Abstract
A total of 14 faecal samples were screened to determine the occurrence and intensity of intestinal helminth parasites in Asiatic lions kept at Dhaka Zoo, Bangladesh. The identified parasite eggs included cestode (*Spirometra* sp.) and nematodes (*Toxascaris leonina*, Hook worm and *Strongyloides* spp.). The overall incidence of parasitic infection was 92.9%, of which 78.6% (11/14) of the samples were positive for *Spirometra* sp., 57.1% (8/14) for *Toxascaris leonina*, 28.6% (4/14) for hookworm and 21.4% (3/14) for *Strongyloides* spp. This study emphasizes the need for the establishment of routine control programmes against helminthosis in zoo animals so as to safeguard the health of captive wild animals, and of humans working with and visiting these animals.

Introduction
Dhaka zoo located just besides Dhaka city, exhibiting the wild animals in natural settings using modern method of keeping many animal species in close proximity to one another. Under natural conditions, excessive infections of helminths seldom occur, whereas in caged animals, as in a zoo, the stress to which the animals are subjected weakens their immunological system, making them more susceptible to parasite infection (Perez Cordon *et al.* 2008).

Helminth diseases are an important problem for zoo animals (Isaza *et al.* 1990; Singh *et al.* 2006a). Occurrence of parasites in animals housed in zoological gardens might vary according to the type of husbandry practices, disease prophylaxis and treatment administered (Lim *et al.* 2008).

Intensive husbandry of animals produces conditions which facilitates the spread of parasites. The frequent use of anthelmintics often results in evolution of resistant strains. Moreover, the nutritional status of captive animals can also enhance or diminish their resistance to diseases (Geraghty *et al.* 1982). Gastrointestinal parasites of zoo animals include zoonotic species to humans and raise public health concerns (Singh *et al.* 2006b, Levecke *et al.* 2007).

Despite of above facts, prevalence data of gastrointestinal parasites in zoological gardens remain scarce. Only a couple of studies have comprehensively examined parasites in captivity worldwide (Ippen and Henne 1982; Barutzki *et al.* 1985; Ghoshal *et al.* 1988; Kumar *et al.* 2005). Therefore, this present study attempts to determine the occurrence and intensity of helminth parasites of Asiatic lions, captive at Dhaka Zoo, Bangladesh. The findings of this study may prove essential for the development of a better understanding of the helminth parasite fauna of these animals and for the prevention of spread of infectious parasitic diseases among animals within the zoo or to humans.

Materials and Methods

Study sites and animals
This study was conducted at Dhaka Zoo, Bangladesh. Its present collection includes about 1,669 animals belonging to 120 different species, including 54 species of mammals (392 animals), 57 species of birds (1208 animals) and 9 species of reptiles (69 animals). This zoo covers an area of over 75.53 hectares. For the prevention of parasitic diseases, zoo animals are treated thrice a year (April, August and December) with appropriate anthelmintics on rotation basis. Animals are checked by the zoo veterinarian and the animal caretaker/ handlers; fecal analysis is performed routinely and positive animals are treated accordingly.

Sampling and parasitological analysis
A total of 14 (08 males and 06 females) Asiatic lions are present at Dhaka zoo. The 20-25 grams of faecal sample from individual lion was collected immediately after defecation or in the next morning. The samples were collected in separate polythene bags and preserved in 10% formalin until further processing in March-April 2010. The correctly labeled and properly numbered polythene bags containing the faecal samples with all required information were brought to the laboratory of the department of Parasitology, Bangladesh Agricultural University, Mymensingh for microscopic examination. Parasite eggs were tentatively identified according to the morphology and then quantitative estimation was done by using the Stoll’s ova dilution technique to determine eggs per gram (EPG) (Soulsby 1982).

Results
In the present study, a total of 14 Asiatic lions (*Panthera leo persica*) were examined for the presence of intestinal helmint parasites. The overall prevalence of parasitic infection was 92.9%, of which 78.6% (11/14) of the samples were positive for *Spirometra* sp., 57.1% (8/14) for *Toxascaris leonina*, 28.6% (4/14) for hookworm and 21.4% (3/14) for *Strongyloides* spp. (Table 1). It was observed that, all the male Asiatic lions (8/8) were infected by one or more species of intestinal parasites whereas 83.3% (5/6) females were infected (Table 2). All the aforesaid species of parasites were identified in males but *Strongyloides* spp. was absent in females. Mixed parasitic...
infections were recorded in 7 out of 13 positive samples (Table 3). The most frequent cases (three) of multiple parasitism were Spirometra spp. + Toxascaris leonina + Hook worm, whereas, Spirometra sp. + Toxascaris leonina + Strongyloides spp. and Spirometra spp. + Toxascaris leonina + Hook worm + Strongyloides spp. was observed only once and the mixed infection comprising of Spirometra sp. + Toxascaris leonina was found twice. In the present study, EPG (Egg per Gram of Faeces) was also determined. The range of EPG was 100-20000 for Spirometra sp., 100-10000 for Toxascaris leonina, 100-500 for hookworms and 100-300 for Strongyloides spp.

