Research in
Agriculture, Livestock and Fisheries
ISSN : P-2409-0603, E-2409-9325
An Open Access Peer-Reviewed International Journal

Article Code: 0333/2021/RALF
Article Type: Research Article

Res. Agric. Livest. Fish.
Vol. 8, No. 1, April 2021: 145-155.

MORPHOLOGICAL IDENTIFICATION AND PREVALENCE OF GASTROINTESTINAL HELMINTHS IN BACKYARD CHICKEN FROM SELECTED AREAS OF BANGLADESH

S. M. Abdullah[1], Amrito Barman[1], Md. Yakub Ali[1], Md. Saiful Islam[2] and Uday Kumar Mohanta[1*]

[1]Department of Microbiology and Parasitology, and [2]Department of Anatomy, Histology and Physiology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, P.O. Box 1207, Dhaka, Bangladesh.

***Corresponding author:** Uday Kumar Mohanta; E-mail: uday_vet01@yahoo.com

ARTICLE INFO

Received
10 March, 2021

Revised
20 April, 2021

Accepted
26 April, 2021

Online
May, 2021

Key words:

Helminths
Backyard chicken
Morphology
Prevalence
Bangladesh

A B S T R A C T

Poultry industry is the promising sub-sector in livestock, which has been expanding day by day. Among the poultry species, backyard chicken is one of the appropriate incomes generating species in rural areas of Bangladesh. Parasitic infections have been considered as one of the major hindrances for backyard chicken rearing. Therefore, the present study aimed to investigate the prevalence of gastrointestinal helminths of backyard chicken in some selected areas of Bangladesh through their detailed morphological identification. A total number of 108 gastrointestinal tracts were examined for helminths from April 2018 to July 2018. The collected helminths were identified according to the keys and description of Yamaguti (1961) and Soulsby (1982). A high rate of helminth infection (100%) was observed in backyard chickens in Bangladesh. One cestode, *Raillietina tetragona* (67.59%); two nematodes, *Ascaridia galli* (43.51%) and *Heterakis gallinarum* (28.70%); and two trematodes, *Catatropis verrucosa* (21.29%) and *Echinostoma revolutum* (6.48%) were encountered during the study. Most of the helminths were recovered from small intestine followed by caecum. All five species of helminths were found from Dhaka and Pabna, but surprisingly no trematodes were found from Bandarban. Out of 108 chickens, 29.62% were infected with single species of helminths while the rest 70.38% harbored mixed infections. This work strongly suggests that helminths create serious problems in backyard chicken as they hamper production and therefore, appropriate control strategies are needed to design for better production.

To cite this article: Abdullah S. M., A. Barman, M. Y. Ali, M. S. Islam and U. K. Mohanta, 2021. Morphological identification and prevalence of gastrointestinal helminths in backyard chicken from selected areas of Bangladesh. Res. Agric. Livest. Fish., 8 (1): 145-155.

INTRODUCTION

Poultry, especially chicken is one of the most intensively reared domesticated species, and is the most profitable livestock production businesses (FAO, 1987). Its importance in developing countries through improving the nutritional status and income generation of many small farmers have been recognized by various scholars and rural development agencies in the last two decades (Eyinnaya, 1992). The purposes of backyard chicken production are for women empowerment and income generation through egg production and hatching, consumption, cultural and religious ceremonies (Moges et al., 2010). Like other countries, poultry production is a promising sector in Bangladesh which is increasing day by day contributing approximately 37% of total animal protein (Ahmed and Islam, 1990). However, Poultry diseases are the major hindrances for poultry production, resulting in decrease 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 disease of backyard chicken, and are considered as the major cause of ill-health and loss of productivity in different parts of the world (Yimer et al., 2001). This problem is seen wherever poultry are raised, whether in large commercial operations or in small backyard flocks, economic losses due to the parasites can be significant (Fatihu et al., 1991). 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 factor for the poultry industry (Soulsby, 1982).

