PREVALENCE AND DETAIL MORPHOLOGICAL IDENTIFICATION OF ENDOPARASITES OF MURINE RODENTS IN DHAKA CITY, BANGLADESH: SPECIAL REFERENCES TO PUBLIC HEALTH SIGNIFICANCE

A Thesis

by

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MASTER OF SCIENCE IN PARASITOLOGY

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY

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CERTIFICATE

*This is to certify that the thesis entitled "PREVALENCE AND DETAIL MORPHOLOGICAL IDENTIFICATION OF ENDOPARASITES OF MURINE RODENTS IN DHAKA CITY, BANGLADESH: SPECIAL REFERENCES TO PUBLIC HEALTH SIGNIFICANCE" submitted to the Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **Master of Science in Microbiology and Parasitology**, embodies the result of a piece of bona fide research work carried out by **Amrito Barman**, Registration No. 12-04767 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.

Dated:

Place: Dhaka, Bangladesh

Dr. Uday Kumar Mohanta
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Dedicated

To

My Beloved Parents

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The Author

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ACRONYMS AND ABBREVIATIONS

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

PREVALENCE AND DETAIL MORPHOLOGICAL IDENTIFICATION OF ENDOPARASITES OF MURINE RODENTS IN DHAKA CITY, BANGLADESH: SPECIAL REFERENCES TO PUBLIC HEALTH SIGNIFICANCE

ABSTRACT

Rodents are common pests that transmit various deadly pathogens. Here we study the helminth parasites of rodents from different ecological niches. The Gastrointestinal helminths were investigated from a total of 70 rodents, namely *Bandicota bengalensis* (sand rice rat, n=20), *Rattus rattus* (black rat, n= 15), *Rattus norvegicus* (brown rat, n=25) and *Mus musculus* (house mouse, n=10). The rats were live-captured from houses in the slum areas (n=20) and stationary shops (n=20), residential buildings (n=15) and rice fields (n=15). The overall prevalence of helminth infection was 71.42%. The highest prevalence was found in *R. norvegicus* (84%), followed by *B. bengalensis* (75%), *R. rattus* (66.66%) and *M. musculus* (40%). Among different areas of Dhaka city, the highest prevalence rate was recorded in slum areas (85%). The parasites detected from the rodents were *Taenia taeniformis* (35%), *Hymenolepis diminuta* (47.14%), *Moniliformis moniliformis* (42.85%), *Heterakis spumosa* (60%) and *Gongylonema neoplasticum* (34.28%). To the best of our knowledge, *G. neoplasticum* is going to be reported for the first time from rodents in Bangladesh. Except *H. spumosa*, all the parasites recovered have public health significance. Therefore, proper attention needs to be paid for the prevention of rat borne zoonosis through the control of rodents.

CHAPTER 1

INTRODUCTION

Rodents are represented by many families, of which Family Muridae embraces all the small rodents such as rats, mice and rat-like rodents. Their predatory and depredatory habits have a noticeable impact on human economies through their role as the major vectors of human and domestic animal diseases worldwide (Anantaraman, 1966; Huq *et al.*, 1985). In developing countries, conditions are more suitable for survival and propagation of rodents but awareness to control these carrier is minimum than those in developed countries. They act as reservoir hosts of some parasites infecting humans and livestock (Gofur *et al.*, 2010). Rodents may spread numerous diseases worldwide, many of which have zoonotic potentials. Disease transmission mainly occurs through the contamination of human foods with saliva, urine and feces of rodents (Priyanto *et al.*, 2014). Studies on the parasites of rodents in most countries reported that they harbor a number of helminth parasites, such as cestodes (*Taenia taeniformis*, *Hymenolepis diminuta*, *Hymenolepis nana*, *Raillietina celebensis*, *Oochoristica symmetrica* etc.), nematodes (*Heterakis spumosa*, *Gongylonema neoplasticum*, *Mastophorus muris*, *Syphacia muris*, *Citellina dispar*, *Trichuris* spp., *Aspiculuris tetraptera*, *Syphacia obvelata* etc.) and acanthocephala, *Moniliformis moniliformis* (Singh, 1962; Gupta and Trivedi, 1985; Agnieszka *et al.*, 2006; Singla *et al.*, 2008; Khanum *et al.*, 2009). The rodents borne endoparasites such as, *T. taeniformis, H. nana*, *H. diminuta*, *G. neoplasticum, M. moniliformis* etc. are reported as transmissible to human, and constitute a public health problem (Marangi *et al.*, 2003). Human hymenolepiasis caused by *H. diminuta* is rare, and being diagnosed a total of 500 human cases worldwide (Wiwanitkit, 2004). The worm is cosmopolitan but more common in warmer climates. The infections caused by *H. diminuta* are asymptomatic, but there may be abdominal pain, diarrhea and irritability in human. Due to such rare phenomenon among humans, diagnosis, analysis and description of *H. diminuta* in each case gives new knowledge (Cohan, 1989; Rohela *et al.,* 2012). Humans with *T. taeniformis* can be a source of infection to other humans or animal intermediate hosts by shedding eggs and gravid proglottids through feces. The infection of *T. taeniaeformis* have been reported in human from Sri Lanka, Argentina, Czechoslovakia, Denmark etc. (Ekanayaka *et al*., 1999). Chronic headaches and seizures, nausea, vomiting, vertigo, ataxia, confusion or other changes in mental status, behavioral abnormalities, progressive dementia etc. are observed due to infection of cysticerci of *T. taeniformis* in human.

The parasites, *G. neoplasticum*, observed in stomach wall are natural and common parasite among rodents, would seem to cause human infection because of the ubiquitous distribution of rodents. In human, a fewer than 60 cases have been reported worldwide. A case from the Alsace region, which appeared to be the first case of human gongylonemosis described in France (Bernard *et al.,* 2013). *M. moniliformis*, acanthocephala or thorny-headed worm, is normally a parasite of the rat, the hamster, the white mouse, the cat, and the dog in most parts of the world. *M. moniliformis* is also recognized as a zoonotic parasite of public health concern. This species may rarely infect humans. The first case of human infection with *M. moniliformis* was reported in Iran during in 1970 from a 18-month-old child from Zabol (Salehabadi *et al.,* 2008). Anorexia, vomiting accompanied by foamy diarrhea, irritability, cough and sweating are generally observed in human (Sahba *et al.,* 1970). The intestinal parasite, *H. spumosa*, is a common nematode of rodents throughout the world (Milazzo *et al.*, 2003; Villafane *et al.,* 2008; Kataranovski *et al.,* 2010; Pakdel *et al.,* 2013). However, tissue reaction to this nematode has been rarely documented and most studies described little pathogenic effects of this parasite (Villafane *et al.,* 2008; Kataranovski *et al.*, 2010).

The presence of rodents, therefore, represents a potential epidemiological problem (Spatafora and Platt, 1982). Although emerging rodent-borne diseases have been captured worldwide attention, but little has been documented on this aspect in Asia. The endoparasitic infections, which are harbored and transmitted by human and animal population have not been as thoroughly investigated as the microbial infections, especially in Bangladesh. In Bangladesh, very few studies (Huq, 1969; Shaha, 1974; Bhuiyan *et al.,* 1996; Khanum *et al.*, 2001; Alam *et al.*, 2003; Khanum and Arefin, 2003; Muznebin *et al.*, 2009; Khanum *et al.*, 2009 and Gofur *et al.*, 2010) have been carried out only on prevalence of the parasites and worm burden of rodents. Unfortunately, none of these studies included detail morphological identification of rodent borne parasites.

Objectives of the study:

- Morphological identification of the endoparasites found in murine rodents.
- Prevalence or investigation of endoparasites in murine rodents in Dhaka city.

