

Spirocercosis in stray dogs of Chittagong Metropolitan area of Bangladesh: an epidemiological and pathological investigation

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Abstract

A total of sixty adult stray dogs (32 male and 28 female) randomly captured from different locality of Chittagong metropolitan area were euthanized during the period of May 2010 to January 2011. The dogs were necropsized to observe the presence of *Spirocerca lupi* in their oesophagus producing characteristic nodular lesions. The associated haematological changes were also determined. Twenty four (40%) of the captured dogs were positive (40.62 % male and 39.28 % female) for spirocercosis having oesophageal nodular lesions. One to three broad based nodular lesions with an average diameter of 2.104 ± 0.139 cm were observed in the caudal oesophagus of spirocerca positive dogs. Minimum 1 and maximum 12 worms found harboring in each nodule with an average number of 3.75 ± 0.686 . The association between the oesophageal nodular lesions and body condition of the dogs was inconclusive and location-wise prevalence was also not clear. Anaemia was observed in infected dogs with significant reduction in ESR values and MCHC%. Leukocytosis, particularly neutrophilia was common in infected dogs in comparison to the non-infected. Histopathology revealed extensive fibrous tissue proliferation around the adult worms in most of the nodular lesions with large numbers of newly formed blood vessels resembling granulation tissues. A considerable degree of fibroplasia was evident with predominant accumulation of fibroblasts and irregular layers collagen fibers in mature nodules. Accumulation of large numbers of neutrophil was common in nodular lesions, whereas, infiltration of lymphoplasmacytic cells also found in some cases. One lesion showed evidence of fibrosarcoma characterized by proliferation of undifferentiated atypical fibroblasts having abnormal mitotic figures.

Key Words: Fibrosarcoma, Histopathology, Haematology, Nodule, Prevalence, Stray dogs, Spirocercosis, Parasite,

Introduction

Spirocercosis is a cosmopolitan parasitic disease of carnivores caused by a spirurid nematode *Spirocerca lupi* (Mazaki-Tovi *et al.* 2002, Dvir *et al.* 2001, and Lobetti, 2000). It is mostly prevalent in warmer climatic conditions particularly in tropical and subtropical countries (Van der Merwe *et al.* 2008). In infected dogs, spirocercosis produce variety of clinical manifestations depending on the stage of the disease, aberrant migrations and complications. Vomition or regurgitation, dysphagia, dyspnoea, pyrexia and anorexia are the commonly found manifestations (Lobetti, 2000, Jubb *et al.* 1993 and Bailey, 1972). No sex or age predilection for this infection have been recorded, but due to the nematodal lifecycle, dogs below 6 months of age do not develop oesophageal disease or exhibit classic clinical signs (Fox *et al.* 1988 and Wandera, 1976). There seem to be an increased susceptibility for infection in larger breed dogs like

German shepherd and Labrador retrievers (Mazaki-Tovi *et al.* 2002, Lobetti, 2000 and Bailey, 1963).

The *Spirocerca lupi* associated lesions are dependent upon its indirect lifecycle which involves an intermediate (coprophagous beetle) and a variety of paratenic hosts (amphibians, reptiles, lizards, domestic, birds and small mammals) (Anderson, 2000, Soulsby, 1986 and Sharpilo, 1983). Infective larvae within the intermediate host or paratenic host follow a specific migratory route when ingested by final hosts (Dogs); penetrating the gastric mucosa of the dog, migrating along arteries, maturing in the thoracic aorta and then migrate to the caudal oesophagus to form a nodular or tumor-like growth (Urquhart *et al.* 1996 and Soulsby, 1986). Larvae cause necrosis, hemorrhage and neutrophil exudation within the vessel walls in which they migrate. In the thoracic aorta, degenerate elastic and muscle tissue becomes fibroid and mineralized to form permanent

intimal scars with aneurysms of varying size and number. Metaplastic ossification of the aorta, to the extent of bone marrow formation, has also been reported (Kumar *et al.* 1981). Parasites containing oesophageal nodular lesions are the most common findings for definitive diagnosis of spirocercosis in dogs which can be observed by necropsy, radiography and endoscopy. The detection of typical embryonated eggs in fecal smears is difficult and requires special laboratory techniques and facilities for visualization (Fox *et al.* 1988; Cabrera and Bailey, 1964). One to four worm-containing nodules are usually found in the submucosa of the wall of the oesophagus located a few centimeters cranial to the diaphragm (Bailey, 1963 and Chandrasekharan *et al.* 1958). The nodules may vary from <1 to >4 cm in diameter and they can distort the oesophageal wall and extend to the surrounding mediastinal tissues. The worms present in a nodule may vary from a few to greater than 30 in number. The adults are large spiraled pink worm with males up to 54 mm and females up to 80 mm in length (Soulsby, 1982).