Previous researches on gastrointestinal helminths were conducted to observe the prevalence by many authors in Bangladesh (Rabbi et al., 2006; Alam et al., 2014; Ferdushy et al., 2016). These researches had very limited morphological data regarding to the helminths. Identification of the helminths was based on the size and location of helminths. These could only identify the genus of a helminth. Therefore, a study was undertaken to determine the morphological identification and prevalence of gastrointestinal helminths in backyard chicken from selected areas of Bangladesh. The study may provide useful information on the prevalence of helminths parasites in backyard chicken and may aid in the identification of the helminths described here, and thereby, assist in designing effective control strategies.

MATERIALS AND METHODS

Sampling areas

The study was carried out from April to July, 2018. The gastrointestinal tracts of the slaughtered chickens were collected from the rural areas of four districts of Bangladesh, namely Dhaka (36), Bandarban (24), Mymensingh (24) and Pabna (24). Finally, the identification was performed in the Microbiology & Parasitology Laboratory, Sher-e-Bangla Agricultural University, Dhaka.

Collection of samples

Around noon in each sampling day, a batch of 6 intestines was randomly picked up from a group of intestines, slaughtered in the rural market between early morning and at the time of collection. The intestines were placed in separate, labelled polythene bags and transported to the laboratory maintaining a cool chain protocol. Firstly, the whole intestines were separated into three parts, namely small intestine, large intestine and caecum. The entire length of each part of the intestine was incised longitudinally, and the contents were emptied into sieves placed on large clean plastic cups with labelling. Contents were washed in normal saline and examined under light. Larger helminths were collected directly by curved needle or forceps, and smaller ones were isolated through dropper. Worms were counted and grouped on the basis of gross morphology before being stored in plastic bottles containing 70% alcohol according to the method described by Permin and Hansen (1999).

Processing of cestodes and trematodes

In case of trematodes and cestodes, permanent slides were made after the staining of the specimens. For this purpose, trematodes and cestodes were collected from plastic bottles containing 70% alcohol. Then the specimens, preserved in 70% alcohol 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 glass slides with canada balsam. Finally, the slides were kept until the canada balsam dried, and observed under microscope for detailed morphological studies (Jones et al., 1994; Barman et al., 2020).

Processing of nematodes

The nematodes were washed well in water to remove the preservatives, dehydrated in 70-90 % alcohol depending on the thickness of the worm and cleared by submerging them in lactophenol. Then the nematodes were examined under microscope and photographs were taken from different body parts as an aid for identification (Anderson et al., 2009).

RESULTS

Morphological identification

Through the microscopic examination of all 108 samples, five helminths were confirmed. These include two species of nematodes (*A. galli* & *H. gallinarum*), two species of trematodes (*E. revolutum* & *C. verrucosa*) and only one species of cestode (*R. tetragona*).

Ascaridia galli

Adult worms, recovered from small intestines, were yellowish white in color and semitransparent. The males were generally 45 to 60 mm long, while females were longer, ranging from 70 to 90 mm. The oral opening was surrounded by three prominent lips. Two conspicuous papillae were observed on the dorsal lip and one on each of the subventral lips (Fig. 1a). The esophagus was filariform and the intestine was simple where the whole body was enclosed in a tough proteinaceous covering called cuticle. The cuticle was distinctly striated and cuticular alae are poorly developed (Fig. 1b). Morphologically, sexual dimorphism in ascarids characterized by ventrally coiled tail with precloacal sucker was found 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 (Fig. 1c). The posterior end of male was more complex. 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 aid to attach during copulation. The worms had two well-developed unequal spicules at the posterior end (Fig. 1d). All of these morphological characteristics correspond to the properties to the Genus *Ascaridia,* and the Species *A. galli.*

