and American. Their molecular and morphological data combined with previously published data supports the synonymy of *E. robustum, E. miyagawai*, and *E. friedi* previously based on either molecular or morphological evidence.

2.5 Morphological studies on *Catatropis verrucosa*

Kanev *et al*. (1994) had been redescribed the life-cycle of *C. verrucosa* from infected snails collected along the River Danube in Europe. Taxonomic problems were discussed and the main features of the species were listed. Based on experimental life-cycle studies, the following facts were demonstrated. The first intermediate hosts were the prosobranch freshwater snails *Bithynia tentaculata.* The same snails were also first intermediate hosts for *Notocotylus imbricatus*. In all these species, the species characteristics were expressed by the adult morphology only, and the larvae could not be identified by morphological criteria. It was proposed that tri-oculate monostome cercariae found in naturally infected *B. tentaculata* and *B. leachi* be referred to as Cercaria imbricata group. There was no second intermediate snail host in the life-cycle of *C. verrucosa*. The final hosts were birds. The adult worms possess, on the ventral body surface, a median ridge and two lateral rows of 12 (range 11–14) papillae per row.

Birmani *et al*. (2011) identified two trematodes of the genus *Catatropis* recovered from intestine of host bird. During that study on the helminth parasites of Black Coot in Sindh Province of Pakistan, the detailed study of the worms resulted the lack of some diagnostic characteristics for the identification up to the species level. Therefore, these worms were identified up to the generic level.

Rolf and Gudrun (2012) identified five out of 15 notocotylid trematodes free-ranging Northern shovelers (Anas clypeata Linneus) in Pakistan. Out of the 31 flukes, 10 specimens were used morphological studies, 4 others were also examined by scanning electron microscopy and one remaining trematode was cut in serial semi-thin sections for histological evaluation in order to describe a new species. Like all species of this genus, *Catatropis pakistanensis* n. sp has a median ridge starting posterior to the basis of the cirrus sac and extends posterior to the ovary. Bilateral to this ridge there were two rows of 9–10 ventral papillae each. Metraterm and cirrus sac were equally in length. In contrast to most other *Catatropis* spp. the genital opening in *C. pakistanensis* was situated between the oral sucker and bifurcation of the caeca.

2.6 Prevalence of Gastrointestinal Helminths of Poultry

Phiri *et al*. (2007) examined the helminths from gastrointestinal tracts of 125 free-range chickens in Zambia and revealed a 95.2% prevalence rate. The species and their prevalences were: *Allodapa suctoria* (85.6%), *Tetrameres americana* (80.8%), *Ascaridia galli* (28.8%), *Gonglonema ingluvicola* (50.4%), *Raillietina* spp. (81.6%) and *Heterakis gallinarum* (32.8%). No trematodes or *Syngamus trachea* were found. Mixed infections accounted for 88.2% as compared to 7.2% of single infections. Effects of helminthoses on weight gain were investigated in 100 growing chickens randomly assigned to treatment and untreated control groups. There was a significant mean (+/- SEM) weight gain (gms) of 812.8 +/- 51.4 in the treatment group and 623 +/- 57.4 in the control group ($p < 0.01$). The mean (+/- SEM) worm burdens from the control group and the treatment group were 96.3 +/- 5.61 and 22.05 +/- 2.61, respectively.

Komba *et al*. (2013) carried out a study to determine the presence of intestinal helminths in apparently healthy free range local chickens slaughtered at Morogoro live bird market so as to establish the magnitude of the problem. A total of 252 intestines of slaughtered chickens were examined during the survey. Helminths were recovered and identified using standard methods. Two hundred and twenty chickens (87.3%) were infested with helminth species. In that survey, nine different helminth species were recovered, namely; *Ascaridia galli* (10.5%); *Heterakis gallinarum* (5.3%); *H. wasolonche* (3.9%); *Capillaria* spp. (2.6%); *Raillietina echinobothrida* (38.2%); *R. tetragona* (34.2%); *R. cesticillus* (2.6%); *Choanotaenia infundibulum* (1.3%) and *Hymenolepsis cantaniana* (1.3%). The predilection site for the cestodes was the small intestines except for *H. cantaniana* which was also recovered from the large intestines. With the nematodes, *Capillaria* spp. and H. wasolonche were recovered from the caeca, *H. gallinarum* from small and large intestines and *A. galli* from all intestinal parts.

Naphade and Chaudhari (2013) conducted a study of seasonal prevalence of parasitic helminths in broiler chickens from Marathwada region of Maharashtra. The study was conducted during, annual cycle June 2012 to May 2013 from different sampling station and different season to estimate the seasonal prevalence of parasitic helminths. For this study annually 279 broiler chickens were randomly selected from different part of Marathwada region under managed systems and different season. A simple salt floatation method was employed for examination of parasitic helminths. After examined the intestine of the chickens the overall prevalence found 46 (16.48 %) during the annual cycle. The seasonal prevalence percentage of parasitic helminths was highest during summer (19.53 %) followed by rainy (16.51 %) and lowest during winter (13.21 %) season. There was difference found in the seasonal prevalence. The average helminth parasite found in the broiler chickens 15 (5.37 %) cestode and 20 (7.16 %) nematode while the mixed infestations was 11 (3.94 %). It was found that the percentage of seasonal prevalence of cestode (Summer: 6.57 %), (Rainy: 5.12 %), (Winter: 4.46 %), nematode (Summer: 8.60 %), (Rainy: 7.17 %), (Winter: 5.46 %) and mixed infection (Summer: 4.35 %), (Rainy: 4.21 %), (Winter: 3.27 %) was highest during summer followed by rainy and lowest during winter season. The major helminth parasites was found in the broiler chickens include *Raillietina* spp. (4.30 %) and *Ascaridia* spp.(6.81 %).The results of

parasitic helminths were discussed in relation to seasonal variation and found highest during summer followed by rainy and lowest during winter season.

Mekibib (2014) investigated the prevalence of gastrointestinal helminth of scavenging chicken in villages around Hawassa, Southern Ethiopia, from October 2010 to April 2011. A total of 360 faecal samples and 122 postmortem examination were conducted. The overall postmortem and coproscopic prevalence of scavenging chicken gastrointestinal helminthes (GIT) were 88.5% and 77.8%, respectively. In the examined scavenging chicken about 67.5% and 29.2% of the chickens were positive for nematodes and cestodes species, respectively the postmortem examination revealed 51.6% infection with *Heterakis gallinarum* followed by *Ascarida galli* (45.9%), *Raillietina tetragona* (20.5%), *Raillietina echinobothrida* (17.2%), *Capillaria* species (13.1%), *Raillietina cesticillus* (8.2%) and *Hymenolepis cantanian* (3.3%). There was a significant difference in the overall prevalence of GI helminth parasites observed between male and female and between age groups of chickens ($P < 0.05$ and $P < 0.01$, respectively).

Alam *et al*. (2014) observed the prevalence of gastrointestinal helminth infections and the gross pathological lesions produced by them from February 2012 to January 2013 in the Department of Pharmacology of Bangladesh Agricultural University, Mymensingh. In that study, a total of 320 indigenous chickens aged ranging from 2 to 4 months were examined to identify the different types of gastrointestinal helminth infections in indigenous chickens. During routine examination, six species of helminth parasites were recorded, of which five species of nematodes such as *Ascaridia galli, Heterakis gallinarum, Capillaria* spp, *Acuaria hamulosa* and *Dispharynx spiralis*; and one species was cestode called *Raillietina tetragona*. The highest prevalence was observed for *Ascaridia galli* (41.56%) followed in descending order by *Raillietina tetragona* (19.68%), *Heterakis gallinarum* (15.62%), *Acuaria hamulosa* (8.75%), *Capillaria* spp. (4.68%) and *Disopharinx spiralis* (1.56%). The gross pathological lesions were observed in case of *Acuaria hamulosa* and *Heterakis gallinarum* infection. In case of *Acuaria hamulosa* infection keratinization of gizzard mucosa and cross section of adult *Acuaria hamulosa* were seen along with marked infiltration of neutrophils. The results of this study

suggested that both nematodes and cestodes were highly prevalent in indigenous chickens in the studied werea.