CHAPTER 2

REVIEW OF LITERATURE

Rats and mice are worldwide distributed and are the most common rodents found in the city and its surrounding areas. Four species of rodents namely black rats (*Rattus rattus*), brown rats (*Rattus norvegicus*), lesser bandicoot rat (*Bandicota bengalensis*) and house mouse (*Mus musculus*) are very common around human habitats in tropical and sub-tropical regions. Their origin from the far east and distributed around the world through ship trades. The breeding of rodents has been increased rapidly in the recent years because of the abundance of food resources and lack of environmental hygiene in urban areas (Arfa, 1987; Abdel and Eisha, 1997). Rodents impose economic damages, and involve significant impact on public health system. They can cause destruction of food stuffs, electrical equipment and buildings by contamination or gnawing with excreta resulting in significant economic losses (Coomansingh *et al.*, 2009).

Khalid *et al*., 1992; Mafiana *et al*., 1997; Beaver *et al.*, 1984; Milaazzo *et al*., 2010; Zain *et al*., 2012; Hindi and Haddaf, 2013 reported that rodents are hosts to a number of endoparasites and many parasites of rodents are common with human too and some parasites can be transferred from rodents to human, for example *H. nana, C. hepatica, H. diminuta, T. taeniaeformis, Diphyllobothrium* sp., *M. moniliformis, G. neoplasticum* etc. These authors have recorded different species with different prevalence of endoparasites depending on the geographical distribution.

According to Elkady *et al*., 2008; Okoye and Obiezue, 2008; Onyenwe *et al*., 2009 and Sumangali *et al*., 2012, rodents play an important role in the zoonotic cycle of many parasitic diseases and some of which more important than the others such as schistosomiasis, hymenolepiasis and angiostrongliosis. Several studies on the endoparasites of rodents have been conducted in different parts of the world (Seong *et al*., 1995; Mafiana *et al*., 1997; Wahed *et al*., 1999; Kassa and Assefa, 2000; Milazzo *et al*., 2003; Stojcevic *et al*., 2004; Claveria *et al*., 2005; Zihiry, 2006; Waugh *et al*., 2006; Zain *et al*., 2012; Coomansingh *et al*., 2009; Milaazzo *et al*., 2010; Madi *et al*., 2001; Gaherwal *et al.*, 2011; Kataranovski *et al*., 2011; Bashan and Sabra, 2012; Shafiyyah *et al*., 2012; Kiran *et al*., 2013; Amarasingh and Premathilake, 2014; Araujo *et al*., 2014; Guimarães *et al*., 2014; Ogunniyi *et al*., 2014 and Priyanto *et al*., 2014). Many endoparasites including cestodes, trematodes and nematodes have been identified and reported from *R. rattus*, *R. norvegicus*, *B. bengalensis* and *M. musculus* in cities and villages from developed and developing countries.

Amarasingh and Premathilake, (2014) observed that *R. rattus*, captured from the western province of Srilanka harbored liver cysts of *C. fasciolaris* and acanthocephala (*M. moniliformis*). On other hand, Seong *et al*., (1995) recovered *C. hepatica* (11.6%), *H. diminuta* (16.3%), and metacestode of *T. taeniaformis* (51.2%) from *R. norvegicus* in Korea. Recent studies in rodents from Brazil showed occurrence of *M. musculus* and *R. rattus* (Guimarães *et al*., 2014; Porta *et al.*, 2014 and Araujo *et al*., 2014) and they found *A. tetraptera* and *S. obvelata*, *H. diminuta*, *Strongyloides* sp. and *H. diminuta*. Meanwhile, Waugh *et al*., (2006) recorded nine species of gastrointestinal helminths from wild rodents, *R. rattus* and *R. norvegicus*, in Jamaica. The detected endoparasites were *Raillietina* sp. (0.2%), *Trichuris* sp. (0.2%), *Rictularia* sp. (0.7%), *Syphacia obvelata* (1.1%), *Strongyloides ratti* (1.4%), *H. diminuta* (3.8%), *P. muricola* (4.3%), *M. moniliformis* (11.2%) and *N. brasiliensis* (14.2%).

Claveria *et al*., (2005) reported biodiversity of parasites in *Rattus* spp. in Philippines captured in wet markets. They detected *H. diminuta*, *M. moniliformis*, *T. taeniaformis* strobilocercus larvae and *C. hepatica* in liver, *Trichosomoides* *crassicauda* of the urinary bladder, *Sarcocystis* sp. in muscle tissue, and two different species of *Stronglyloid* looking intestinal nematodes.

Coomansingh, (2009) and associates reported that recent significant increases in the population of rodents in Grenada warranted a study to determine the prevalence of helminth endoparasites in 242 *R. norvegicus* rats (Norwegian rats) captured from all six parishes. They observed overall prevalence of helminth parasites as 90.9%. Three nematodes, two cestodes and one acanthocephala were identified. The prevalences of *N. brasiliensis*, *S. muris*, *H. diminuta*, *T. taeniaeformis*, *M. morniliformis* and *T. crassicauda* were 76.8%, 28.1%, 16.1%, 23.1%, 3.7% and 1.2%, respectively. Significant parasite burdens were identified in the captured rodents, some of which are of public health significance due to their zoonotic potentiality.

Egbunu and Dada, (2016) determined the prevalence of intestinal helminth parasites of domestic rats in selected sites around student's hall of residence in The Federal University of Technology, Akure. Twenty-five (25) rats and mice species were captured and standard parasitological methods were used to identify the rodents endoparasites. *R. novergicus* and *M. musculus* were the two species of domestic rodents captured. Nematodes were namely, *S. muris*, *S. ratti*, *N. brasiliensis*, *T. spiralis*, *S. stercoralis*, while cestodes were *H. nana*, *H. diminuta* and *T. taeniaeformis*. Nematodes were more prevalent (72%) than cestodes (52%). Overall prevalence of helminths was 84% in male rodents and 40% in female rodents. Small intestines of the rodents were more parasitised than the large intestine. The prevalence of intestinal helminth parasites in *R. novergicus* and *M. musculus* was 100% and 90%, respectively.

Gofur *et al.*, (2010) reported that 23 out of 30 rodents were infected with endoparasites, two species of helminth parasites such as *H. nana* (26.67%) and *S. muris* (66.67%). *H. nana* was observed in small intestine and *S. muris* in all parts of the alimentary tract. The prevalence of infection was higher in caecum (63.33%) and rectum (63.33%) than small intestine (36.67%).

Kataranovski *et al.,* (2011) recovered seven helminth species from *R. norvegicus* in Belgrade area (Serbia) of which five were nematodes (*H. spumosa*, *Nippostrongylus brasiliensis*, *Capillaria* sp., *T. muris* and *S. muris*) and two cestode species *H. diminuta* and, *Rodentolepis fraternall*. Kiran *et al.*, (2013) reported that the prevalence of cestodes in three species of rodents, *R. rattus*, *R. norvegicus* and *M. musculus*, revealed 36% (n=50) of infection in four cities of Dehradun, from April 2011 to December 2011. Three species, *H. diminuta*, *T. taeniaeformis* and *Diphyllobothrium* sp., were recorded from each infected rodent. *R. norvegicus* were highly infected with cestodes (46.1%) followed by *R. rattus* (37.5%). Mean worm burden of *T. taeniaeformis* was high 3.3 and 2.8, respectively in both rodent species, *R. rattus* and *R. norvegicus*. In their study, they found mixed infection of nematodes and cestodes in 58% of captured rodents.