Heterakis gallinarum

The adult worms, collected from the caecum, differed in length. The females (8 to 13 mm) were generally longer than that of the males (7 to 11 mm). They were small, white in colour and had three well-defined lips with slightly curved head when collected (Fig. 2a). The esophagus was connected with a short narrow anterior portion (pharynx) and ended in a well-developed bulb (Fig. 2b). 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. Male worms had stylet-like tail end that smoothly tapered posteriorly. The worms had two well-developed unequal spicules at the posterior end (Fig. 2c). The preanal sucker could be easily seen, which was round, well-developed and surrounded by a chitinized ring. Eggs in the uterus were ellipsoidal, with a thick, smooth shell, containing a single cell (Fig. 2d). Each of every morphological characteristic is identical to the Genus *Heterakis*, and Species *H. gallinarum.*

Reillietina tetragona

Multiple mature cestodes, measuring 12-25 cm long were isolated from small intestines. These cestodes were whitish in color, elongated, dorso-ventrally flattened. The body was divided into the head region called ‘scolex’, followed by an unsegmented ‘neck’, and then by highly segmented body called ‘strobila’ (Fig. 3a). Each scolex beared an apical rounded rostellum, which is medium and armed with many minute hammer-shaped hooks arranged in single row. The scolex was also surrounded by four ovoid suckers which are lined with several rows of spines (Fig. 3b). The strobila was composed of a series of body segments called proglottids, gradually increasing in size posteriorly. The mature segment was longer than broad and the common genital pores were single and being in front of the anterior 1/3 of the lateral margin of the mature segment. Each mature proglottid beared a set of male and female reproductive organ (Fig. 3c). Testes were located on both sides of the ovary and behind vitellarium. These features matched with the morphology of the Genus *Reillietina*, and Species *R. tetragona*.

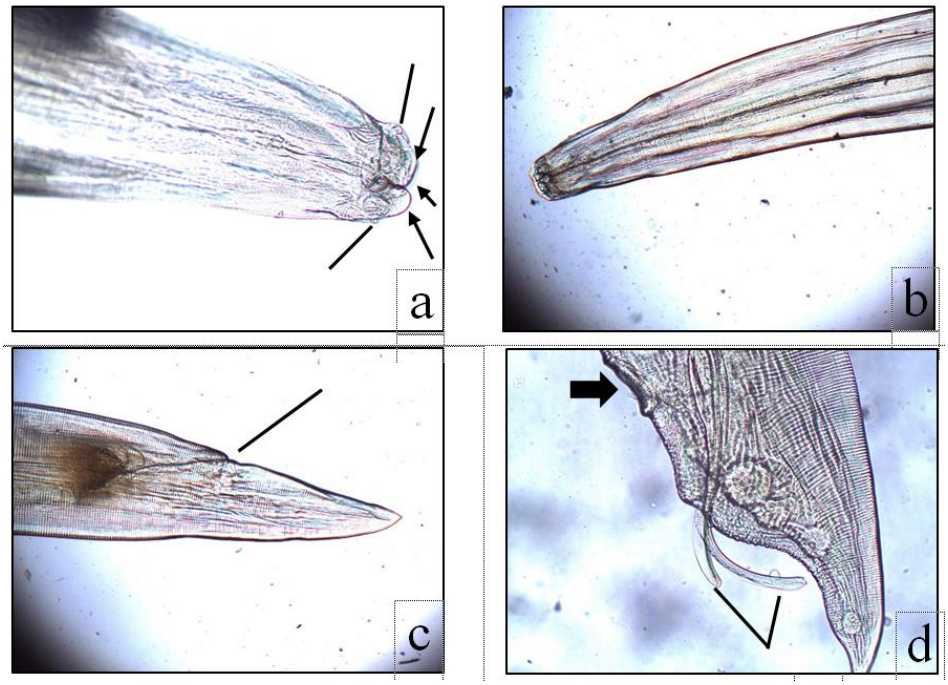

Figure 1. Different body parts of *A. galli.* **a.** Anterior end of the parasite; the arrows indicate the lips and the lines indicate the papillae on the lips. **b.** Filariform esophagus with cuticular striation. **c.** Posterior end of the female; the line indicates anal opening. **d.** Posterior part of the male (10X) the lines indicate the spicules and the block arrow indicates the precloacal or preanal sucker.