Lucas (2014) comprehend the possible role of helminth infections of poultry and the prevalence of helminth infections in slaughtered chickens was investigated. A total of 305 gastrointestinal tracts were collected from slaughtered chickens, 177 and 128 of which were from broilers and layers respectively. The intestines were longitudinally incised and the contents washed into cups for the recovery of worms and worm eggs using standard parasitological methods. Results showed that helminth infections were common in the grown birds. *Ascaridia galli*, *Heterakis* spp. and *Raillietina* spp. were common with the prevalence of 22.3, 0.6 and 6.2% respectively. However, there was a breed discrepancy in prevalence particularly in *A. galli* in which layers had a higher prevalence (34.4%) than broilers (14.1%). It was concluded that the risk of helminths infections was high in grown birds intensively managed in deep litter in the study werea and that it could be the same in similar environments where poultry were managed on deep litter and could compound diagnosis of other health problems of chickens.

Amaral and Alberto (2016) conducted a servey on nematodes of the genus *Ascaridia*, important endoparasite of poultry. It was known *Ascaridia galli* was one of the most common nematode worldwide that (round worm) affecting chickens, however there had never been a study conducted to measure the prevalence of this round worm in Timor Leste. To measure the prevalence of this parasite a survey was conducted in 9 villages across three regions of Timor Leste. The survey revealed that *A. galli* were 5.9% (2.2-12.5%) positive in Covalima municipality, 3.1% (0.6 - 8.9%) in Manatuto Municipality and 15.4% (8.1 - 23.0%) in Lospalos Municipality. The overall prevalence for Timor Leste was 7.8% (5.0 - 11.5%).

Afolabi *et al*. (2016) conducted a survey of intestinal parasites of chickens, carried out in Akure, Ondo State, Nigeria from January to December, 2015. A total of 327 chickens of different breeds were examined for gastrointestinal infections. Fecal samples obtained from these chickens were prepared for microscopy using flotation technique. The results showed that 67 (20.5%) of the 327 chickens examined were infected with various gastrointestinal parasites. Among the infected chickens, the layers were the most

susceptible to gastrointestinal parasites with a prevalence of 88.4%, while broilers were the least susceptible with a prevalence of 7.2%. It was further observed that the highest prevalence of gastrointestinal infection (37.6%) was recorded among the chickens that were kept in an extensive management system, while the lowest prevalence (9.6%) was recorded among the chickens kept in an intensive management system.

Ogbaje *et al*. (2016) conducted a survey to determine the prevalence of gastrointestinal helminthes in local chickens, broilers and layers slaughtered in Makurdi metropolis between September 2007 and April 2008. A total of 440 samples were collected from male and female chickens. Of the total samples examined, 200(45.5%) were from domestic chicken, 140(31.8%) from broilers and 100(22.7%) from layers. Of the total sample examined, 280(63.6%) were infected with one or more species of helminthes. Of the number positive for infections, 103(23.4%) had single infection, 105(23.9%) double infections and 60(13.6%) triple infections. Overall, 165(37.5%) of the samples had *Ascaridia galli*, 122(27.7%) had *Heterakis gallinarum* and 214(48.6%) had various tapeworm species. Out of the 200 samples from domestic chickens, 110(55%) were found infected with *Ascaridia galli,* 80(40%) with *Heterakis gallinarum* and 145(72.5%) with different tapeworm species. Of the 140 gastrointestinal tracts from broilers, 50(35.7%) were infected with *Ascaridia galli,* 40(28.6%) with *Heterakis gallinarum* and 60(42.9%) with various tapeworm species. Out of the 100 gastrointestinal tracts from layers, 5(5%) were infected with *Ascaridia galli,* 2(2%) with *Heterakis gallinarum* and 9(9%) with various tapeworm species. The species of tapeworm encountered were *Raillietina* species, *Choanotaenia* species and *Hymenolepis* species. These respective species constituted 30.9%, 5.2% and 3.6% of the tapeworm burdens.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sampling Area

The intestines of the slaughtered chickens were collected from the rural areas of four districts of Bangladesh, namely Dhaka (36), Bandarban (10), Mymensingh (7) and Pabna (10). This experiment was performed in the Microbiology & Parasitology Laboratory at the Sher-e- Bangla Agricultural University, Dhaka.

3.2 Collection of samples

Around noon on each sampling day, a batch of 6 intestines were randomly picked from a group of intestines of chickens slaughtered between early morning and at the time of collection. On sampling days, the gastrointestinal tracts (GIT) were placed in separate, labelled polythene bags and transported to the laboratory maintaining a cool chain protocol.

The samples were used for the isolation of helminthes. The gastrointestinal tracts were separated into small intestine, caecum and large intestine. The entire length of each intestine was incised longitudinally and the contents were emptied into sieves placed in large clean plastic cups with labelling. Contents were washed in normal saline and examined under a light microscope. Larger helminths were collected directly by curved needle or forceps, and smaller ones were isolated under the microscope. Worms were grouped and counted with morphometrically before being stored in plastic bottles containing 70% alcohol according to the method described by Permin and Hansen (1999).

3.3 Processing of cestodes and trematodes

In case of trematodes and cestodes, stained permanent slides were made. For this purpose, trematodes and cestodes were collected from plastic bottles containing 70% alcohol. Then the specimens were flattened between two glass slides with slight pressure and fixed in 70% alcohol until future works.

After flattening for a week, the specimens were dipped in 50% alcohol for one hour and then into distilled water for another one hour. Then the specimens were transferred in Haematoxylin-Carmine solution and kept overnight for staining. The excessive stain was removed by 3% HCl-Alcohol. The stained specimens were washed with ascending grades of alcohol for hardening, cleared in xylene and mounted in canada balsam. Finally, the slides were kept until the canada balsam dried, and observed under microscope.

3.4 Processing of nematodes

In case of nematodes, the specimens were not stained because the nematodes were very thin and transparent. Before observation, the nematodes were washed well in water to remove the preservatives, dehydrated in 70 - 90 % alcohol as per the thickness of the worm and cleared by submerged them in lactophenol. Then the nematodes were examined under microscope and photographs were taken from different body parts as an aid for identification.

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS

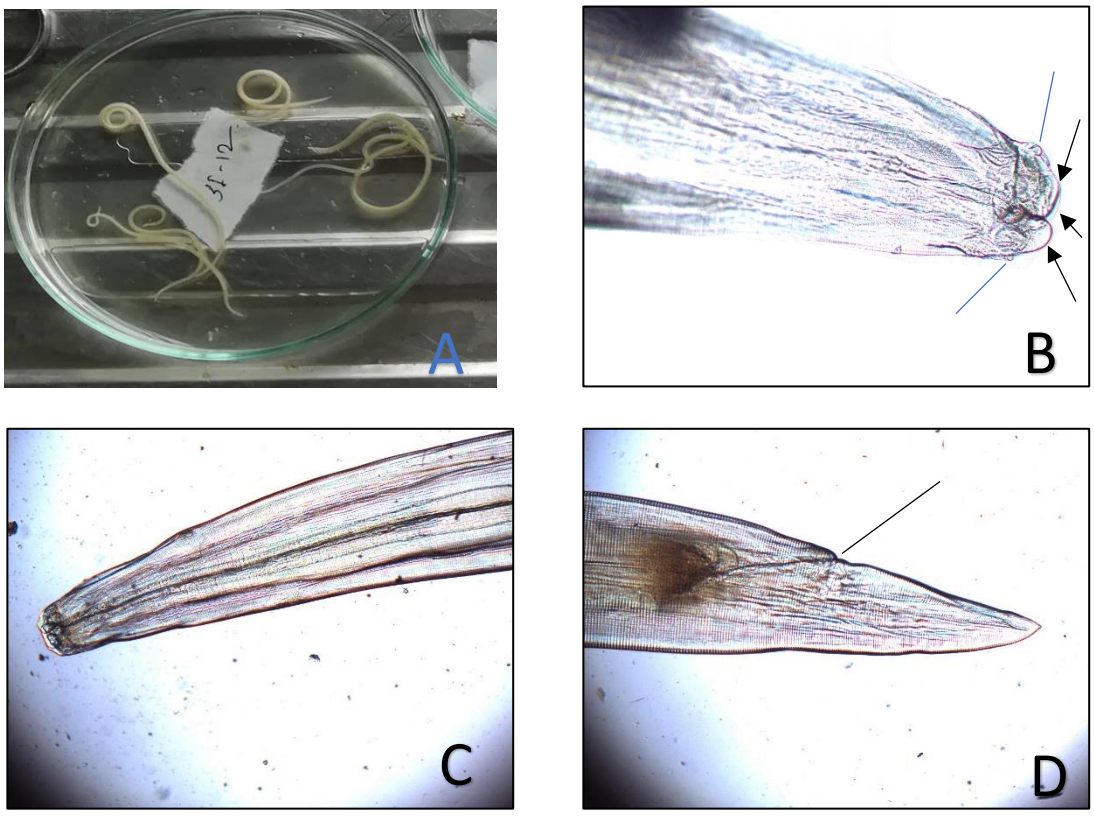
Through the examination of all 63 samples, five helminths were confirmed by observing them under light microscope. These include 2 species of nematode (*A. galli* & *H. gallinarum*), 2 species of trematodes (*E. revolutum* & *C. verrucosa*) and only 1 species of cestode (*R. tetragona*).