**Discussion**

The majority (92.9%) of the Asiatic lions examined in this study were found infected with at least one intestinal parasite species. Most of the helminths species identified in this study have previously been described in captive lions (Kumar et al. 2005; Fagiolini et al. 2010), housed in different zoological garden and are known to be pathogenic to both animals and human beings (zoo veterinarian, animal handlers and visitors). The results of the present study supported the fact that the parasitic diseases caused by intestinal helminths have a higher prevalence in zoo animals of the countries with warm and tropical climates, due to favorable development factors such as light, temperature and moisture (Perez Cardon et al. 2008).

Tapeworms have been found commonly among zoo animals (Chauhan et al. 1973 & Sen Gupta 1974) but only tapeworm Spirometra sp. was detected in this study. Spirometra sp. was the most prevalent parasite (78.6%) and had the highest number of eggs per gram of faeces. Eggs had a fairly thin shell, yellowish in colour, were roughly oval shaped, and slightly pointed at the end, with an operculum on one side. Eggs were filled with cells and no other host species, the availability of mice paratenic host, direct life cycle of the parasite, and the

### Table 1. Prevalence and intensities of intestinal helminth parasite eggs in faecal sample of captive Asiatic Lions.

<table>
<thead>
<tr>
<th>Parasites</th>
<th>No of animals affected (n=14)</th>
<th>Percentage (%)</th>
<th>Intensity Per Gram of Feces (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirometra sp.</td>
<td>11</td>
<td>78.6</td>
<td>100-20000</td>
</tr>
<tr>
<td>Toxascaris leonina</td>
<td>08</td>
<td>57.1</td>
<td>100-10000</td>
</tr>
<tr>
<td>Hook worm</td>
<td>04</td>
<td>28.6</td>
<td>100-500</td>
</tr>
<tr>
<td>Strongyloides spp.</td>
<td>03</td>
<td>21.4</td>
<td>100-300</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13</strong></td>
<td><strong>92.9</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

* Total no. of animals affected is less than the summation of individual infection because same animal was infected by more than one type of gastro-intestinal helminths; n - Total samples examined

### Table 2. Sex related prevalence of intestinal helminth parasites eggs in faecal sample of captive Asiatic Lions.

<table>
<thead>
<tr>
<th>Parasites</th>
<th>No of animals affected (prevalence %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=08)</td>
</tr>
<tr>
<td>Spirometra sp.</td>
<td>7 (87.5)</td>
</tr>
<tr>
<td>Toxascaris leonina</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>Hook worm</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Strongyloides spp.</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>8</strong> (100.0)</td>
</tr>
</tbody>
</table>

* Total no. of animals affected is less than the summation of individual infection because same animal was infected by more than one type of gastro-intestinal helminths; n = Total samples examined

Roundworm and hookworms, which cause visceral or cutaneous larva migrans in humans, are the most common helminths reported in carnivores (Custer and Pence 1981 & Paton and Rabinowitz 1994, Singh et al. 2006b). The present study reported that among the infected Asiatic lions, there was a higher occurrence of Toxascaris leonina (57.1%) compared to hookworms (28.6%) and Strongyloides spp (21.4%). Fagiolini et al. (2010) also reported a higher prevalence of T. leonina than hook worms in lions kept at Giardino Zoologico of Pistoia. The size of the ascarid eggs here was consistent with T. leonina, although Toxocara canis and T. cati have both been found in lions. T. leonina egg is slightly oval shaped with a smooth outer shell whereas both T. cati and T. canis have spherical with rough pitted outer shell though it is difficult to differentiate between these two Toxocara species based on the egg morphology. It has been recorded that Toxocara spp. is the most common roundworm of Felidae as well as Canidae (Abe and Yasukawa, 1996; Lim et al. 2008) including zoo lions (Ippen and Hene 1982; Barutzki et al. 1985; Ghoshal et al. 1988; Kumar et al. 2005). It has also been reported that Toxascaris species was not found in wild lions, but appears frequently in zoo lions (Barutzki et al. 1985; Ghoshal et al. 1988; Tang et al. 1988). In general differences in parasites between wild lions and lions in zoos may be explained by their close proximity to a wide variety of other host species, the availability of mice paratenic host, direct life cycle of the parasite, and the
suitable tropical climatic condition with high moisture that helped *T. leonina* to survive in the zoo environment.