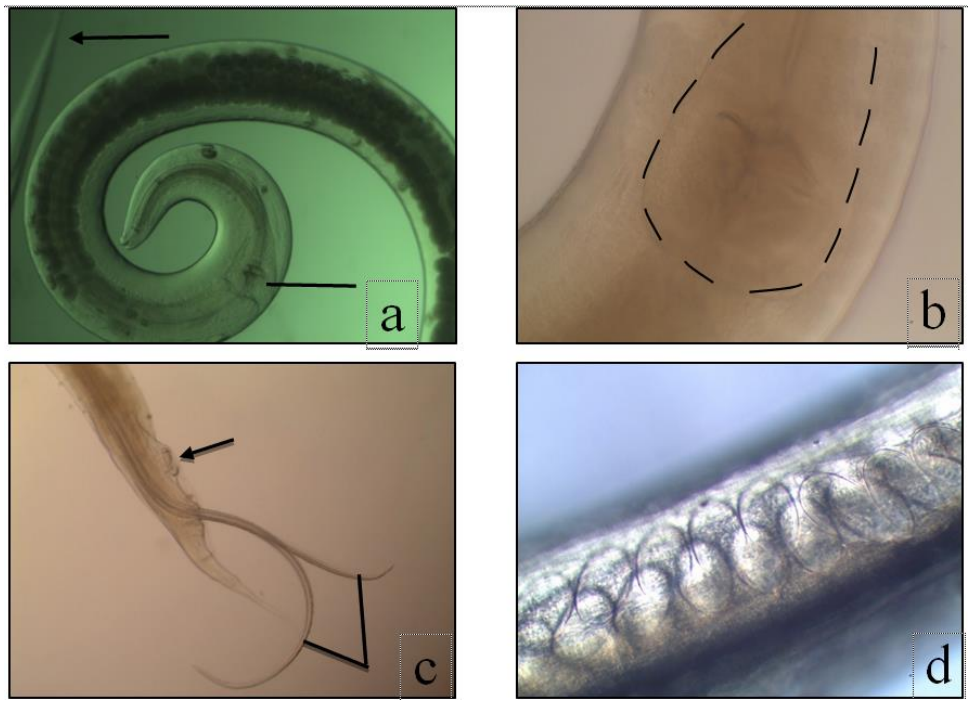

Figure 2. Different body parts of *H. gallinarum.* **a.** Slightly curved anterior end of female; arrow indicates the lips and the line indicates the bulb shaped esophagus. **b.** Bulb shaped esophagus in high magnification (40X). **c.** Posterior end of the male (10X); the arrow shows the preanal sucker and the line shows the unequal spicules. **d.** Multiple eggs in the uterus of female.