4.1 Morphological observation

4.1.1. Morphology of *A. galli*

The specimens were recovered from both small and large intestines. Adult worms were yellowish white in color and semitransparent (Figure 1A). The oral opening is surrounded by three prominent lips which are trilobed (Figure 1B). These features match with the Order Ascaridida. The esophagus was filariform and the intestine is simple, which are the special feature of Family Ascarididae. The whole body was enclosed in a tough proteinaceous covering called cuticle. The cuticle was distinctly striated and the cuticular alae were feebly developed (Figure 1C). Two conspicuous papillae occured on the dorsal lip and one on each of the subventral lips (Figure 1B). A pair of so-called neck papillae occured on the sides of the body near the anterior end. Morphologically sexual dimorphism in ascarids was characterized by ventrally coiled tail with precloacal sucker in males, and a blunt and rounded posterior end in females. The posterior portion of female also possessed a single large anal opening just before the extremity and possessing one pair of papillae just near to its tip. The tail end was rather straight and blunted (Figure 1D). The posterior end of male was comparatively elaborate and more complex (Figure 1E). There were two prominent apertures, anus towards the posterior end and precloacal

or preanal sucker immediately anterior to the spicules. The precloacal sucker was supported by a sclerotized ring which serves the functions as an aid to attach during copulation. The worms had two well-developed unequal spicules at the posterior end (Figure 1F). The uteri of females were packed with eggs which were oval to ellipsoidal, with a thick, smooth shell, containing a single cell. All of these morphological characteristics are corresponded to the Genus *Ascaridia,* and the Species *A. galli.*



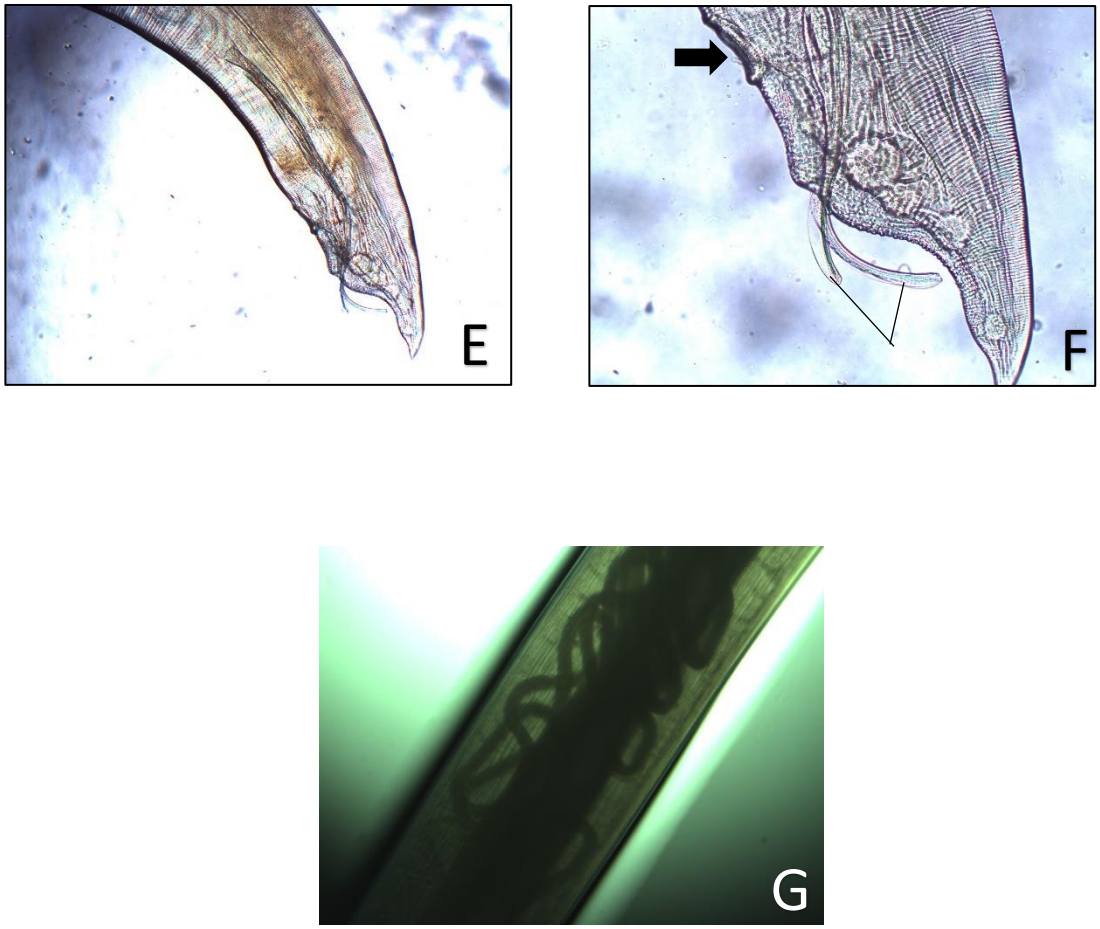


Figure 1: Different body parts of *A. galli*, **A.** Gross sample of *A. galli.* **B.** Anterior part of the parasite; the black arrow indicates the lips and the blue lines indicate the papillae on the lips. **C.** Cuticular striation. **D.** Posterior part of the female; the black line indicates anal opening. **E.** Posterior part of the male (4X). **F.** Posterior part of the male (10X); the black lines indicate the unequal spicules and the block arrow indicates the precloacal or preanal sucker. **G.** Uterus of the female.

4.1.2 Morphology of *H. gallinarum*

The adult worms were collected from large intestine, specially from the caecum. Adult worms were small and white in colour and had three well-defined lips, which are the general characters of the Order Ascaridida. The head end was slightly curved (Figure 2A). The esophagus was engaged with a short narrow anterior portion (pharynx) and ended in a well-developed bulb containing a valvular apparatus (Figure 2B, 2C). These are the common features of Family Heterakidae. The cuticle was usually with lateral flanges. Alae, which ran almost the entire length of the body, were ridges formed by the thickening of the cuticle. Adult female and male caecal worms differed in length, with the female (10 to 15 mm) generally being larger than that of the male (7 to 13 mm). The tail end of female was elongated, gradually tapered. The anal opening was at the posterior part of body. The vulva of the female was located at the middle of the body (Figure 2D). There were three bends in the vagina after the vulva, angled posteriorly, anteriorly and once again posteriorly. Male worms had stylet-like tail end that smoothly taper. The worms had two well-developed unequal spicules at the posterior end (Figure 2E). Gubernaculum is absent. The preanal sucker was easily seen, round, well-developed, surrounded by a chitinized ring. Eggs in the uterus were ellipsoidal, with a thick, smooth shell, containing a single cell (Figure 2F). Each of every morphological characteristic are almost identical to the Genus *Heterakis*, and Species *H. gallinarum.*