Milazzo *et al*., (2003) and Milaazzo *et al*., (2010) studied helminth fauna of commensal rodents, *M. musculus* and *R. rattus* in Sicily, Italy. Parasites recovered from black rat, *R. rattus* were one species of digenea (*Brachylaima* sp.), two species of cestode (*H. diminuta, H. nana*), seven species of nematodes (*S. muris, A. tetraptera, M. muris, H. spumosa, C. hepatica, Eucoleus gastricus* and *N. brasiliensis*) and one unidentified species of acanthocephala. Katranovski *et al.*, (2010) showed helminth fauna of *R. norvegicus* from the Belgrade, Serbia. They recovered *H. diminuta* (30.46%) and *Rodentolepis fraternal* (12.5%), *H. spumosa* (36.75%), *N. brasiliensis* (16.22%), *Capillaria* sp.(5.96%), *T. muris* (5.96%), *S. muris* (4.30%) and *Strongylus* sp. larvae (0.33%).

Muznebin, (2009) with other researchers found five helminth parasite species from two taxonomic groups from *R. norvegicus*. The cestodes were *Vampirolepis nana*, *H. diminuta* and nematodes were *C. dispar*, *H. spumosa* and *S. muris*. They found the highest prevalence in *H. diminuta* but the highest intensity was recorded in *V.*

nana. The prevalence and intensity of *V. nana*, *H. diminuta*, *C. dispar*, *H. spumosa* and *S. muris* were 56.25% (65.11±13.23), 72.92% (4.37±0.89), 62.50% (19.63 ±2.10), 66.67% (9.06±1.85) and 64.58% (24.65±2.60), respectively.

Paul *et al.*, (2016) investigated the prevalence of gastrointestinal helminths in rodents, in Maiduguri municipal between February and June 2015. They collected rodents randomly from residential sites within Maiduguri metropolis by trapping, using mechanical and glue board traps. Out of 85 rodents sampled in the study, a total of 7 (8.2%) were positive for gastrointestinal helminths. The only species of endoparasite identified was *H. diminuta*. There was no significant difference in prevalence of intestinal helminths among different sexes and age groups (P>0.05).

Singla *et al.* (2005) estimated the prevalence of endoparasitic infections of rodents in Punjab State, India. Three species of wild rodents, namely, *R. rattus* (*n* = 42), *B. bengalensis* (*n* = 34) and *T. indica* (*n* = 15), were live-captured from houses and crop fields. Various organs examined revealed that the highest rate of helminth parasites infection detected in *R. rattus* (40.5%), followed by *B. bengalensis* (35.3 %) and *T. indica* (20.0%), with an overall infection rate of 35.2%. Metacestodes (1–6) of *C. fasciolaris* were observed in the liver in all three rodent species.

Sumangali *et al.*, (2012) showed that urban rodents played important role in public health through being reservoirs of many zoonotic diseases. Screening of rodents for endoparasites from Peradeniya and Pilimathalawe in Kandy district was carried out to assess their potential as reservoirs of zoonoses. They caught live rodents by using single-catch rodent traps from July 2006 to February 2007. Three rodent species, *R. rattus* (n = 17), *M. musculus* (n = 2) and *B. indica* (n = 2) were examined. Five zoonotic endoparasites were identified, namely, *H. diminuta, M. moniliformis*, *Raillietina* sp. and *C. fasciolaris*. Cestodes were the predominant parasitic group (52.4%), of which *C. fasciolaris* (42.7%) was the most common type followed by *Strongyloides* sp. (19.0%). Among the infected rodents, 23.8% had mixed infections with *H. diminuta* and *M. moniliformis* and *H. diminuta*, *Raillietina* sp. and *C. fasciolaris*. Although *R. madagascariensis* was recorded in *R. rattus* in 1954, none of the rodents examined in their study was infected with *Raillietina.* However, one bandicoot rat was infected with *Raillietina* sp. in their study. They identified that urban rodents carried zoonotic infections, and in developing countries where the communities are socio-economically challenged, urbanization is in favour of the spread of these infections to humans.

Wahed *et al*., (1999) identified many endoparasites in wild rodents from Qalyobia Governorate. The species and their infection rates were *H. diminuta* (23.8%), *C. fasciolaris* (7%). While Elkady *et al*. (2008) recorded 10 species trematodes, 4 species cestodes and 10 species nematodes in rodents collected from Dakahlia Governorate. On other hand, Madi *et al*., (2001) found only one species of cestode in brown rats from urban area of Doha, Qatar. Zihiry (2006) recovered *H. diminuta* and two species of nematodes *Protospirura magna* and *Pterygodermatites tani* from *R. norvegicus* in Basrah, Iraq. Rodents gathered from Taif Governorate, Saudi Arabia have been found infected with *H. nana*, *H. diminuta* (Bashan and Sabra, 2012). Kassa and Assefa, (2000) recorded *H. diminuta* (30.7%) and *H. nana* (12.9%) among household rodents in Addis Ababa.

Zain *et al*., (2012) identified several species of parasites from urban rodents population in Kuala Lumpur, Malaysia. Eleven species of endoparasites comprising seven nematodes (*H. spumosa*, *M. muris, N. brasiliensis, S. muris, P. tani*, *G. neoplasticum*, *Angiostrongylus malaysiensis*), three platyhelminthes (*H. nana*, *H. diminuta* and *T. taeniaeformis*) and one Acanthocephala (*M. moniliformis*). On other study from Malaysia, Shafiyyah *et al*., (2012) identified *N. brasiliensis* (80.3%), *H. nana* (23.4%), *C. hepatica* (13.9%) and *H. diminuta* (2.9%) in wild rodents.

CHAPTER 3

MATERIALS AND METHODS

3.1. Sampling area and trapping of rodents

A total of 70 rodents were trapped from houses in the slum areas (n=20), different stationary shops (n=20), residential buildings (n=15) and rice fields (n=15). All the rodents were trapped alive using wire box bait traps with front spring door. Baits included meat, parts of fishes, breads, tomato, cucumber and cheese. Traps were set just before sunset, and were collected in the next morning. The rodents were brought to the laboratory of Microbiology and Parasitology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207 for dissection and collection of endoparasites (Figure 1).

Figure 1: Trapping of rodents and measuring of rodent for identification

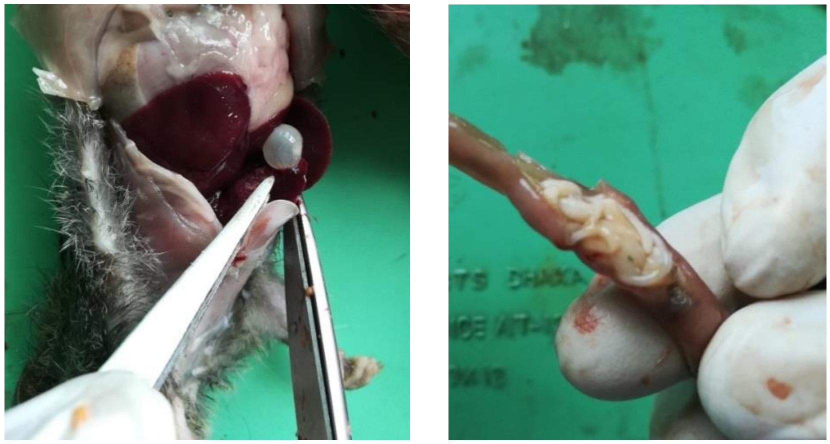
3.2. Animal dissection

Each of the rodents was put separately in a glass flask and anesthetized with a cotton plug soaked in chloroform until it dies. The body cavity of individual rodent was slit open from throat to anus revealing the esophagus, lungs, stomach, heart, small intestine, large intestine, liver and urinary bladder (Figure 2). The viscera were removed without damaging, and dissection were done separately under the dissecting microscope and examined for helminths. The contents of the intestine were also examined carefully for helminth parasites. The recovered endoparasites were washed with normal saline and fixed in 70% alcohol.