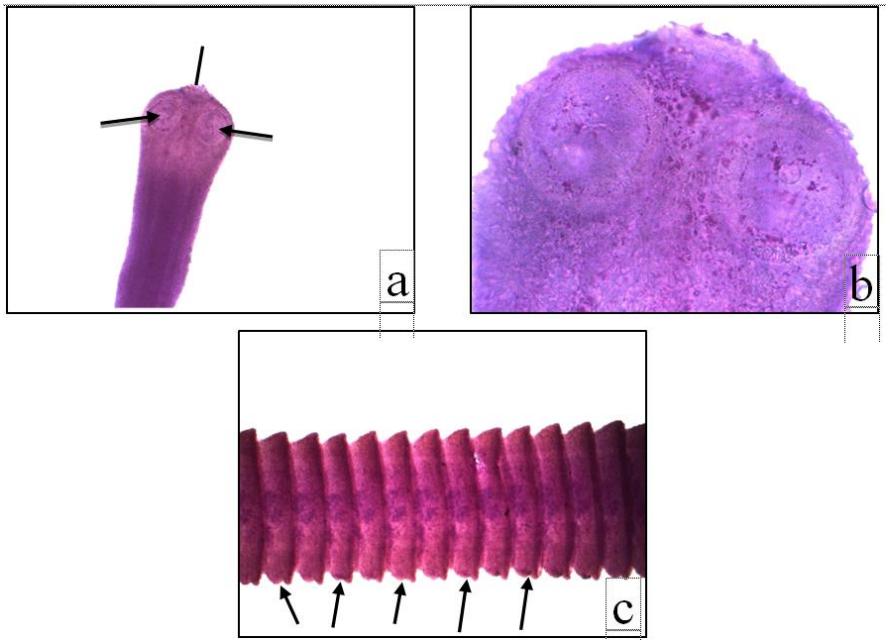

Figure 3. Different body parts of *R. tetragona.* **a.** Anterior end of the parasite (10X); the line indicates rostellum and the arrows indicate the suckers. **b.** Scolex in high magnification (40X); **c.** The broad segment having common genital pores on each (black arrows); Each mature proglottid has a set of reproductive organ in the middle part.

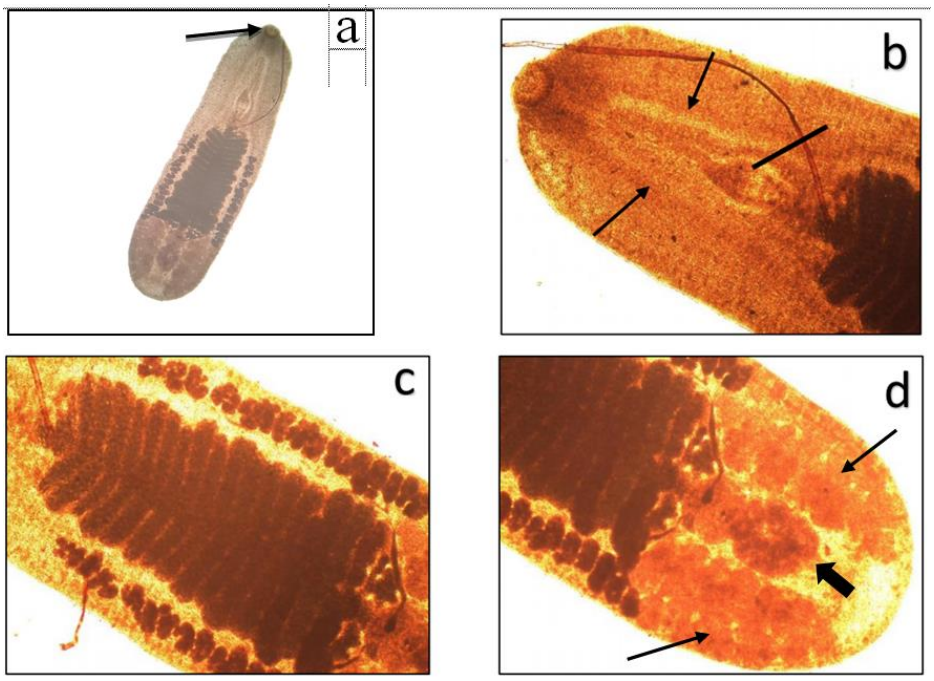

Figure 4. Different body parts of *C. verrucosa.* **A.** Whole fluke under 4X magnification; the arrow indicates the oral sucker. **b.** Anterior end of the fluke; the arrows indicate caecal bifurcation and the line indicates the cirrus sac. **c.** Uterine loops (18 in numbers) of the parasite (10X); both side of the loops covered by vitellaria. **d.** The reproductive organs of parasites; the arrows indicate testes and the block arrow indicates the ovary.