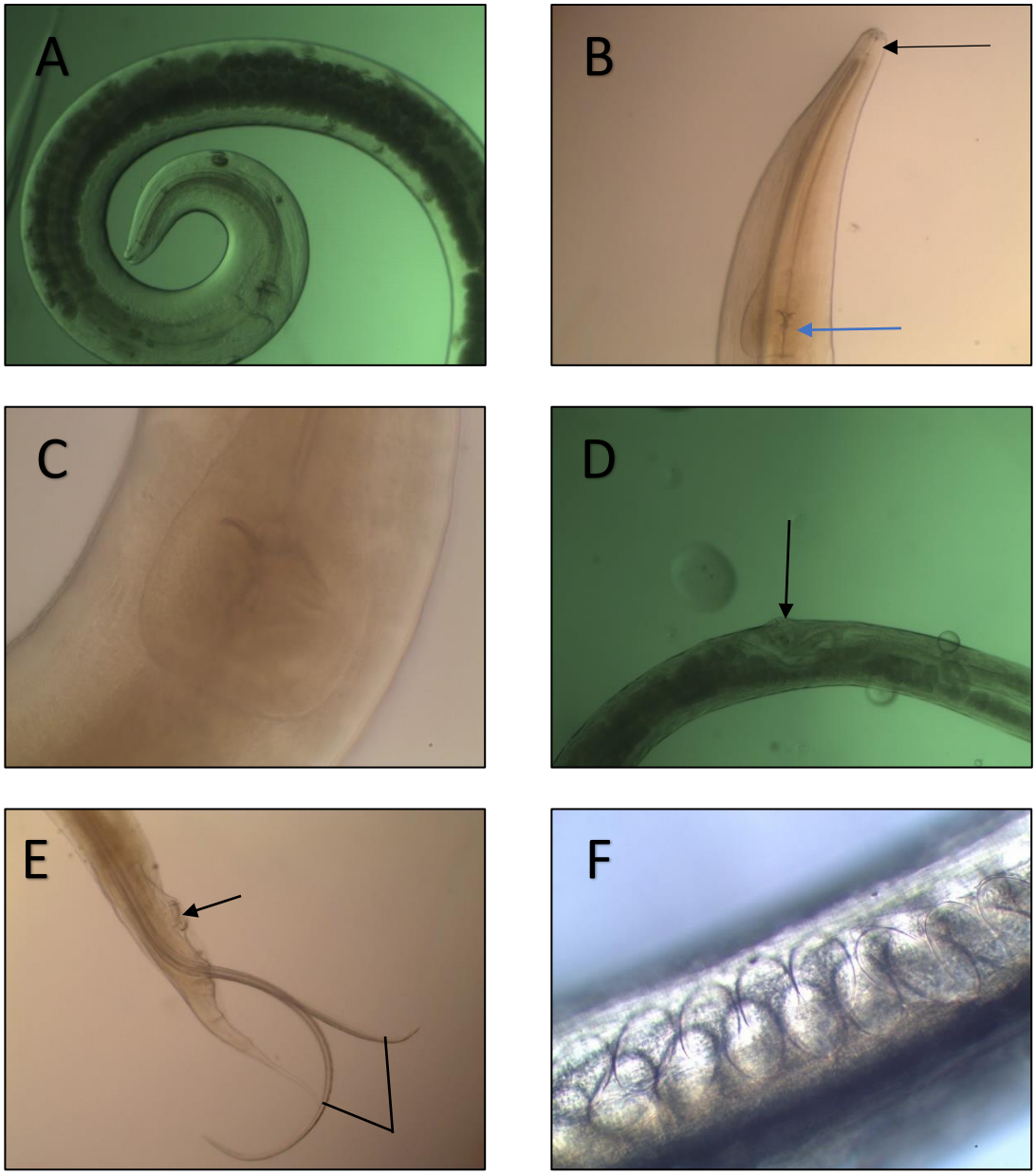


Figure 2: Different body parts of *H. gallinarum*, **A.** Slightly curved head end. **B.** Anterior part of the parasite; the black arrow indicates the lips and the blue arrow indicates the bulb shaped esophagus. **C.** Bulb shaped esophagus in high magnification (40X). **D.** Vulvar opening of the female (the black line). **E.** Posterior part of the male (10X); the black arrow shows the preanal sucker and the black line shows the unequal spicules. **F.** Multiple eggs in the uterus of female.

4.1.3 Morphology of *R. tetragona*

Multiple mature cestodes, measuring 12-30 cm long were isolated from both small and large intestines. These cestodes were whitish in color, highly elongated, dorso-ventrally flattened (Figure 3A). They had multiple segments, bearing four suckers on their scolices. These are the identical features of the Order Cyclophyllidea. The body is divided into the head region called 'scolex', followed by an unsegmented 'neck', and then by highly segmented body proper called strobila. The strobila is composed of a series of ribbon-like body segments called proglottids, gradually enlarging from the anterior end towards the posterior. The scolex bears an apical rounded rostellum, which is medium and armed with many minute hooks, arranged in single row. The hooks are hammer-shaped. This is surrounded by four ovoid suckers which are lined with several rows of spines (Figure 3B, 3C). These are the morphological features of Family Davaineidae. The mature segment is longer than broad and the common genital pores are single and being in front of the anterior 1/3 of the lateral margin of the mature segment. Each mature proglottid has a set of male and female reproductive organ (Figure 3D). Testes are located on both sides of the ovary and behind vitellarium. Each egg capsule contained 4-12 eggs and many calcareous corpuscles, each of which is surrounded by a membrane. These features are matched with the identical morphological characteristics of the Genus *Reillietina*, and Species *R. tetragona*.

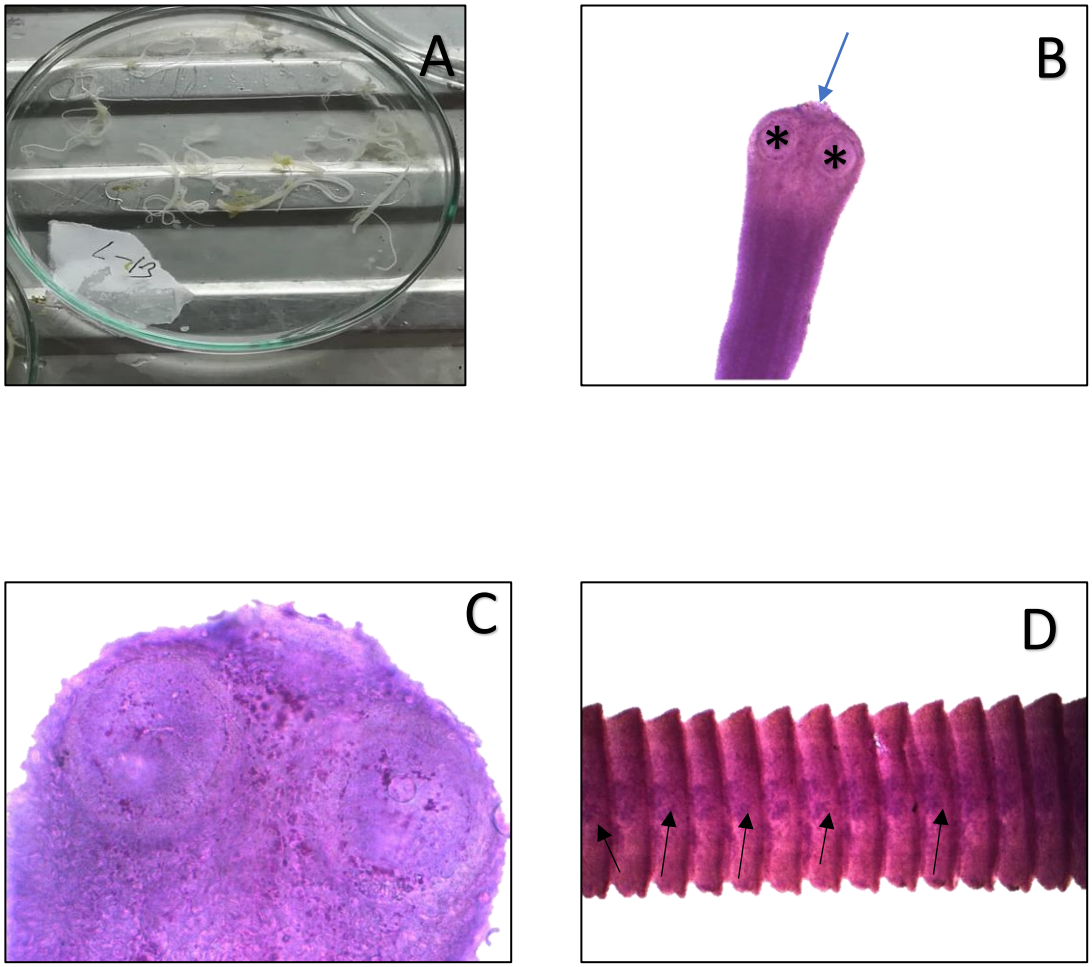


Figure 3: Different body parts of *R. tetragona.* **A.** Gross sample of parasite after collection. **B.** Anterior part of the parasite (10X); the blue arrow indicates rostellum and the "*" indicate the suckers. **C.** Scolex in high magnification (40X); **D.** The broad segment having common genital pores on each (black arrows); Each mature proglottid has a set of reproductive organ in the middle part.

