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Original papers

Prevalence and detail morphological identification of helminths of murine rodents in Dhaka city, Bangladesh

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ABSTRACT. Rodents are common pests that transmit various deadly pathogens to humans. Here we have studied the helminth parasites of rodents from different ecological niches in Dhaka city, Bangladesh. The gastrointestinal helminths were investigated from a total of 70 rodents, namely *Bandicota bengalensis* (20), *Rattus rattus* (15), *Rattus norvegicus* (25) and *Mus musculus* (10). The rodents were live-captured from houses in the slum areas (20), stationary shops (20), residential buildings (15) and rice fields (15). The overall prevalence of helminth infection was 71.43%. 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 recorded in slum areas (85%). Out of 50 rodents, 36 (72%) had mixed endoparasitic infection whereas only 14 (28%) rodents had single infection. The prevalence of endoparasitic infection in male (66%) rodents was higher than that of female (34%). The parasites detected from the rodents were *Heterakis spumosa* (60%), *Hymenolepis diminuta* (47.14%), *Moniliformis moniliformis* (42.85%), *Taenia taeniaeformis* (35%) 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 rodent borne zoonosis through the control of rodents.

Keywords: prevalence, morphological identification, helminths, murine rodents, Dhaka city

Introduction

Rodents represent by many families, of which subfamily Murinae under the family Muridae, embraces the small rodents such as rats, mice and rat-like rodents. Their predatory and depredatory habits have a noticeable impact on human health through their role as the major vectors of human and domestic animal diseases worldwide [1,2]. 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. Rodents act as reservoir hosts of some parasites infecting humans and livestock [3]. Rodents may spread numerous diseases worldwide, many of which have zoonotic potentials. The rodent borne helminths such as, *Taenia taeniaeformis*, *Hymenolepis nana*, *Hymenolepis diminuta*, *Gongylonema neoplasticum*, *Moniliformis moniliformis* etc. are reported as transmissible to human, and constitute a public health problem [4].

The infection of *T. taeniaeformis* has been reported in man from Sri Lanka, Argentina, Czechoslovakia, Denmark, etc. [5]. Human hymenolepiosis caused by *H. diminuta* is rare, and being diagnosed a total of 500 human cases worldwide [6]. *Moniliformis moniliformis*, acanthocephala or thorny-headed worm, is normally a parasite of the rodent, the hamster, the white mouse, the cat, and the dog in most parts of the world. This acanthocephalan parasite is also recognized as a zoonotic parasite which may rarely infect humans. The parasite, *G. neoplasticum*, observed in stomach

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%)
Total		70	50 (71.42%)

wall is natural and common parasite among rodents. In human, a fewer than 60 cases of *G. neoplasticum* have been reported worldwide. The intestinal parasite, *H. spumosa*, is a common nematode of rodent throughout the world [7,8–10].

Although emerging rodent-borne diseases have been captured worldwide attention, but little has been documented on this aspect in South Asia. The endoparasitic infections, which are harbored and transmitted by human and animal has not been as thoroughly investigated as the microbial infections, especially in Bangladesh. In Bangladesh, very few studies [3,11–17] 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.

Therefore, the aim of this present study was to carry out the prevalence and detail morphological identification of helminths of rodents to assess the load of rodent borne helminth infections in Dhaka city, Bangladesh.

Materials and Methods

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). Traps were set just before sunset and collected in the next morning. The trapped rodents were brought to the laboratory for dissection and collection of helminths. Each of the rodents was put separately in a glass flask and anesthetized with a cotton plug soaked in chloroform until it dies. 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 helminths were washed with normal saline and fixed in 70% alcohol.

Cestodes and acanthocephalans 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 for future works. For staining, specimens were transferred to 50% alcohol and washed in distilled water. Then specimens were 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.

The recovered parasites were studied under microscope and photo graphs of major identifying organs were taken. For cestodes and acanthocephalans, hooks, suckers, rostellum, proglottids, pseudo-segmentation, ovary, testes, proboscis, receptacle, lemnisci were considered for identifying

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

organs while lips, oesophagus, pre-cloacal genital suction cup, spicule, papillae, tail, verruciformes or humps, eggs, L1 larva were considered for the identifying keys of nematodes. The species confirmation was made by following the keys and description published previously [18–22].

Results

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

Among 70 rodents, 50 (71.42%) were infected with helminths, of which *R. norvegicus* had the highest helminth infection (84%), followed by *B. bengalensis* (75%), *R. rattus* (66.66%) and *M. musculus* (40%) (Table 1).