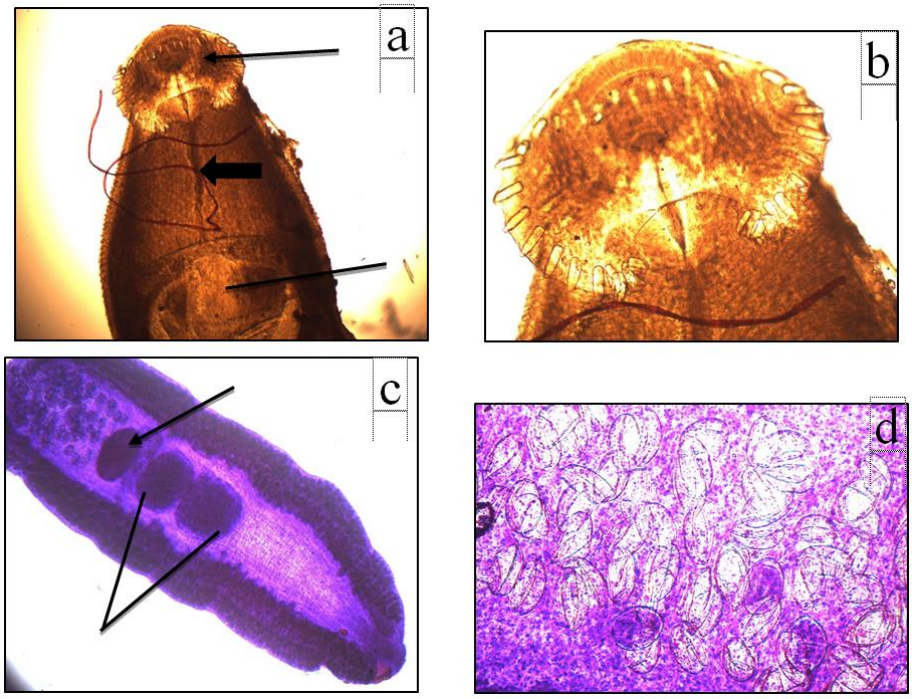

Figure 5. Different body parts of *E. revolutum.* **a.** Anterior end of the fluke; the black arrow indicates the oral sucker, the line indicates the ventral sucker and the block arrow indicates esophagus. **b.** The 37 spines are distinct around the oral sucker (10X). **c.** Posterior part of the fluke; the arrow indicates the ovary and the lines indicate the testes. **d.** Numerous eggs in the uterus (40X).

Catatropis verrucosa

A large number of minute flukes were recovered from the caecum of the poultry intestine which was 1.5-2.0 cm long and upto 0.5 cm wide. The specimens had small muscular body which was dorsoventrally flattened and narrowing anteriorly. The pharynx was absent and the esophagus was very short (Fig. 4a). Cup-shaped oral sucker was terminal while there was no ventral sucker. 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 (Fig. 4b). There were two testes which were irregularly lobed, located on extracaecal field in posterior third of the body. Ovary was trilobed, situated at the testicular level (Fig. 4d). Uterus had a number of closely packed loops, overlapping cirrus sac, reaching anteriorly up to the level of Mehli's gland. Uterine loops were 18 in number (Fig. 4c). Vitellaria were arranged extracaecally but at some places it overlapped the caeca, which are the special morphological features of the Genus *Catatropis*, and Species *C. verrucosa.*

Echinostoma revulotum

The echinostomes were recovered from the large intestine of chicken. The body was muscular, dorsoventrally flattened and 'C' shaped in appearance. 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, 6 lateral spines on each side and 15 dorsal spines) (Fig. 5b). Adult flukes had a short forebody and the ventral sucker was large (Fig. 5a). 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 (Fig. 5c). The cirrus sac was oval and located transversely between the level of caecal bifurcation and anterior border of ventral sucker. Both intestinal caeca ran upto the posterior end of the fluke (Figure 5d). Multiple eggs were located in the uterus. The eggs were oval, large, thin shelled, operculated and contained unsegmented ovum (Fig. 5d) which is the special morphological characteristics of the Genus *Echinostoma,* and Species *E. revolutum*.

Prevalence

The study was carried out in a total of 108 gastrointestinal tract of backyard chicken. All examined intestine were found infected with various species of gastrointestinal helminths, comprising two species of nematodes, two species of trematodes and only one species of cestode. These parasites were found in different locations of the gastrointestinal tracts of backyard chicken.