4.1.4 Morphology of *C. verrucosa*

A large number of minute flukes were recovered from the caecum of the poultry intestine. Small muscular body was dorsoventrally flattened, attenuated anteriorly and broadly rounded posteriorly. Pharynx was absent and the esophagus was very short (Figure 4A). These are the identifying feature of Order Plagiorchiida. Cup-shaped oral sucker was terminal (Figure 4C). There was no ventral sucker which are the morphological feature of Family Notocotylidae. Adult flukes were 1.5-2.0 cm long and 0.5-1.0 cm wide. Long caeca were bifurcated, smooth, extending posteriorly between the uterine loops and vitelline follicles, then passed through the testes and ovary, and finally terminated blindly at the level of excretory pore. Cirrus sac was elongated, containing prostatic cells and coiled seminal vesicle. Genital pore was median, closely posterior to the caecal bifurcation (Figure 4A). There were two testes which are irregularly lobed, located in extracaecal field in posterior third of the body. Ovary was trilobed, situated at the testicular level (Figure 4D). Uterus had a number of closely packed loops, overlapping cirrus sac, reaching up to the level of Mehlis gland. Uterine loops were 18 in number (Figure 4B). Vitellaria was fairly composed of large follicles arranged extracaecally but at some places it overlaps the ceca, extending from the anterior third of the testes up to the anterior third uterine loop, which are the special morphological characteristics of the Genus *Catatropis*, and Species *C. verrucosa.*

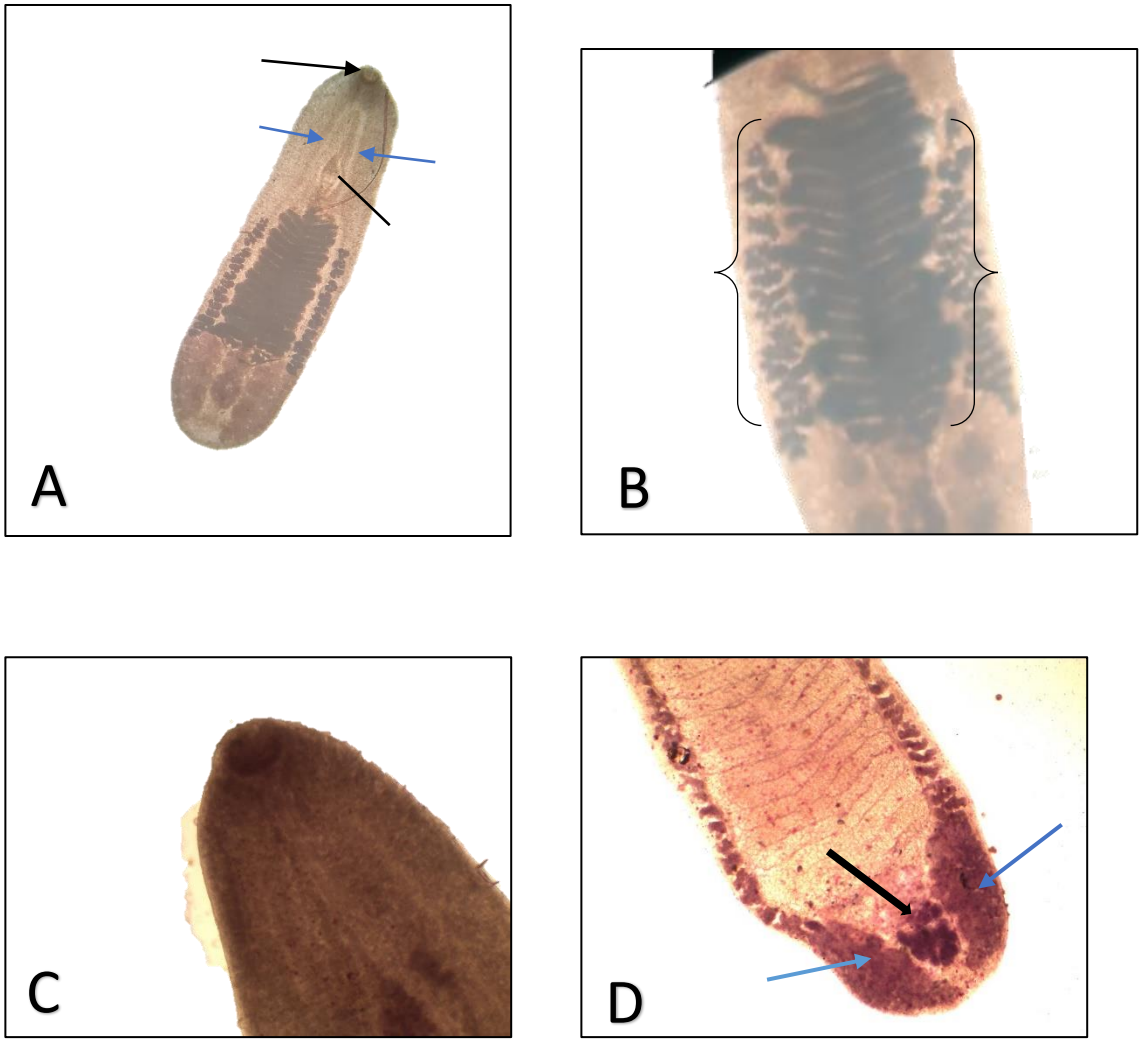
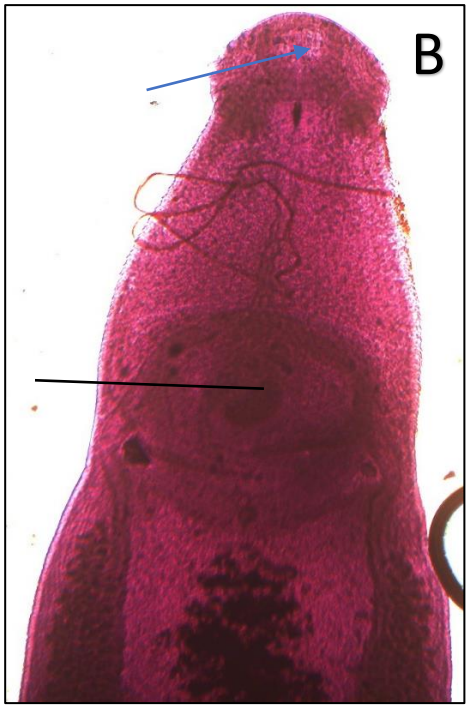


Figure 4: Different body parts of *C. verrucosa*. **A.** Whole fluke under 4X magnification; the black arrow indicates the oral sucker, the blue arrows indicate caecal bifurcation and the broken line indicates the cirrus sac. **B.** Uterine loops (18 in numbers) of the parasite (10X); both side of the loops covered by vitellaria. **C.** Cup shape oral sucker (10X); **D.** The reproductive organs of parasites; the blue arrows indicate testes and the block arrows indicates the ovary.