Figure 2: Dissection of rodent showing internal organ

3.3. Processing of cestodes, acanthocephala and nematodes

Cestodes and acanthocephala were collected in a separate glass petridishes, containing normal saline and washed three times to remove any debris. The flatworms were then flattened between two glass slides with slight pressure and fixed in 70% alcohol until future works. For staining, specimens were transferred to 50% alchol and washed in distilled water. Then specimens put in haematoxilin solution for 24hrs. The excessive stain was removed by 3% HCl. The stained specimen was dehydrated with ascending grades of alcohol (from 70% to 100%), cleared by xylene and mounted with Canada balsam. The nematodes were washed well in saline water to remove the preservative, and examined under microscope using lactophenol.



(A) (B)

Figure 3: Internal organs infected with helminth parasites. **A.** Liver having cyst containing *T. taeniaformis*. **B.** Small intestine infected with *H. diminuta*

CHAPTER 4

RESULTS AND DISCUSSION

Results

Through examination of 70 different types of rodents (*R. rattus*, *R. norvegicus*, *B. bengalensis* and *M. musculus*), a number of different endoparasites were recovered. The endoparasites include two species of cestodes (metacestode of *T. taeniformis* and *H. diminuta*), one species of acanthocephala (*M. moniliformis*) and two species of nematodes (*H. spumosa* and *G. neoplasticum*).

4.1. Morphological identification

4.1.A. Morphology of Metacestode of *Taenia taeniaeformis*

The results of this study showed the rodent livers infected with single and multiple hepatic cysts (Figure 4A). Some of these cystic structures were found less than 5mm in diameter. Liver with this size of cyst was suspected to be less than one month of infection, where the larva scolex was present. In cases of more than one month of infection, the cysts were biggest and clearest (Figure 4B). The tapeworm beared double rows of anchor shaped hooks with distinctly large four lateral suckers on the scolex indicating a cestode of the family Taenidae (Figure 4C, 4D). At the anterior end of the strobilocerci, the protoescolex consisted of an evaginated rostellum armed with a double and alternating ring of large and small hooks, which are also the morphological traits of the Family Taeniidae. The hooks were arranged in a circular pattern with a large double circlet of 30 to 48 hooks belongs to Genus *Taenia* and Species *T. taeniaeformis* (Figure 4C). Behind the scolex, there was the neck region. The third region was the strobilus which had pseudo-segmentation throughout the whole body (Figure 4E), and there was a bulged terminal portion at the posterior end of the parasites (Figure 4F) which are the characteristic features of metacestode of *T. taeniaeformis*.

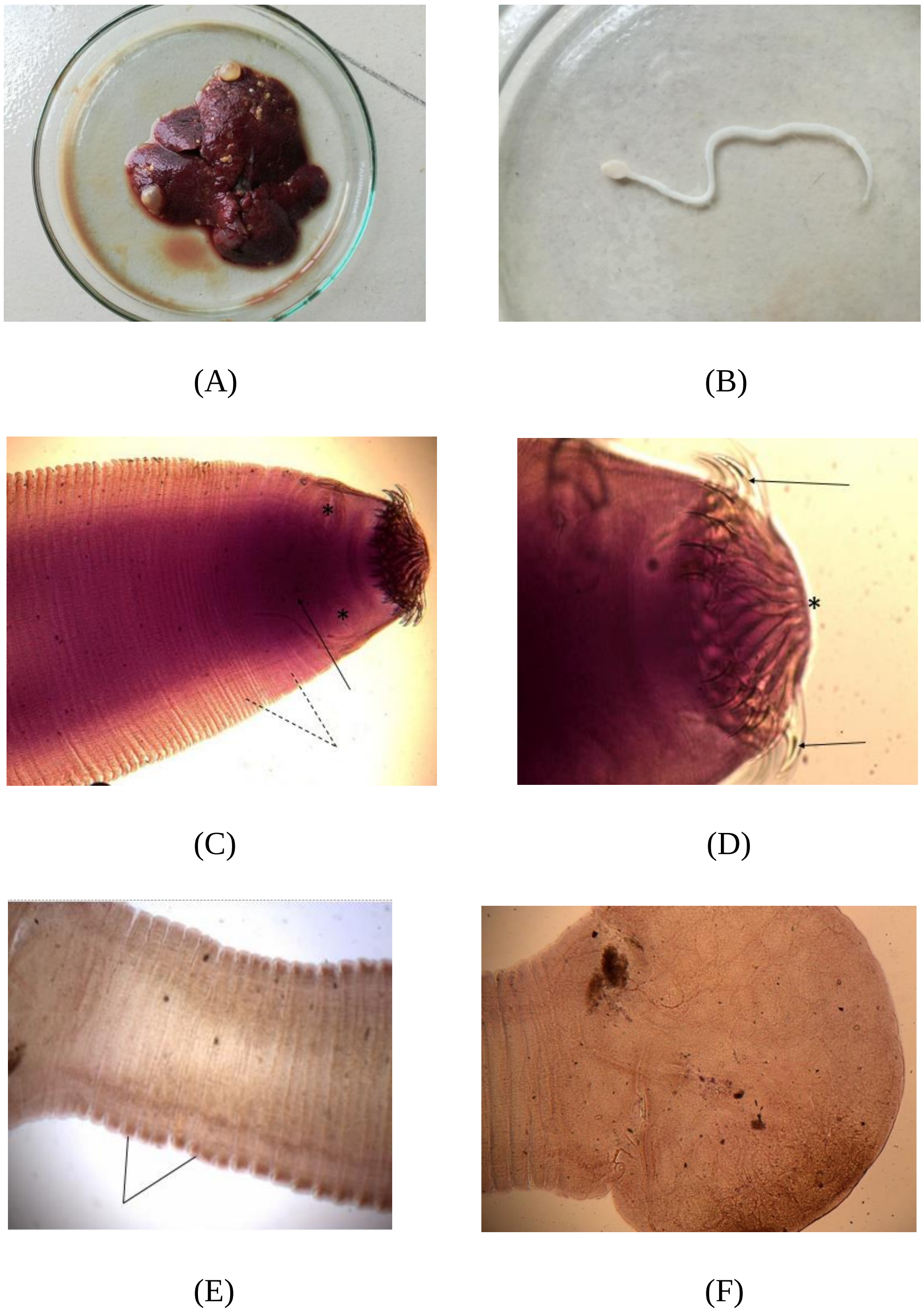


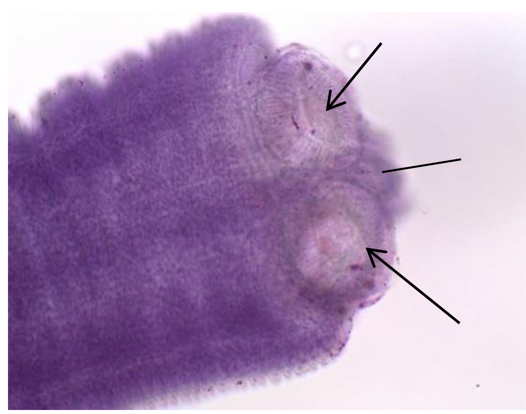
Figure 4: Different body parts of metacestode of *T. taeniformis*. **A.** The cyst of *C. fasciolaris*, a larval form in the liver of rodents. **B.** Metacestode of *T. taeniformis*, *C. fasciolaris*, found after the rupture of the cyst. **C.** Scolex region. "*" indicates suckers. The black arrow shows anterior end of strobilocercus. The broken lines represent immature pseudo–

segmentation just below the neck region. **D.** Protoscolex region. “*” indicates rostellum with hooks. The black arrows show double crown anchor shaped hooks. **E.** Strobilocercus. The black lines indicate mature pseudo-segmentation without internal organ. **F.** Bulged posterior portion (terminal bladder) of the parasite