The highest prevalence was found in rodents in the slum areas (85%), followed by stationary shops (75%), local rent houses (66.67%) and rice fields (53.33%) (Table 2).

Both male and female rodents examined were infected with one or more species of helminths. Thirty six out of 50 infected rodents (72%) had mixed endoparasitic infection, and only 14 (28%) had single infection (Table 3).

The prevalence of *H. spumosa* was the highest (60%) in rodents, followed by *H. diminuta* (47.14%), *M. moniliformis* (42.85%), *T. taeniaeformis* (35%) and *G. neoplasticum* (34.28%) (Table 4).

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

City structure	Trapped rodents	Infective rodents (%)
Houses of slum areas	20	17 (85.00%)
Rice fields	15	8 (53.33%)
Local rent houses	15	10 (66.67%)
Stationary shops	20	15 (75.00%)
Total	70	50 (70%)

Morphological identification

Metacestode of *Taenia taeniaeformis*

The results of this study showed that livers of some rodents had single or multiple hepatic cysts of (Fig. 1A) less than 5mm in diameter. In cases of more than one month of infection, the cysts were biggest and clearest (Fig. 1B). The tapeworm beared double rows of anchor shaped hooks with distinctly large four lateral suckers on the scolex (Fig. 1C, 1D). At the anterior end of the strobilocerci, the protoscolex consisted of an envaginated rostellum armed with a double and alternating ring of large and small hooks. The hooks arranged in a circular pattern with a large double circlet of 30 to 48 hooks (Fig. 1C). Behind the scolex, there was the neck region. The third region was the strobilus which had pseudo-segmentation throughout the whole body (Fig. 1E), and there was a bulged terminal portion at the posterior end of the parasites (Fig. 1F) with these characteristic morphological features, the isolates were identified as *C. fasciolaris*, the metacestode of *T. taeniaeformis*.

Hymenolepis diminuta

Single and multiple mature *H. diminuta*,

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. taeniaeformis*	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%)

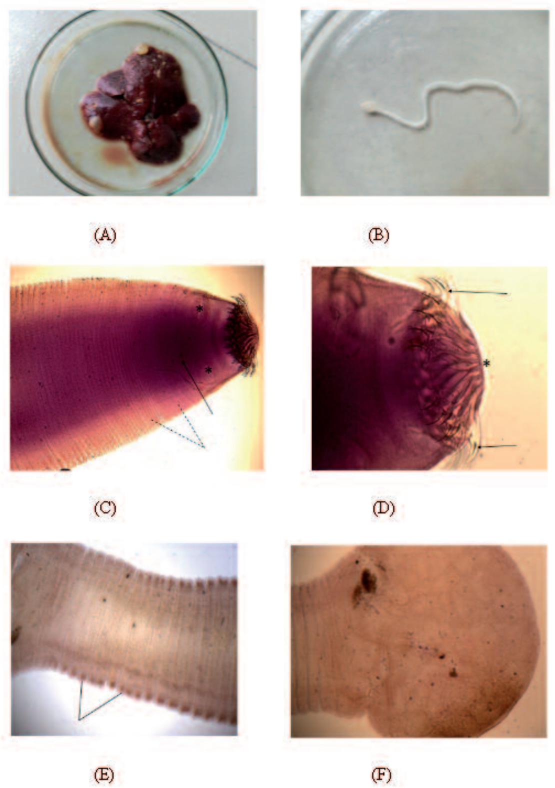

Figure 1. Different body parts of metacestode of *T. taeniaeformis*. A. The cyst of *C. fasciolaris*, a larval form in the liver of rodents. B. Metacestode of *T. taeniaeformis*, *C. fasciolaris*, found after the rupture of the cyst. C. Scolex region. "*" indicates suckers. The black arrow shows anterior 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.

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 (Fig. 2A). The scolx had retractable rostellum without hooks (Fig. 2A, 2B) and the strobila started with short and narrow proglottids, followed by mature ones (Fig. 2C). Each mature segment contained three ball like testes and one ovary (Fig. 2D). These morphological properties matched with those of *H. diminuta*.