4.1.5 Morphology of *E. revulotum*

The echinostomes were recovered from the large intestine of poultry. The Body was muscular, dorsoventrally flattened and c shaped in appearance (Figure 5A). Pharynx was absent and the esophagus was very short All flukes were 6-8 mm long and 1.5-2 mm wide and had a well-developed head collar bearing 37 spines (5 angle spines and 6 lateral spines on each side and 15 dorsal spines) (Figure 5D, 5E). The esophagus was shorter than the diameter of the ventral sucker (Figure 5B). These morphological properties matched those of the members of 37 collar spined echinostomes (*E. revolutum* group). Adult flukes had a short forebody and did not have constriction at the level of the ventral sucker. There are two testes, arranged in a tandem position, located at the posterior part of the body. Testes were elongated with smooth margin and slightly separated from each other. The anterior testis was shorter and wider than the posterior testis. The ovary was oval, median, and transversely located between the posterior end of the uterus and cranial margin of the anterior testis (Figure 5C). The cirrus sac was oval and located transversely between the level of intestinal bifurcation and anterior border of ventral sucker. Multiple eggs are located in the uterus. The eggs are oval, large, thin shelled, operculated and contain unsegmented ovum (Figure 5F) which are the special morphological characteristics of the Genus *Echinostoma*, and Species *E. revolutum.*


A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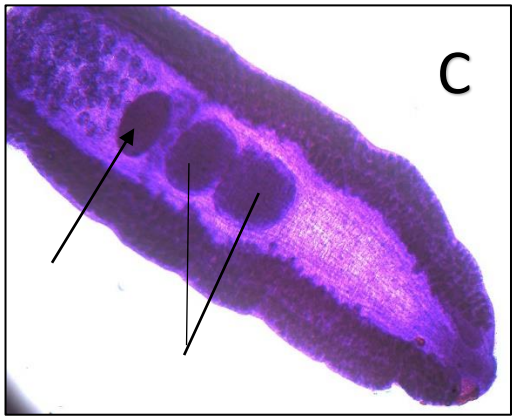
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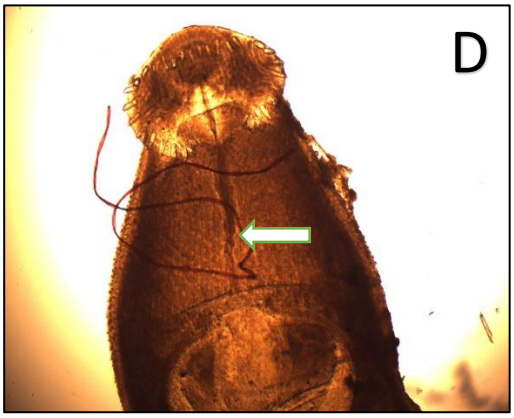
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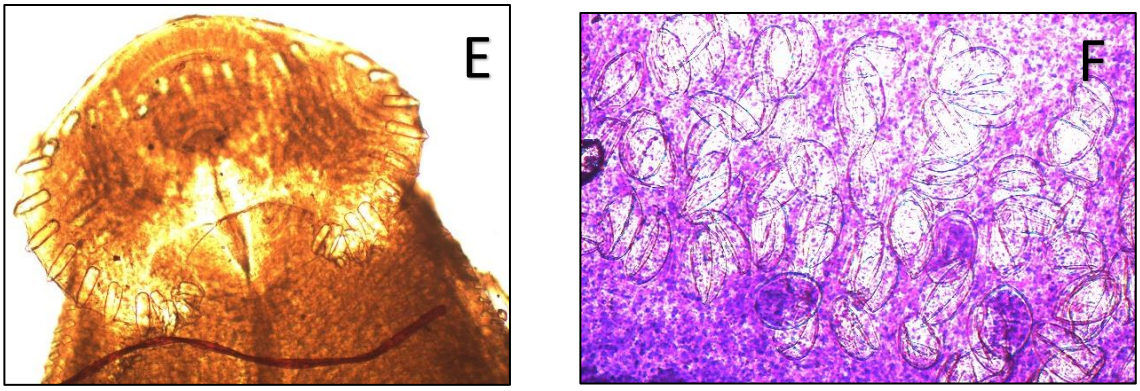

Figure 5: Different body parts of *E. revolutum.* **A.** Gross sample of the fluke occurring c-shaped. **B.** Anterior part of the fluke; the black arrow indicates the oral sucker and the broken line indicates the ventral sucker. **C.** Posterior part of the; the black arrow indicates the ovary and the broken lines indicate the testes. **D.** Oral sucker with short esophagus (white block arrow) and caecal bifurcation. **E.** The 37 spines are distinct around the oral sucker. **F.** Numerous eggs in the uterus.

4.2 Prevalence

4.2.1 Overall prevalence of helminths in backyard chicken

The study was carried out in a total of 63 gastrointestinal tract of backyard chicken. Out of the 63 examined samples, all of those were infected with various species of gastrointestinal helminths, comprising two species of nematode, two species of trematodes and only one species of cestode. Those parasites were found in different locations of the gastrointestinal tracts of backyard poultry.

The nematode parasites encountered were *A. galli* and *H. gallinarum* out of which *A. galli* (47.61%) was the most prevalent and *H. gallinarum* (38.09%) was the least. The trematode parasites recovered included C. *verrucosa* and *E. revolutum* out of which *C. verrucosa* (23.80%) was most prevalent followed by *E. revolutum* (7.93%). The cestode parasites encountered was *R. tetragona* (73.01%). The overall prevalence is shown in Table 1.

Table 1: Location and overall prevalence of helminths in backyard poultry

Class of helminths	Name	Location	No. of infected chicken	Prevalence
Nematode	*A. galli*	Small intestine & Large intestine	30	47.61%
	H. gallinarum	Caecum & Large intestine	24	38.09%
Trematode	*C. verrucosa*	Caecum	15	23.80%
	E. revolutum	Small intestine & Large intestine	5	7.93%
Cestode	*R. tetragona*	Small intestine	46	73.01%

4.2.2 Prevalence of helminths in different geography

The samples were collected from Dhaka (N=36), Pabna (N=10), Bandarban (N=10), Mymensingh (N=7). Total 5 species of helminths were recovered from Dhaka namely *A. galli* (44.44%), *H. gallinarum* (38.88%), *C. verrucosa* (33.33%), *E. revolutum* (13.88%) and *R. tetragona* (72.22%) which were given in the Table 2.

Table 2: Prevalence of gastrointestinal helminths in Dhaka (N=36)

Helminths	No. of infected chicken	Prevalence
Nematode		
A. galli	16	44.44%
H. gallinarum	14	38.88%
Trematode		
C. verrucosa	12	33.33%
E. revolutum	5	13.88%
Cestode		
R. tetragona	26	72.22%

Ascaridia galli (57.14%), *H. gallinarum* (42.85%), *C. verrucosa* (28.57%) and *R. tetragona* (100.00%) were collected from Mymensingh (Table 3) and *A. galli* (60.00%), *H. gallinarum* (50.00%), *C. verrucosa* (30.00%) and *R. tetragona* (60.00%) were collected from Pabna (Table 4). In those areas, there were no *E. revolutum.*

Table 3: Prevalence of gastrointestinal helminths in Mymensingh (N=7)

Helminths	No. of infected chicken	Prevalence
Nematode		
A. galli	4	57.14%
H. gallinarum	3	42.85%
Trematode		
C. verrucosa	2	28.57%
Cestode		
R. tetragona	7	100.00%

Table 4: Prevalence of gastrointestinal helminths in Pabna (N=10)

Helminths	No. of infected chicken	Prevalence
Nematode		
A. galli	6	60%
H. gallinarum	5	50%
Trematode		
C. verrucosa	3	30%
Cestode		
R. tetragona	6	60%

Ascaridia galli (40.00%), *H. gallinarum* (20.00%) *R. tetragona* (80.00%) were collected from Bandarban (Table 5). In those areas, no trematode was found.

Table 5: Prevalence of gastrointestinal helminths in Bandarban (N=10)

Helminths	No. of infected	Prevalence
Nematode		
A. galli	4	40%
H. gallinarum	2	20%
Cestode		
R. tetragona	8	80%

4.2.3 Organ Preferences of gastrointestinal helminths

The results showed that most of the parasites prefer to colonize the small intestine than the large intestine. Some of the parasites were found in the caecum. No parasite was recovered in the crop and gizzard. *A. galli*, the largest nematode of poultry, was mostly found in small intestine and *H. gallinarum* was found mostly in the caecum. Only cestode, *R. tetragona* was collected from small intestine.