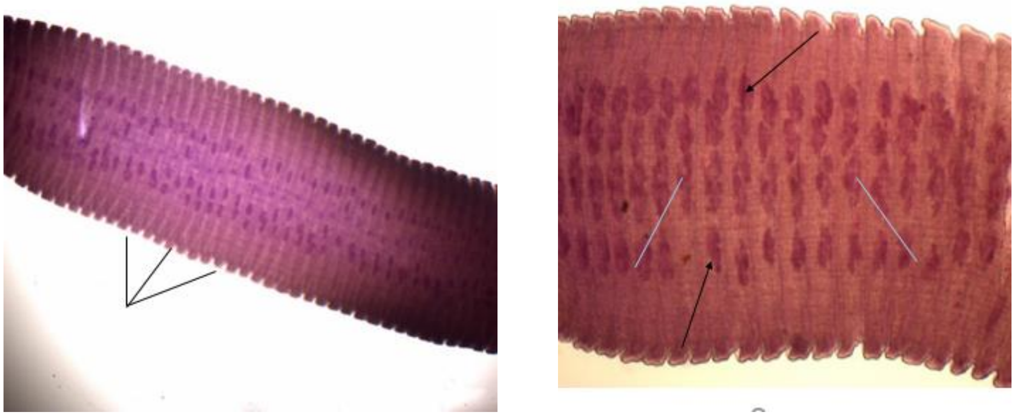
4.1.B. Morphology of *Hymenolepis diminuta* (Rat tapeworm)

Single and multiple mature *H. diminuta*, measuring 15- 60 cm long were isolated from the small intestine of rodents The scolex was spherical, and had four suckers located bilaterally on the dorsal and ventral surface (Figure 5A), which is identifying character of Order Cyclophyllidea. The scolx had retractable rostellum without hooks (Figure 5A, 5B) and the strobila started with short and narrow proglottids, followed by mature ones (Figure 5C). These are the identifying traits of the Family Hymenolepididae. Each mature segment contained with three ball like testes and one ovary (Figure 5D), matching to the morphological characteristics of Genus *Hymenolepis* and Species *H. diminuta*.

(A)



(B)

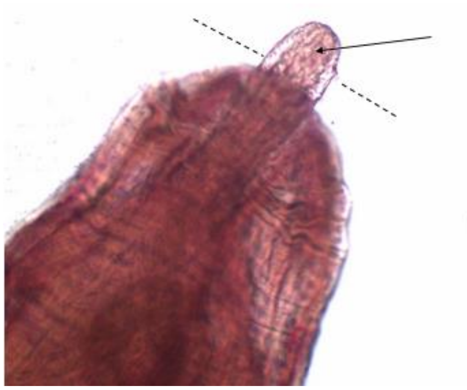


(C) (D)

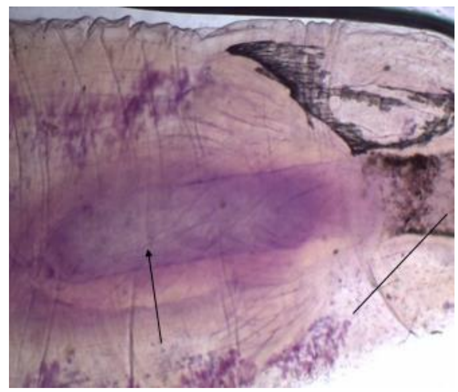
Figure 5: Different body parts of *H. diminuta*. **A.** Scolex region. Black lines indicate armed suckers. **B.** The black arrows indicate suckers. The black line indicates retractable unarmed rostellum. **C.** The black lines show mature proglottids with internal organs. **D.** Mature proglottids. The black arrows indicate testes. The white lines indicate ovary

4.1.C. Morphology of *Moniliformis moniliformis*

The worm was recovered from intestinal tract of examined Rodents. At the anterior end of the body, there was a retractable proboscis, and the second region, posteriorly, was the trunk. These morphological properties belonged to the Order Moniliformida. *M. moniliformis* was sexually dimoprhic, cylindrical worm. The adult male was generally 4 to 5 cm long, while female was longer, ranging from 10 to 30 cm. The proboscis was covered with hooks (Figure 6A), and was attached to the trunk by a neck. The proboscis retracted into a proboscis sheath which also known as receptacle (Figure 6B). The lemnisci, which arose near the point where the proboscis sheath attached to the trunk, and floated free within the body cavity (Figure 6C). These morphological features matched with these in the Family Moniliformidae. In male, there were two testes arranged in tandem (Figure 6D). Male had copulatory bursa, used to hold the female during copulation, and had cement glands. At the posterior end of the female body cavity, there was a selector apparatus, and then there was an uterus , which connected via a short vagina to a vulva. Occasionally a cement cap will be seen over the vulva, which was deposited by the male after copulation, these were the morphological structures of Genus and Species *Moniliformis* and *M. moniliformis*, respectively.

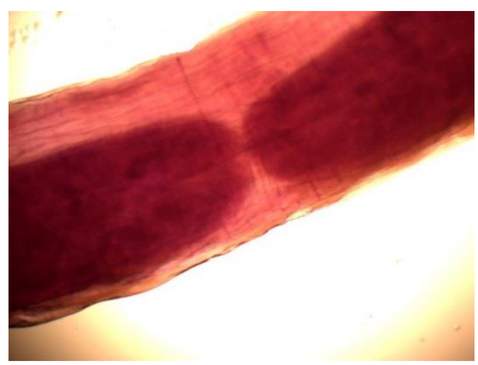


(A)



(B)

(C)



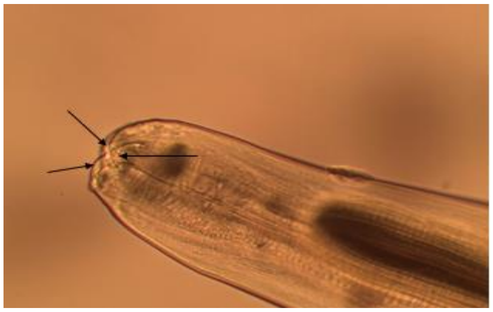
(D)

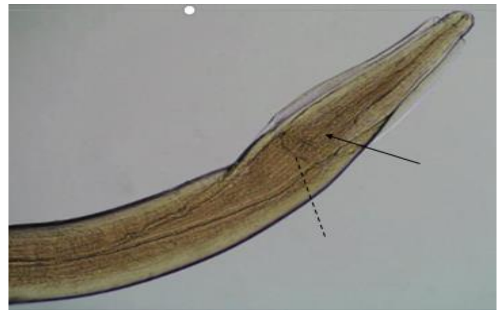
Figure 6: Different body parts of *M. moniliformis*. **A.** Proboscis covered with hooks. The black arrow indicates cylindrical retractable proboscis. The black broken lines indicate hooks. **B.** Proboscis sheath or receptacle indicated by the black arrow. The black line indicates proboscis. **C.** Lemnisci near the point where the proboscis sheath attaches to the

trunk, shown by the broken black arrows. **D.** Two testes arranged in tandem position