Moniliformis moniliformis

The worms were 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. The adult

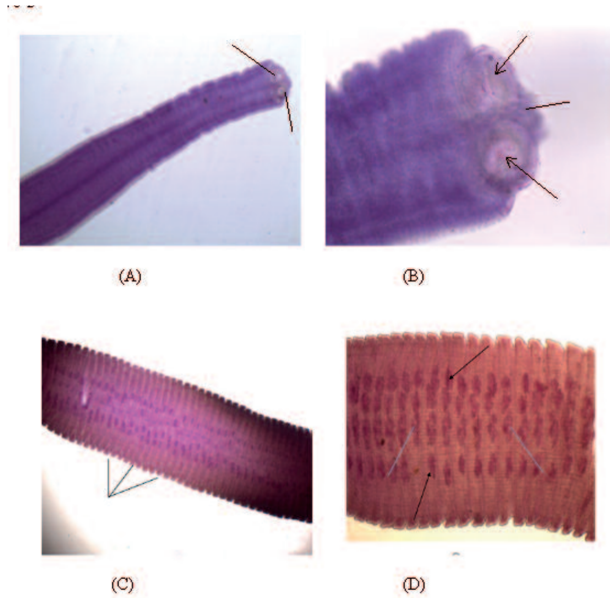

Figure 2. Different body parts of *H. diminuta*. A. Scolex region. Black lines indicate armed suckers. B. The black arrows indicate suckers. The black line indicates retractable unarmed rostellum. C. The black lines show mature proglottids with internal organs. D. Mature proglottids. The black arrows indicate testes. The white lines indicate ovary.

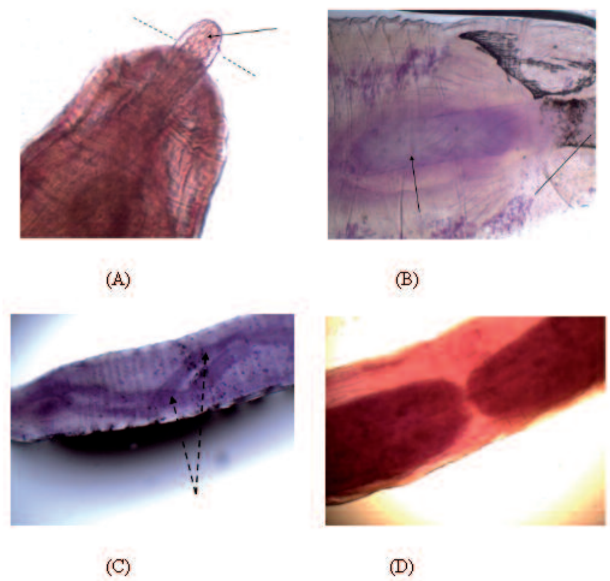

Figure 3. Different body parts of *M. moniliformis*. A. Proboscis covered with hooks. The black arrow indicates cylindrical retractable proboscis. The black broken lines indicate hooks. B. Proboscis sheath or receptacle indicated by the black arrow. The black line indicates proboscis. C. Lemnisci near the point where the proboscis sheath attaches to the trunk, shown by the broken black arrows. D. Two testes arranged in tandem position.

Figure 4. 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.

male was generally 4 to 5 cm long, while female was longer, ranging from 10 to 30 cm. The proboscis was covered with hooks (Fig. 3A), and was attached to the trunk by a neck. The proboscis retracted into a proboscis sheath which also known as receptacle (Fig. 3B). The lemnisci, which arose near the point where the proboscis sheath attached to the trunk, and floated free within the body cavity (Fig. 3C). In male, there were two testes arranged in tandem (Fig. 3D). 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 a uterus, which was connected via a short vagina to a vulva. From these morphological properties the specimen were identified as *M. moniliformis*.

Heterakis spumosa

The specimens of *H. spumosa* were collected

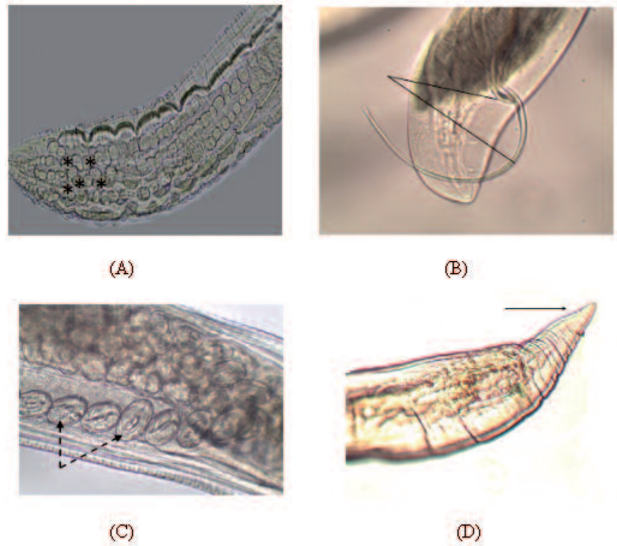

Figure 5. Different body parts of *G. neoplasticum*. A. Anterior part of the parasite. "*" indicates the numerous cuticular humps also known as verruciforms. B. Posterior part of male. The black lines indicate unequal spicules. C. Elliptical eggs of female. The broken black arrows show elliptical eggs containing embryos with the L1 larva. D. Posterior part of the parasite. The black arrow indicates blunt tail.