Table 6: Site preferences of different gastrointestinal helminths (N=63)

Preferred sites	Parasites	No. infected	Percentage
Small intestine	*A. galli*	28	44.44%
	E. revolutum	3	4.76%
	R. tetragona	46	73.01%
Large intestine	*A. galli*	8	12.69%
	H. gallinarum	9	14.28%
	E. revolutum	2	3.17%
Caecum	*H. gallinarum*	22	34.92%
	C. verrucosa	15	23.80%

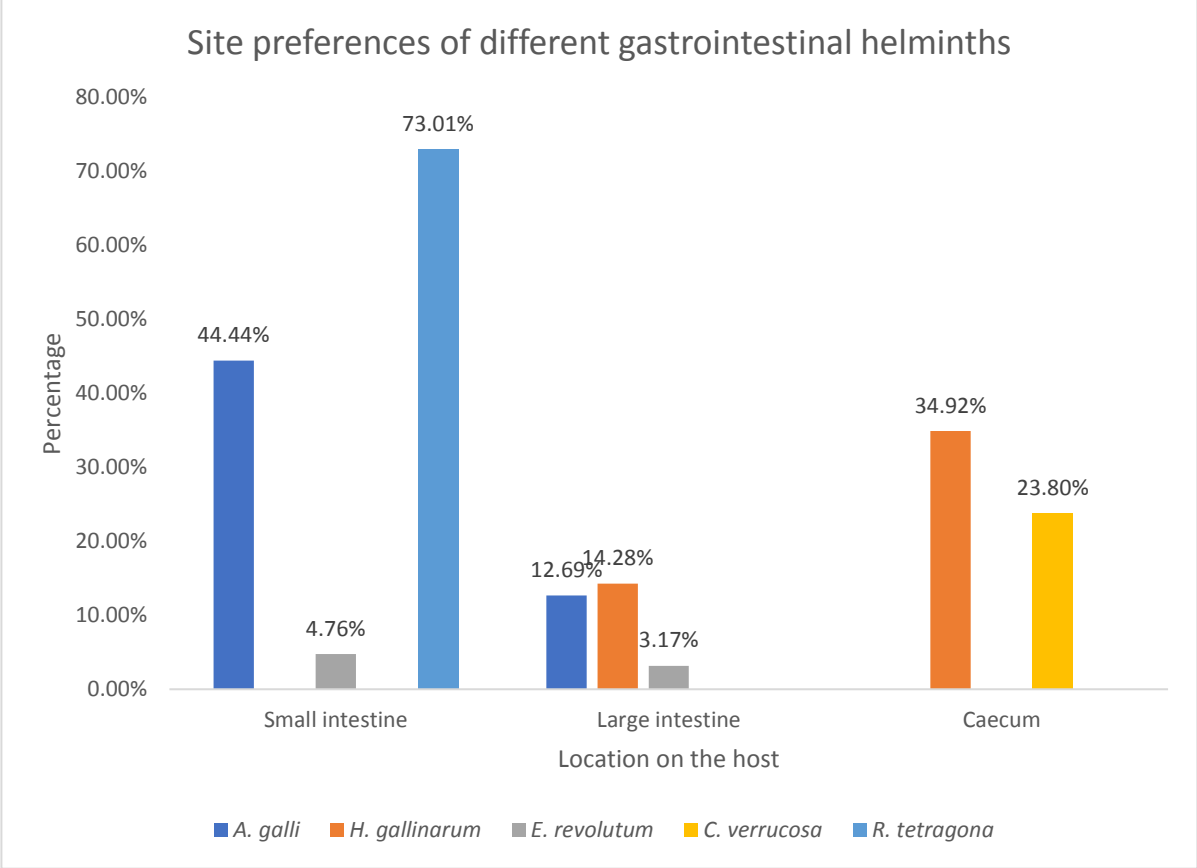

Figure 6: Site preferences of different gastrointestinal helminths

4.2.4 Single and mixed type of infection

Examined gastrointestinal tract of poultry were infected by one or more species of helminth parasites. Among the 63 chicken, 16 were infected with single species of helminths (25.39%) and rest 47 were infected with multiple species of helminths. In case of the multiple infection, 28 chicken were infected with two species of helminths (44.44%) and 19 chicken were infected with more than two species of helminths (30.15%).

Table 7: Percentages of single and multiple type of infection (N=63)

Type of infection	No. infected chicken	Percentage
Single		
One species	16	25.39%
Mixed		
Two species	28	44.44%
> two species	19	30.15%

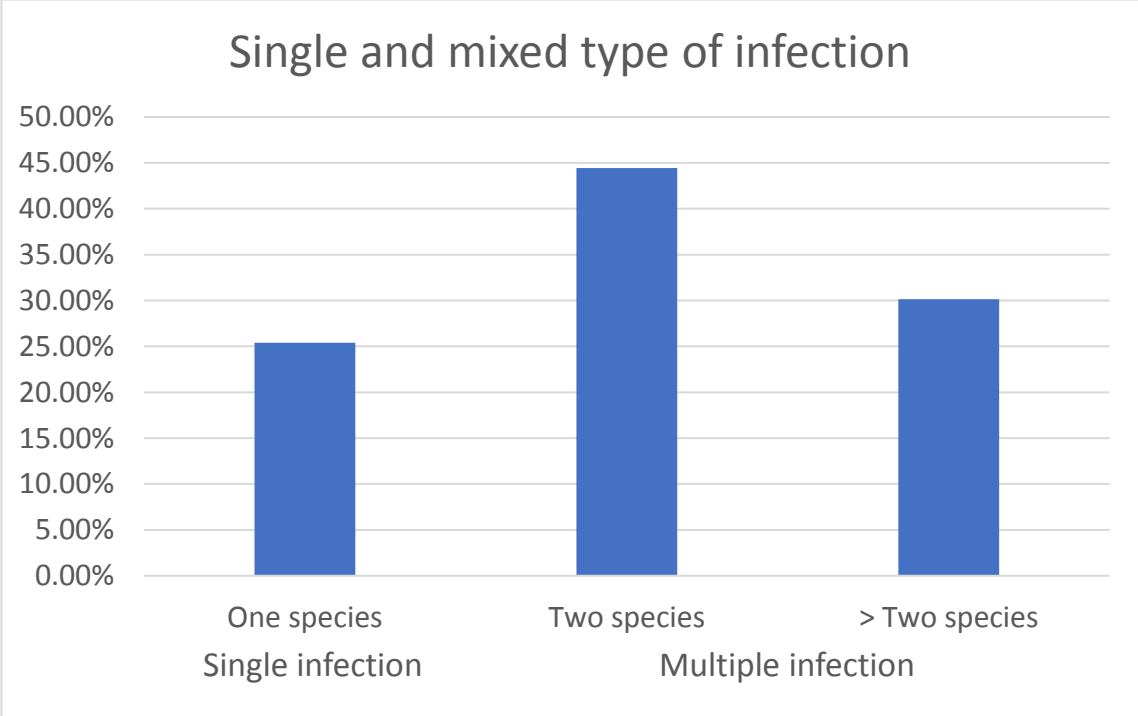

Figure 7: Percentages of single and multiple type of infection

DISCUSSION

After the extensive study of the gastrointestinal tracts of backyard chicken, *Gallus domesticus*, for helminth parasitism in different areas of Bangladesh, various species of helminth parasites were recovered with a very high prevalence (100%).

Total five species of helminth parasites were recorded of which two species were nematodes, two species were trematode and one species belonged to cestode. Similar studies were conducted by earlier scientists. Ferdushy *et al*. (2014) reported that 84.6% gastrointestinal helminth infection in Narsingdi district of Bangladesh. However, Rabbi *et al*. (2006) reported relatively higher prevalence (100%) of gastrointestinal helminth infection in indigenous chickens in Mymensingh district. This result is similar to our study because the results of Rabbi *et al*. (2006) were based on a non-random sample of 80 indigenous chicken's viscera. The smaller size of the sample and randomness is responsible for the higher prevalence of gastrointestinal helminths other than probable regional variation. There are lots of report having more than 50% prevalence rate from the different parts of the world. Mekibib *et al*. (2014) also reported similar prevalence of gastrointestinal helminth infections in scavenging chickens from Ethiopia. Yadev and Tandon (1998) revealed that 90.9% of helminth infections in subtropical high rainfall area of India. Eshetu *et al*. (2001) found that 91.01% chickens infected with gastrointestinal helminthes from Amhara region Ethiopia. Nokana *et al*. (1991) during their survey of helminth parasites in backyard flocks in Michigan by litter examination also showed relatively high contamination rates. Wakelin (1964) in Britain found 59.2%, Romanenko *et al*. (1985) in Roostov recorded 100% and Guclu (1994) in Turkey found 59% birds affected with helminth parasites. Similar studies were also performed by earlier scientists (Ssenyonga, 1982; Samad and Rahman, 1985; Huq, 1986; Kang and Suh, 1987).