4.1.D. Morphology of Intestinal nematode, *Heterakis spumosa*

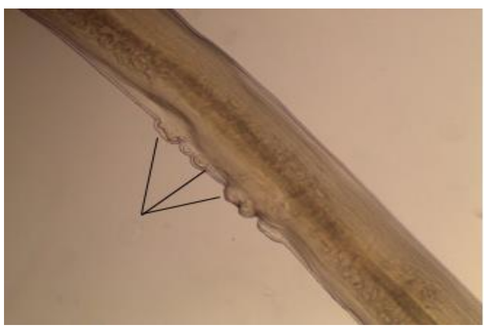
The specimens of *H. spumosa* were collected from the large intestine, specifically in the blind and initial portion of the colon. The adults *H. spumosa* were whitish in color, having 3 small unequal lips in the oral cavity, which are the special features of Order Ascaridida (Figure 7A). Oesophagus was cylindrical and long, ending in a distinct bulb with a "Y" shaped structure inside (Figure 7B). This morphological character resembles with the Family Heterakidae. In male, large pre-cloacal genital suction cup and spicule were present at the posterior end (Figure 7C). There were 3 pairs of lateral papillae (the proximal and distal papillae located dorsally, whereas the middle papilla was double and located ventrally) at the tip of the tail (Figure 7D). Females had 5 cuticular processes associated with vulva: first one anterior to the vulvar opening, second one posterior to it, and other three located posteriorly to the latter (Figure 7E). There was an elongated, sharp, pointed tail posteriorly (Figure 7F). Ovoid eggs with sharp shell were present. Eggs were slightly longer than they were wide and morulated (Figure 7G). Those special morphological features are corresponding to the Genus *Heterakis* and Species *H. spumosa*.


(A)


(B)


(C)


(D)


(E)


(F)

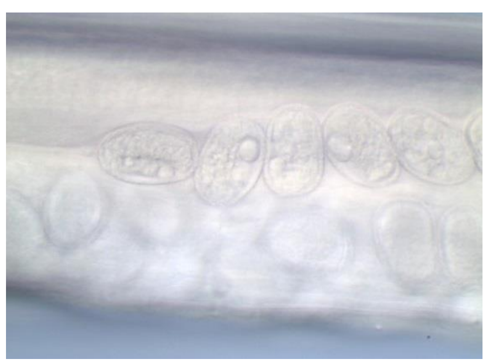

(G)

Figure 7: Different body parts of *H. spumosa*, **A.** Mouthpart. The black arrows indicate three unequal lips. **B.** Oesophagus, indicated by the black arrow. The black lines show bulb shaped oesophagus. **C.** Posterior part of male. "*" indicate pro-cloacal genital suction cup. The black broken line indicates spicule. **D.** Three pairs of lateral papillae, represented by the broken lines. **E.** Posterior part of female, The black lines indicate five cuticular processes associated with vulva. **F.** Posterior part of female, The black arrow indicates elongated, sharp and pointed tail. **G.** Embryonated eggs of female parasite

4.1.E. Morphology of *Gongylonema neoplasticum*

The anterior extremity of the worm was covered by verruciformes or humps which were abundantly found in female but were fewer in male (Figure 8A) These are the characteristics of the Genus *Gongylonema*. There was a pair of lateral cervical papillae. The buccal opening was small and extended in the dorsoventral direction. Around the mouth, a cuticular elevation enclosed the labia, and eight papillae were located laterodorsally and lateroventrally. Two large lateral amphids were seen. On the lateral sides of the female's tail, phasmidal apertures were observed. The males have two unequal spicules at the posterior end (Figure 8B). The caudal end of the male was asymmetrically alate and bore 10 pairs of papillae and two phasmidal apertures. The elliptical eggs had embryos with the L1 larva still inside the pregnant female (Figure 8C). The female had blunt tail (Figure 8D). These are the morphological characteristics of Genus and Species of *Gongylonema* and *G. neoplasticum*, respectively.

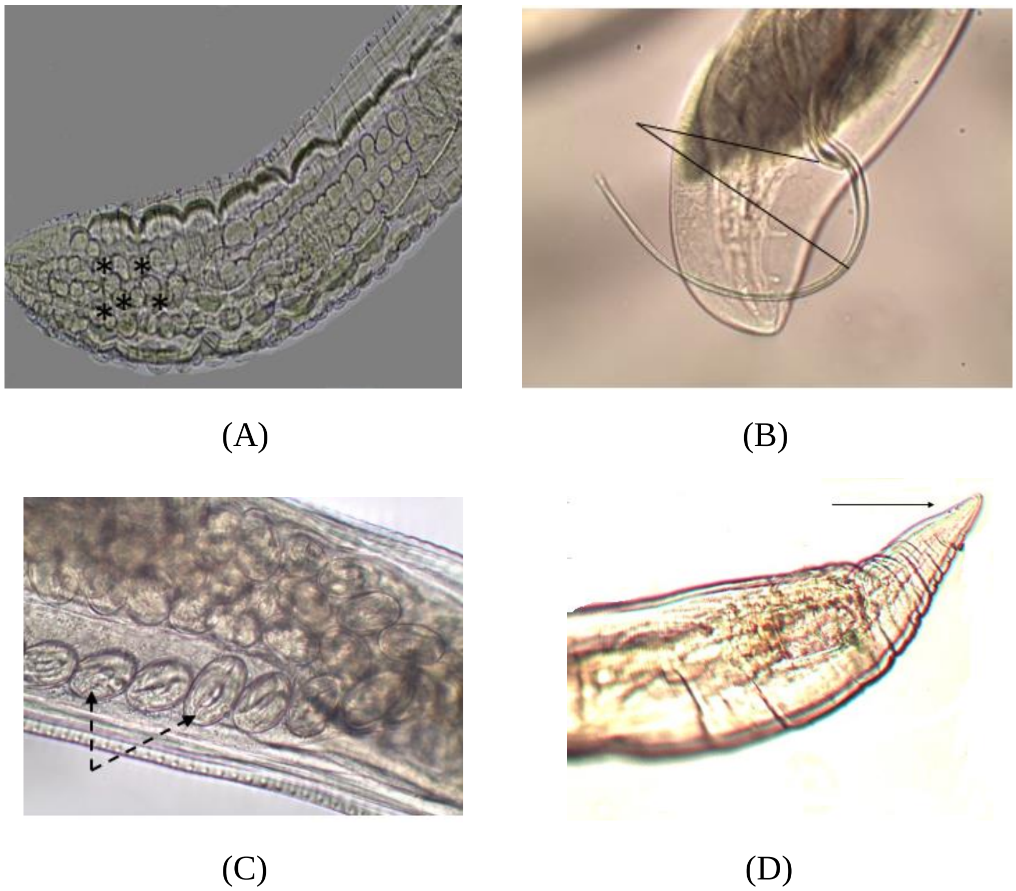


Figure 8: Different body parts of *G. neoplasticum*, **A.** Anterior part of the parasite. "*" indicates the numerous cuticular humps also known as verruciforms. **B.** Posterior part of male. The black lines indicate unequal spicules. **C.** Elliptical eggs of female. The broken black arrows show elliptical eggs containing embryos with the L_1 larva. **D.** Posterior part of the parasite. The black arrow indicates blunt tail

4.2. Prevalence

4.2. A. Prevalence of endoparasites in different rodents

The study was carried out in a total of 70 animals of which the number of *R. norvegicus, B. bengalensis, R. rattus* and *M. musculus* were 25, 20, 15, 10, respectively. Among 70 rodents, 50 (71.42%) were infected with helminths. *R. norvegicus* had the highest helminthic infection (84%), followed by *B. bengalensis* (75%), *R. rattus* (66.66%) and (40%) in *M. musculus* (Table 1).