Overall prevalence of helminths in backyard chicken

The encountered nematodes were *Ascaridia galli* and *Heterakis gallinarum,* of which *A. galli* (43.51%) was the most prevalent followed by *H. gallinarum* (28.70%). The recovered trematodes were included *Catartropis verrucosa* and *Echinostoma revolutum* where *C. verrucosa* (21.29%) was the most prevalent followed by *E. revolutum* (6.48%). The cestode encountered was only one species, *Raillietina tetragona* (67.59%). The results also showed that the huge number of the helminths prefer to colonize the small intestine than the large intestine. Some of the helminths were found in the caecum. No helminth was recovered in the crop and gizzard. The overall prevalence with their predilection site is given in Table 1.

Table 1. Location and overall prevalence of helminths in backyard poultry (N=108)

Class of helminths	Name	Location	No. of infected chicken	Prevalence (%)
Nematode	*A. galli*	Small intestine	47	43.51%
	H. gallinarum	Caecum	31	28.70%
Trematode	*C. verrucosa*	Caecum	23	21.29%
	E. revolutum	Large intestine	7	6.48%
Cestode	*R. tetragona*	Small intestine	73	67.59%

Table 2. Prevalence of gastrointestinal helminths in different geographies

Name of the helminths	Different geographies							
	Dhaka (n=36)		Mymensingh (n=24)		Pabna (n=24)		Bandarban (n=24)	
	No. of infected chicken	Prevalence (%)	No. of infected chicken	Prevalence (%)	No. of infected chicken	Prevalence (%)	No. of infected chicken	Prevalence (%)
A. galli	16	44.44	9	37.50	12	50.00	10	41.66
H. gallinarum	11	30.55	8	33.33	7	29.16	5	20.83
C. verrucosa	9	25.00	6	25.00	8	33.33	-	-
E. revolutum	5	13.88	-	-	2	8.33	-	-
R. tetragona	27	75.00	15	62.5	14	58.33	17	70.83

'-' indicates not prevalent

Table 3. Percentages of single and multiple type of infection (n=108)

Type of infection	No. infected chicken	Prevalence (%)
Single		
One species	32	29.62%
Mixed		
Two species	47	43.51%
> two species	39	36.11%

Prevalence of helminths in different geographies

The samples were collected from Dhaka (n=36), Mymensingh (n=24), Pabna (n=24) and Bandarban (n=24). Helminths were recovered from different geography were given in the Table 2. Total five species of helminths were collected from Dhaka and Pabna. From the samples of Mymensingh, *E. revulotum* was not found whereas no trematode was from Bandarban.

Single and mixed type of infection

Examined gastrointestinal tract of chicken were infected by one or more species of helminth parasites (Table 3). Among the 108 intestines, 32 were infected with single species of helminths (29.62%) and rest 86 were infected with multiple species of helminths. In case of the mixed infection, 47 chicken were infected with two species of helminths (43.51%) and 39 chicken were infected with more than two species of helminths (36.11%).

DISCUSSION

In Bangladesh, there are very limited morphological data regarding precise identification of the helminths in backyard chicken. Only the gross morphology and the predilection site in host had been used as keys for identification of the helminths. Therefore, detailed morphological observations have been conducted in this study to provide a basis for identification of the helminths in backyard chicken. Both male and female *A. galli* possesses all the salient features of the ascarid nematodes described by Kung (1949), Kajerova et al. (2004), Hodová et al. (2008). But the morphometric characters regarding the length of the body and tail had minor deviation from the previous reports. These deviations may be due to the difference in host species, geographic locations, methods of preservations, etc. Morphological and morphometric characteristics of adult *H. gallinarum* including trilobed lips and bulb shaped esophagus are the confirmatory characteristics, fully matched with previous studies (Abou Znada, 1993; Rahman and Manap, 2014; Sheikh et al., 2016). Whitish, highly elongated, dorso-ventrally flattened cestodes were identified as *R. tetragona* isolated from small intestine of poultry. The detailed moephological properties of these cestodes were identical to the findings of many authors (Soulsby, 1982; Ramadan and Zanda, 1991; Ahmed and Nabila, 2004). The very small trematode, *C. verrucosa,* recovered from the caecum of the poultry, were dorsoventrally flattened, attenuated anteriorly and broadly rounded posteriorly, containing 18 uterine loops. The shape and orientation of testes and ovary along with the other morphological characteristics of this fluke were very similar to the previous reports (Odening, 1966; Dvorjadkin, 1987; Kanev et al., 1994). Morphological identification of *Echinostoma* species in the 37-collar-spined 'revolutum' group was 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, 1994; Chantima el al., 2013; Mohanta et al., 2018).