from the large intestine, specifically in the blind and initial portion of the colon. The adults *H. spumosa* were whitish in color, having three small unequal lips in the oral cavity (Fig. 4A). Oesophagus was cylindrical and long, ending in a distinct bulb with a "Y" shaped structure inside (Fig. 4B). In male, large pre-cloacal genital suction cup and spicule were present at the posterior end (Fig. 4C). 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 (Fig. 4D). 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 (Fig. 4E). There was an elongated, sharp, pointed tail posteriorly (Fig. 4F). These morphological features correspond to the species *H. spumosa*.

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 (Fig. 5A). There was a pair of lateral cervical papillae. The buccal opening was small and extended in the dorso-ventral direction. Around the mouth, a cuticular elevation enclosed the labia, and eight papillae were located latero-dorsally and latero-ventrally. Two large lateral amphidswere seen. On the lateral sides of the female's tail, phasmidal apertures were observed. The males have two unequal spicules at the posterior end (Fig. 5B). 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 in female (Fig. 5C). The female had blunt tail (Fig. 5D). These morphological characteristics proved that the specimen were *G. neoplasticum*.

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 an overview on the intestinal parasitic infection of rodents in Bangladesh. Five species of helminths were reported, namely, *H. diminuta*, *C. fasciolaris* (larval form of *T. taeniaeformis*), *M. moniliformis*, *H. spumosa and G. neoplasticum*. Except *H. spumosa*, all have zoonotic and medical importance. To our best of knowledge *G. neoplasticum*, a rodent borne nematode is going to be reported in this study for the first time in Bangladesh.

City structures and poor sanitary conditions are accounted higher rodents capture and helminth infection. The results of the study have revealed that 71.42% rodents were infected with helminths where *R. norvegicus* had the highest infection (84%). 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.

In this study, highest number of infected rodents was found from the houses in the slum areas (85%). The pronounced growth of urban slum settlements, most of which has occurred in tropical regions of the world with poor resources in 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 boosted parasitic infection by rodents, assisting the epidemic transmission of infectious diseases to humans [23–25]. Slum communities are characterized by untended refuse, open sewers, and overgrown vegetation, which promote rodents infection. There is high possibility for transmission of parasites to human because of continuous contact with hosts. In rice fields availability of infected rodents are slightly less than other selected areas due to lack of other hosts.

In the present study, the prevalence of helminths in male and female rodents was 66% and 33%, respectively. The higher prevalence in males may be due to the fact that males are more active than females. Males 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 [26–28]. Also due to ecological and physiological causes, males are more infected than females. Sexually mature male rodents are often more susceptible to infection and carry higher parasite burdens in the field.

The cestodes recorded in this study include *H. diminuta* and metacestodes of *T. taeniaeformis* (*C. fasciolaris*). *Hymenolepis 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. Another cestode, *C. fasciolaris* (metacestode of *T. taeniaeformis*), had a prevalence of 35%. A higher prevalence was reported in rodents from India [29] and Sri Lanka [18]. Because of host specificity, favourable transmissible cycle *C. fasciolaris*, there was high prevalence of *T. taeniaeformis* in rodents. In the present study, *M. moniliformis* was the only acanthacephalan isolated from the rodents with incidence rate of 42.85%. Human cases with these parasites have reported from many countries [29].

In this study, the prevalence of *G. neoplasticum* was 34.28%. *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 to the mucosa, forming a characteristic sinuous pathway. *Gongylonema neoplasticum* is going to be reported for the first time from rodents in Bangladesh. *Heterakis 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. Due to host availability, favourable condition and direct life cycle, they are easily infecting the rodents. These types of parasites affect not only human health but also livestock.

In conclusion, 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 play important role in the epidemiology of zoonotic helminth infection. The information presented here may improve our understanding on 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 towards rodents control should be implemented in Dhaka city and other parts of Bangladesh.

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

Ethical approval. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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