There are a lot of variation in prevalence from different parts of Bangladesh. Some helminths are relatively higher in some areas such as *R. tetragona* in Mymensingh (100%) and *C. verrucosa* is not found from Bandarban and Pabna. This may be due to the very low amount of smple. Therefore, the remarkable prevalence of infection observed in backyard poultry from different parts of Bangladesh can be attributed to a number of factors like the type of management and production system, exposure to intermediate

hosts, inadequate or no use of anthelmintics, the climatic conditions which alter the population dynamics of the parasite. Backyard poultry of Bangladesh are reared in semi-scavenging system, in which they collect maximum of their food from the nature. Their food enterprise includes different types of seeds, kitchen wastages, insects, slugs, earthworm *etc*. They act as intermediate or paratenic hosts of many bio-parasites (Soulsby, 1982). Besides, backyard poultry can easily ingest the infective stage of many geo-parasites during taking food from the environment. The paratenic host of A. *galli* and *H. gallinarum* is earth worm which is very common and a favourite feed item for poultry in Bangladesh. Several snails like *Bithynia tentaculata* and *Lymnaea* act as intermediate hosts of *C. verrucosa* and *E. revolutum*, respectively. In case of *R. tetragona*, the ant of the genera *Tetramorium*, *Pheidole,* and house fly, *Musca domestica* act as intermediate host which are also available in our country, especially in the rural areas. Indigenous chickens are very fond of scavenging various insects from the nature which might contribute to the transmission of various helminth infection in chicken. Therefore, the environmental and climatic conditions of the study area seem to be very favorable for such a high intensity of infection, and also a major ecological factor like dispersal and migration plays a significant role in maintaining such a high prevalence of helminth infection in these study areas.

The present study reveals that single type infections were more prevalent than multiple type infections. A good number of backyard poultry were harboring more than one type of helminth species which is in agreement with the observation of Edgar (1953); Kononenko and Khaizade (1983); Mpoame and Agbede (1995); Permin *et al*. (1999); Poulsen *et al*., (2000); Mukaratirwa *et al*., (2001); Magwisha *et al*., (2002); Phiri *et al*. (2007) and Luka and Ndams (2007). Majority of the host birds harboured multiple type of infection by helminthes may be due to the prevailing environmental conditions and free-range management systems which are very favourable to many species of helminth parasites described by Ramadan and Znada (1992) and Watt (1996).

Our results indicate differences in predilection sites for the different helminth species recovered, with some species being recovered from more than one location. A similar observation was reported in the previous studies by Hussen *et al*., (2012) and Molla *et al*.,

(2012). This specificity in parasite distribution in the gastrointestinal tract is thought to be an attribute of differences in physicochemical environments in various regions of the gut. Among other factors, availability of suitable food and attachment sites; and presence of certain specific stimuli such as pressure differences are also known to dictate parasite site segregation along the gastrointestinal tract (Nkwengulila and Mwita, 2004). It may be due to the interactive competition for site and food among co-habiting species as among the factors determining parasite distribution in the gastrointestinal tract (Holmes 1973; William and Jones, 1994).

The most prevalent helminth parasite recorded during the present study was *R. tetragona* (73.01%), followed by *A. galli* (47.61%), *H. gallinarum* (38.09%), *C. verrucosa* (23.80%) and *E. revolutum* (7.93%). The results are in the agreement with the work of many authors (Qureshi, 1950; Wilson *et al*., 1994; Mpoame and Agbede, 1995; Permin *et al*., 1996; Eshetu *et al*., 2001). However, Schou *et al*. (2006), Luka and Ndams (2007) reported a higher prevalence of *A. galli.* The variations in prevalence can be attributed due to the environmental conditions in the area and unavailability of intermediate hosts. The environmental conditions like temperature and moisture favour the larval development in contaminated droppings and facilitate transmission.

In Bangladesh, there are very limited morphological data regarding to the helminths of backyard chicken. Identification of the helminths are based on the size of helminths and the location of host. In this sense, only the genus can be identified. Therefore, a detailed morphological study has been conducted.

Ascaridia galli possesses all the salient features of the ascarid nematodes. The body is cylindrical, highly extended, covered with a collagenous cuticle, anterior end with distinct lips, and the posterior with anus. In addition, marked sexual differences between male and female can be easily diagnosed by the relatively longer females with straight blunt tail end; whereas males are comparatively shorter with an elaborate, curved tail end (Ackert, 1940). The posterior components in *Ascaridia*, especially those of papillae and spicules, are the identifying characters between different species (Kung, 1949). The distinguishing characters of *A galli* is the difference in well-developed spicules in length (Kajerova *et*

al., 2004). The morphological features of *A. galli* also reflect all the characters of the generic diagnosis (Yamaguti 1961, Hodová *et al*., 2008)

There are many reports on the specificity *H. gallinarum* to backyard poultry (Skrjabin *et al*., 1961; Fedynich *et al*., 2005; Sherwin *et al*., 2013). Morphological characteristics of adult male and female *H. gallinarum* nematodes resulted in new data on their morphometric structure that can facilitate the species identification. Morphologically, male *Heterakis* nematodes are more distinct. One spicule of *H. gallinarum* male is visually longer than the other one. Microscopy study of the structure and position of tail papillae in males of those species shows significant differences. In males of *H. gallinarum* there are two preanal, six adanal, and four postanal pairs of papillae. (Abou Znada, 1993; Rahman & Manap, 2014; Sheikh *et al*., 2016).

Whitish, highly elongated, dorso-ventrally flattened cestodes were recovered from intestine of poultry. The mature segment is longer than broad and the common genital pores are single. Each mature proglottid has a set of male and female reproductive organ. Testes are located on both sides of the ovary and behind vitellaria. The morphological features and general measurements of recovered *R. tertragona* in the present investigation were agreed with the findings of many authors (El – Azzazy, 1979; Soulsby, 1982; Mahdy and Olfat, 1988; Ramadan and Abouzanda, 1989; El – Gayer and Amal 1992; Sayed and Gehan, 1996 and Ahmed-Nabila, 2004).

The echinostomes were recovered from the large intestine of poultry. Morphological identification of *Echinostoma* species in the 37-collar-spined 'revolutum' group is difficult due to the contradictory data from different hosts and geographical locations. In this study, the identification of *E. revolutum* was done by description of different authors (Kanev, 1885; Kanev, 1994 Kanev, 1995; Faltnkova, 2015). The morphological characteristics of *E. revolutum* in this study was similar with those reported previously (Kanev, 1994 and Faltnkova, 2015).

Catatropis verrucosa was recovered from the caecum of the poultry intestine which were small muscular body, dorsoventrally flattened, attenuated anteriorly and broadly rounded posteriorly. Cup-shaped oral sucker was terminal with no ventral sucker. Long caeca were

bifurcated and cirrus sac was elongated. Uterine loops were 18 in number. The shape and location of testes and ovary along with the other morphological characteristics of this fluke are very similar to the authors (Joyeux, 1922; Dubois, 1951; Odening, 1966; Kanev and Vasiliev, 1986; Dvorjadkin, 1989; Looss, 1893 and Kanev *et al*., 1994)

CHAPTER 5

CONCLUSION

The present study was aimed to study the morphology and the prevalence of gastrointestinal helminths in backyard poultry in different areas of Bangladesh (Dhaka, Bandarban, Mymensingh and Pabna). Different species of helminth parasites were recovered with a moderately high prevalence (100%). The smaller size of the sample and non-randomness is responsible for the higher prevalence of gastrointestinal helminths. In our study multi-species infection of helminths were observed in most of the chickens which suggest that the environmental condition and the nature of the poultry rearing system are very favorable for the transmission and persistence of the helminth parasites in rural areas of Bangladesh. This condition of high worm burdens can make the bird more prone to bacterial and viral infection as well more easily available to the predators as the bird become very much unthrifty and weak. Therefore, further large-scale studies are needed to measure the impact of helminth infection on the health and productivity of the rural scavenging chickens in rural areas of Bangladesh.

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