Table 1: Prevalence of helminth infection according to the sex of the hosts

Host	Sex of the host	No. of host examined	Infected (%)
B. bengalensis	Male	13	10 (76.92%)
	Female	7	5 (71.43%)
	Total	20	**15 (75.00%)**
R. rattus	Male	9	7 (77.78%)
	Female	6	3 (50.00%)
	Total	**15**	**10 (66.66%)**
R. norvegicus	Male	15	13 (86.66%)
	Female	10	8 (80.00%)
	Total	**25**	**21 (84.00%)**
M. musculus	Male	6	3 (50.00%)
	Female	4	1 (25.00%)
	Total	**10**	**4 (40.00%)**

4.2.B. Prevalence of single and mixed infection in rodents

Both male and female rodents examined were found to be infected with one or more species of endoparasites. Thirty six out of 50 rodents (72%) had mixed endoparasitic infection, and only 14 (28%) had single infection (Table 2). In case of 33 male rodents, only 9 (27.27%) were infected with single endoparasites. However, 24 (72.72%) male rodents were infected with mixed helminth infection. Furthermore, out of 17 female rodents, 5 (29.41%) were infected with single endoparasites. Whereas, 12 (70.59%) were infected with mixed endoparasitic infection. Among the infected rodents (50), the percentage of male and female was 66% and 34%, respectively (Table 2).

Table 2: Percentages of single and mixed infection in examined males and females rodents

Sex	**Type of infection**		**Total**
	Single (%)	**Mixed (%)**	
Males	9 (27.27%)	24 (72.72%)	33
Females	5 (29.41%)	12 (70.59%)	17
Total	**14 (28.00%)**	**36 (72.00%)**	**50**

4.2.C. Prevalence of endoparasites in rodents collected from different areas in Dhaka city

The highest number of infected rodents came from the houses in the slum areas, contributing to (85%), followed by stationary shops (75%), local rent houses (66.66%) and rice fields (53.33%) shown in the Table 3.

Table 3: Prevalence of parasites in rodents collected from different areas in Dhaka city

City structure	Trapped rats	Infective rats (%)
Houses of Slum areas	20	17 (85.00%)
Rice fields	15	8 (53.33%)
Local rent Houses	15	10 (66.66%)
Stationary shops	20	15 (75.00%)

4.2.D. Quantitative indices of helminth infection and prevalence of helminth infection in male and female rodents

The prevalence of each species of helminth parasites were varied greatly from one another in different rodents. The prevalence of *H. spumosa* was the highest (60%) in rodents, followed by *H. diminuta* (47.14%), *M. monliformis* (42.85%), *T. taeniformis* (35%) and *G. neoplasticum* (34.28%) (Table 4). A total of 20 rodents were infected with *T. taeniformis*, where 13 (65%) were male rodents and 7 (35%) were female rodents. Thirty three rodents were infected with *H. diminuta*, 25 (75.26%) were male rodents and 8 (24.24%) were female rodents. Thirty rodents were infected with *M. monliformis,* whereas 20 (66.67%) male rodents and 10 (33.33%) female rodents. In this study, *H. spumosa* was recovered highest from rodents. Out of total 42 rodents, 28 (66.67%) male rodents and 14 (33.33%) female rodents were infected with *H. spumosa*. *G. neoplasticum* newly found in Bangladesh, in total of 24 rodents, 18 (75%) male and 6 (25%) female rodents were infected with *G. neoplasticum* (Table 4).

Table 4: Quantitative indices of helminth infection and prevalence of parasitic species in male and female rodents

Helminth species	**No. of infected rodents (%)**	**No. of Male rodents (%)**	**No. of Female rodents (%)**
Metacestodes of *T. taeniformis*	20 (35.00%)	13 (65.00%)	7 (35.00%)
H. diminuta	33 (47.14%)	25 (75.76%)	8 (24.24%)
M. moniliformis	30 (42.85%)	20 (66.67%)	10(33.33%)
H. spumosa	42 (60.00%)	28 (66.67%)	14(33.33%)
G. neoplasticum	24 (34.28%)	18 (75.00%)	6 (25.00%)

Discussion

In present study all the trapped rodents belonged to four different species such as *R. norvegicus*, *R. rattus*, *B. bengalensis* and *M. musculus*. These rodents are peri-domestic and omnivorous very often seen in buildings, streets, in sewage channels, crops fields, waste disposal sites, farms, slaughter houses, food storage and around houses of the different cities of Bangladesh. The present study gives the overview on the intestinal parastic infection of rodents in Bangladesh. Five species of endoparasites were reported, namely, *H. diminuta, C. fasciolaris* (larval form of *T. taeniaformis*), *M. moniliformis, H. spumosa and G. neoplasticum*. Except *H. spumosa*, all have zoonotic and medical importance. In this study *G. neoplasticum*, a rodent nematodes, has been reported for the first time in Bangladesh to our best of knowledge.

Most of the above parasites have been previously reported in rats from different parts of the Bangladesh (Huq, 1969, Shaha, 1974; Bhuiyan *et al.,* 1996; Khanum *et al.,* 2001; Khanum *et al.,* 2009; Alam *et al.,* 2003; Khanum and Arefin, 2003;

Muznebin *et al.,* 2009 and Gofur *et al.,* 2010). Furthermore, many authors reported similar parasites in different parts of the world (Wahed *et al*., 1999; Seong *et al*., 1995; Madi *et al*., 2001; Soliman *et al*., 2001; Stojcevic *et al*., 2004; Waugh *et al.*, 2006; Coomansingh *et al*., 2009; Bashan *et al*., 2012; Pakdel *et al*., 2013; Priyanto *et al*., 2014; Porta *et al*., 2014). The existence of rodents which act as reservoir hosts to different type of the parasites in close association to human activities may facilitate the transmission of zoonotic parasites (Sumangali *et al*., 2012; Amarasingh and Premathilake, 2014; Guimarăes *et al.,* 2014).

City structures and poor sanitary conditions are accounted higher rodents captured and helminth infection. The results of the study have revealed that 71.42% rodents were infected with helminths and *R. norvegicus* had the highest infection rate 84%. Despite of heavy infection and marked hepatic cysts in the liver, all animals appeared healthy and agile. Among host species, *R. norvegicus* had the highest helminth infection, 61.5%. Huq *et al*., (1985) reported 96.41% prevalence in *R. rattus*. Bhuiyan *et al.* (1996) reported that about 83.33% *B. bengalensis* and 82.08% *R. rattus* were found to be infected. Gofur *et al.,* (2010) reported that about 76.67% *R. norvegicus* were infected with helminth parasites in Bangladesh. From the point of view, Nama and Parihar, (1980) recorded 63.5% rodents infected with helminths in Jodhpur city. High prevalence rate (58.5%) of endoparasitic infection was also recorded in black rat collected from Palestine (Hindi and Haddaf, 2013) and 40.5% from India (Singla *et al*., 2008). In Bangladesh, due to biological and ecological properties, most of the research showed that the infection rate of *R. norvegicus* was higher than any other rodents.

The present study reported that the prevalence of endoparasites in male and female was 66% and 33.33%, respectively. Gofur *et al.* (2010), reported that prevalence of helminth parasites in male and female was 80% and 73.33%, respectively. Senussi, (2016) showed that males had higher parasitic infection rate (24%) than females (20%) in *R. rattus*. This result was in consistent with those recorded by

Mafiana *et al.,* (1997); Katranovski *et al*., (2010); Hindi *et al*., (2013) and Porta *et al.,* (2014). The prevalence of parasitic infection varied from rodent to rodent depending on climate, physiological and behavioral structure. The prevalence of helminth infection in male rodents were higher than female. The reason behind the above satiation could be attributed to that males are more active than females. They have larger house territories which could increase their exposure to infection. While, reproductive females show a stronger site-specific organization and the male hormone testosterone has negative effects in the immune functions (Calhoum, 1962; Grossman, 1989; Folstad and Karter, 1992). Also, due to ecological and physiological cause male are more infected than female. Sexually mature male rodents are often more susceptible to infection and carry higher parasite burdens in the field.