*Strongyloides* and hookworm nematodes were previously reported in free ranging as well as in captive wild felids (Muller-Graf 1995; Gonzalez et al. 2007; Lim et al. 2008 & Fagiolini et al. 2010). In the present investigation, the hookworm eggs found were oval to ellipsoidal, thin walled with 8-16 cell morula and the empty space within the egg. The species was not differentiated to either *Ancylostoma duodenale* or *Ancylostoma tubaeforme*. Frequent hook worm infection among the captive Asiatic lions might be due to the nature of spreading infection as animals usually get infected through percutaneous, prenatal, and trans-mammary transmission (Hendrix and Robinson 2006). The eggs of *Strongyloides* spp. were thin shelled, transparent with embryo or larvae inside. The species of the parasites could not be differentiated morphologically, but Soulsby (1982) described *S. stercoralis* and *S. cati* in dogs and cats. Frequent dung removal and the treatment of infected animals are amongst most important parasite control strategies (Citino 2003). Dung removal is not easily applicable in most areas of the Dhaka Zoo, especially where lions are kept in free range conditions. This could be one of the causes for the higher prevalence observed, even though the animals at this zoo live in large cages, often open exhibits and are treated thrice a year with an anthelmintic drug. On the contrary, cleaning and dung removal, along with routine fecal parasite analysis and the selective treatments performed at the Dhaka Zoo, were more effective measures for the control of these helminth infections.

In this study, it was found that males (100%) were more susceptible to parasitic infection than females (83.3%). The exact cause of higher incidence of parasitic infection in male could be associated with hormonal phenomenon as the elevated plasma testosterone (T) levels were generally found associated with increased parasitism (Roberts et al. 2004), the causal mechanisms underlying this pattern were not well understood. There are two primary, nonexclusive hypotheses proposed to link T levels with parasite load (Klukowski and Nelson 2001). One possibility was that T increased the probability of encountering parasites by stimulating movement and extending daily activity period. Alternatively, T might increase susceptibility to infestation via suppression of the immune system (Klein, 2004). This mechanism has received considerable attention in light of the immunocompetence handicap hypothesis (Folstad and Carter 1992).

In this study, mixed infection was observed in 7 out of 13 lions. A number of reports unfolded the multiparasitism in primates mostly on deer (Levecke et al. 2007; Perez Cordon et al. 2008 & Kanungo et al. 2010). Gregarious nature and suitable environmental condition might be the cause of mixed parasitic infection in free range Monkeys (Mutani et al. 2003). As several lions of different ages and sexes are housed in a single cage and are supplied with same meat and water, chances of mixed infection in such case are pronounced.

In the present study, EPG (Egg per Gram of Faeces) was also determined by Stoll’s ova counting technique. The range of EPG was 100-20000 for *Spirometra* sp., 100-10000 for *Toxascaris leonina*, 100-500 for hookworms and 100-300 for *Strongyloides* spp. Out of 11 positive cases of *Spirometra* sp., 4 samples were having EPG between 10,000 and 20,000. On the other hand, only single case with occurrence of 10,000 EPG for *Toxascaris leonina* was there. After the coprological diagnosis, the parasitized animals were treated by the veterinarian of the Dhaka zoo, using appropriate drugs and the correct treatment procedures to avoid resistance. Some measures were taken against the most frequent parasites, based on cleaning and disinfecting the area, the troughs and the feeders as well as providing the information concerning the care of the animals to the professionals. 

In conclusion, findings of this study revealed that intestinal helminth parasites and multiple infections are highly prevalent in captive Asiatic lions and some of these enteroparasites can be transmitted to humans. This study emphasizes the need for the establishment of routine control programmes of these helminths in zoo animals as to safeguard the health of housed animals, and of humans working with and visiting these animals. The prevalence of parasites recorded in the examined zoo indicate that husbandry procedures, the routine monitoring of parasitic diseases and the use of selective anthelmintics can represent useful measures for the control of intestinal helminth infections in zoological gardens.

**Acknowledgements**

The authors are very grateful to the Curator of Dhaka Zoo, for giving permission to collect faecal samples within the zoo and animal caretaker for providing guidance and technical assistance during fecal sample collection. Special thanks also go to the Head, department of Parasitology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh to permit using the Laboratory.

### Table 3. The most frequent multiple infections by intestinal helminth parasites in faecal sample of captive Asiatic Lions.

<table>
<thead>
<tr>
<th>Parasites</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Parasites: <em>Spirometra</em> sp. + <em>Toxascaris leonina</em></td>
<td>2</td>
</tr>
<tr>
<td>3 Parasites: <em>Spirometra</em> sp. + <em>Toxascaris leonina</em> + Hook worm <em>Spirometra</em> sp. + <em>Toxascaris leonina</em> + <em>Strongyloides</em> spp.</td>
<td>3 + 1</td>
</tr>
<tr>
<td>4 Parasites: <em>Spirometra</em> sp. + <em>Toxascaris leonina</em> + Hook worm + <em>Strongyloides</em> spp.</td>
<td>1</td>
</tr>
</tbody>
</table>
References