After the extensive study of the gastrointestinal tracts of backyard chicken for helminths in different areas of Bangladesh, various species of helminth parasites were recovered with a very high prevalence (100%). Similar observations were published in previous studies. Rabbi et al. (2006) reported a high prevalence (100%) of gastrointestinal helminth infection in indigenous chickens in Mymensingh district. Ferdushy et al. (2016) also reported that 84.6% chicken were infected with helminth in Narsingdi district of Bangladesh. However, Yadev and Tandon (1991) reported 90.9% helminth infections in subtropical high rainfall area of India, neighbour of Bangladesh.

The remarkable high prevalence of helminth infection in backyard chicken from different parts of Bangladesh can be attributed by 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, etc. More interestingly, backyard poultry of Bangladesh are reared in semi scavenging system, in which they collect maximum of their food from the nature. The paratenic host of *A. galli* and *H. gallinarum* is earthworm which is very common and a favourite feed item for backyard chicken in Bangladesh. On the other hand, several snails like *Bithynia* spp. and *Lymnaea* spp. act as intermediate hosts of *C. verrucosa* and *E. revolutum*, respectively. In case of *R. tetragona*, the ant of the genera *Tetramorium* spp., *Pheidole* spp., and house fly, *Musca domestica* act as intermediate host, which are also available in rural area of Bangladesh. Indigenous chickens are very much fond of scavenging various insects from the nature which might contribute to the transmission of various helminth infections to chicken. Therefore, the climatic conditions along with the availability of intermediate hosts might contribute to the very high prevalence of helminth infection in backyard chicken in Bangladesh.

There was a lot of variation in prevalence from different parts of Bangladesh. Cestodes were relatively higher (67.59%) in all areas but no trematode was found in Bandarban district. These results are in the agreement with the work of many researchers (Wilson et al., 1994; Mpoame and Agbede 1995; Permin et al., 1997; Eshetu et al., 2001). However, Schou et al. (2006) reported a higher prevalence (67%) of *A. galli* in Vietnam. The variations in prevalence can be attributed due to the environmental conditions and availability of intermediate hosts mentioned earlier. The present study reveals that mixed infections were more prevalent than single type infections. A good number of backyard chicken (77.62%) harbored more than one type of helminth species, which is in agreement with the observation of 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). Multiple helminth infection may be due to the semi scavenging rearing system, which allows them to pick up multiple infections (eggs, larva, metacecaria, etc.).

CONCLUSION

The present work was aimed to study the morphology and the prevalence of gastrointestinal helminths in backyard poultry in different areas of Bangladesh (Dhaka, Mymensingh, Pabna and Bandarban). Different species of helminths were recovered with a very high prevalence (100%). The smaller size of the sample might be responsible for the higher prevalence of gastrointestinal helminths. In our study, multi-species infection of helminths was 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. Due to the high worm burdens, the birds may become more susceptible to bacterial and viral infection; therefore, they may become more easily reachable to the predators as the bird become very much unthrifty and weak. So, 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.

COMPETING INTEREST

The authors declare that there is no conflict of interest about this publication.

ACKNOWLEDGEMENTS

This project was financially supported by the Ministry of National Science and Technology (NST), Bangladesh.

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