The present study reported that the highest number of infected rodents found from the houses in the slum areas (85%), followed by stationary shops (75%), local rent houses (66.66%) and rice fields (53.33%). The pronounced growth of urban slum settlements, most of which has occurred in tropical regions of the world with poor resources developing countries like Bangladesh. Over the past 50 years, the urban ecology has been transformed creating new habitats for rodents. Lack of access to proper services and poor housing and sanitation condition in slum communities, boost parasitic infection by rodents, assisting the epidemic transmission of infectious diseases in humans (Glass *et al*., 1989, 2005; Childs *et al*., 1991; Ko *et al*., 1999). Slum communities are characterized by untended refuse, open sewers, and overgrown vegetation, which promote rodents infection. There is high possibility of transmission of parasites to human because of continuous contact with hosts. Due to availability of food stuff, low maintenance, lack of hygienic measurement the presence of infected rodents are showable in the stationary shop. The study showed that *M. musculus, R. rattus* and *B. bengalensis* were highly available in local rent houses. In rice fields availability of infected rodents are slightly less than other selected areas due to lack of hosts.

The cestodes recorded in this study include *H. diminuta* and metacestodes of *T. taeniaformis*. *H. diminuta* is a cestode frequently noticed in rodents and humans. Human become infected with *H.diminuta* from accidental ingestion of insects that harbor cysticercoid stage of the parasite in their body cavities. In present study, *H. diminuta* was recovered from the examined rodents with high prevalence (47.14%). However, many other studies observed lower infection rates (Wahed *et al*., 1999; Kassa and Assefa, 2000; Milazzo *et al*., 2003; Milazzo *et al*., 2010; Bashan and sabra., 2012; Kiran *et al*., 2013; Hindi and Haddaf, 2013). Munzebin *et al.*, (2009) reported 72.92% and Gofur *et al.*, (2010) reported 53.33% from *R. norvegicus* from Dhaka city. Kumarasingh *et al*., (2006) recorded high prevalence of cestodes such as *H. diminuta* (38%) from Kandy district. In a recent paper reporting the eight human infections of *H. diminuta* in Spain, a summary of the prevalence of infection in different populations around the world ranged from 0.001% to 5.5% (Tena *et al.*, 1998). The first human case of *H. diminuta* in Iran was reported in 1968, from Mashad area in the northeast of the country in a 10 years old child (Motakef, 1968). In 1972, in a rural area along the Persian Gulf in southern Iran, five cases of infection were found among 635 persons examined (Ghadirian and Arfaa, 1972). Human cases of hymenolepiasis caused by *H. diminuta*, has been reported in the Heilongjiang Province, China (Kang *et al.*, 1994).

Another cestode recovered from the examined rodents was *C. fasciolaris* (metacestode of *T. taeniaformis*), having prevalence of 35%. *C. fasciolaris* is a metacestode of *T. taeniaeformis*, feline tape worm, which is commonly found in liver of intermediated hosts, such as mice, rats, cats, muskrats, squirrels, rabbits, other rodents, bats and human. A higher prevalence rates were reported in rodents from India (Singla *et al*., 2008; Kiran *et al*., 2013); Srilanka (Sumangali *et al*.,

2012) and Nigeria (Ogunniyi *et al*., 2014). Due to hosts specificity, favourable transmissible cycle *C. fasciolaris*, there was high prevalence of *T. taeniformis* in rodents.

In the present study, *M. moniliformis* was the only acanthacephalan isolated from the rodents with incidence rate 42.85%. Human cases with these parasites have been reported from many countries (Faust and Russel, 1964; Muller, 1975; Salehabadi *et al*., 2008). Commansingh *et al.*, (2009) reported 2.7% from wild rats in Grenada city. Wide range of mammals, including rodents are susceptible to *M. monilifomis.*

In this study, the prevalence of *G. neoplasticum* was 34.28%. Chaisiri *et al*., (2017) reported that 10.7% *G. neoplasticum* found from rodents in Cambodia. *Gongylonema* spp. are heteroxenous parasites of the upper digestive tract of many species of birds and mammals. They are most often described in ruminants, but also in rodents, bears, monkeys and human. The adult worms occur in the stomach and in the oesophagus where they burrow and migrate in the mucosa, forming a characteristic sinuous pathway. *G. neoplasticum* is going to be reported for the first time from rodents in Bangladesh.

H. spumosa is a nematode of invasive rodents, mainly affiliated with *Rattus* spp. of Asian origin. Despite the ecological importance and cosmopolitan distribution, little information is available on the genetic characteristics and infectivity to experimental animals of this roundworm in Bangladesh. In this study, the prevalence of *H. spumosa* was approximately 60%. Munzebin *et al.*, (2009) reported that 66.67% *H. spumosa* were found from *R. norvegicus* in Dhaka city. Nematode *H. spumosa* are considered to be highly prevalent in *Rattus* spp. on Honshu Island, Japan and estimated the prevalence of *H. spumosa* in *Rattus* spp. to be approximately 50 to 60 % in Sagamihara, east central Honshu (Villiam *et al.*, 2014). Due to host availability, favourable condition and direct life cycle, they are easily infected the rodents. As so far all the recorded endoparasites except *H. spumosa* in this study from *R. rattus*, *R. norvegicus*, *B. bengalensis* and *M. musculus* have been reported in human from different part of the world. More over the increase population of rats in the city may increase the risk of infection with plague disease because they act as reservoir host. These types of parasites affect not only human health but also livestock. Parasitic infection of livestock, crops are major ailments impend the development of these industries in Bangladesh. The status of helminth infection and species diversity in rodents, which indicates that habitat alteration might affect helminth infection and diversity in rodent hosts. Generalized linear models revealed that host attributes (host species and maturity) and environmental factors (geographical location and habitat) were explanatory variables for helminth infection in these rodents.

CHAPTER 5

SUMMARY AND CONCLUSION

This study was performed in the Dhaka city aimed to find out the prevalence and morphological identification of endoparasites found in four types of rodents such as *B. bengalensis, R. rattus*, *R. norvegicus* and *M. musculus* are common in the city and suburbs. A total of 70 rodnets were captured by box bait traps, and the overall results showed 71.42% infected by one or more types of endoparasites. The results showed that the overall incidence of the endoparasites in *R. norvegicus* was the highest (84%), followed by *B. bengalensis* (75%), *R. rattus* (66.66%) and *M. musculus* (40%).

Both male and female rodents were infected with one or more types of endoparasites. Only 14 (28%) rodents were infected with single type of intestinal parasites while, 36 (72%) rodents were infected with multiple types of endoparasites. The highest endoparasites infection rate observed in houses of slum areas (85%), followed by stationary shops (75%), local rent houses (66.66%) and rice fields (53.33%). The higher prevalence was observed in male rodents (33%) than female rodents (17%). The results showed that the prevalence of *H. spumosa* was the highest 60%, followed by *H. diminuta* 47.14%, *M. moniliformis* 42.85%, *T. taeniformis* 35% and *G. neoplasticum* 34.28%. The nematode *G. neoplasticum* is going to be reported for the first time from rodents in Bangladesh. Humans and animals are at risk from zoonotic helminths through rodents. Human activities that disturb the ecosystems where these rodents live which play important role in the epidemiology of zoonotic diseases. The information presented here improves our understanding of the major parasitic infections that rodents harbor and can transmit to human and animal populations in Bangladesh. In order to avoid unpleasant situations adequate preparations of rodent control should be implemented in Dhaka city and other parts of Bangladesh.

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