Histologically *Spirocerca lupi* associated oesophageal nodules are composed predominantly of fibrocytes with ample mature collagen at initial stage where with the time of nodular maturation; actively dividing fibroblasts become predominant. These fibroblasts are located between numerous immature capillaries, peripheral to the worms and their migratory tracts. The development of oesophageal osteosarcomas and fibrosarcomas has been reported in dogs infected with *Spirocerca lupi*, particularly in endemic areas (Moulton, 2002 and Bailey, 1972). The infected dogs usually show mild anaemia in almost 50% cases (normocytic normochromic anaemia) of early disease conditions (Ranen *et al.* 2004 and Mazaki-Tovi *et al.* 2002). In advanced conditions when nodules transform into neoplasia microcytic hypochromic anaemia become evident. Leukocytosis is more likely found associated with oesophageal osteosarcomas though monocytosis is also reported in some cases. In some recent studies no correlation found between the white cell counts and the spirocercosis associated inflammatory conditions such as pneumonia or spondylitis (Ranen *et al.* 2004 and Mazaki-Tovi *et al.* 2002).

Stray dogs are free roaming ownerless or feral dogs that are not dependent upon human for supervision. Chittagong is a port city located on the banks of the Karnaphuli River in the southeast part of Bangladesh. Tropical monsoon climate and thousands of free roaming stray dogs attributes this densely

populated metropolitan as an endemic place for spirocercosis. A very few reports on spirocercosis in Bangladesh are available; but no epidemiological or pathological investigation has been conducted yet. Therefore this study was undertaken to find out the prevalence of spirocercosis in the stray dogs of Chittagong metropolitan area emphasizing on epidemiology and pathology of this disease.

Materials and Methods

Collection of animals and Necropsy: A total of sixty (60) stray dogs from different thanas of Chittagong metropolitan area were randomly captured and humanely euthanized during the period from May-2010 to February-2011. The capture was conducted under permission of Chittagong City Corporation. The necropsy was conducted in the Pathology and Parasitology laboratory of CVASU at the earliest possible time of euthanasia following standard method described in Coles (1986). For studying the oesophageal lesions the junction of the stomach and duodenum was ligated and oesophagus was separated from other parts of gastro-intestinal tract. Gross pathological changes in oesophagus were carefully recorded, photographed and parasites were collected. From the gross lesions, smaller tissue sections of 4 mm³ thickness were fixed in 10% neutral buffered formalin for histopathological study.

Histopathological study: Only one nodule preferably the most mature one was selected for each dog for histopathology. Formalin fixed tissue samples were washed and dehydrated in graded ethanol and embedded in paraffin wax. Fixed tissues were sectioned at 5 µm thickness and stained with hematoxylin and eosin as per standard method (Luna, 1968).

Haematological study: Haematological parameters such as Red blood cells (RBCs), white blood cells (WBCs) count and haemoglobin concentration (Hb) were manually done according to Campbell (1995). Packed cell volume (PCV) was determined according to Howlett *et al.* (2002). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as mentioned by Campbell (1995). Thin blood smears were immediately prepared and stained with Diff Quick stain (EMD Chemicals, Inc., Gibbstown, New Jersey 08027, USA) and 200 leukocytes were differentiated in smears prepared from each animal.

Statistical analysis : The obtained data was imported, stored and coded accordingly using Microsoft Excel-2000. Chi Square test was done for

Table-1. Association of spirocercosis with different variables

Traits	Variable	Sample	Positive	% Positive	Chi2 Value	P value
Sex (N=60)	Male	32	13	40.62	0.27	0.59
	Female	28	11	39.28		
Location (N=60)	West khulshi	15	6	40.00	0.57	0.90
	Pahartali	17	6	35.29		
	Halishahor	17	8	47.05		
	Pachlaish	11	4	36.36		
Season (N=60)	Summer	28	14	50.00	2.18	0.13
	Winter	32	10	31.25		
Body condition (N=60)	Good	21	9	42.82	1.33	0.51
	Fair	20	6	30.00		
	Poor	19	9	47.36		

determination of association between the variable by using statistical software STATA/IC-11. The associations of spirocercosis with Haematological values were measured by One way ANOVA using statistical software SPSS-11.5. Significance was determined in 95% confidence interval when $p < 0.05$.

Results

Based on oesophageal lesions spirocercosis were diagnosed in 24 (out of 60) captured stray dogs. Among the positive dogs 13 (40.62%) were male and 11 (39.28%) were female. The prevalence of the infections was various in different areas and ranged from 35.29% in Pahartali to 47.05% in Halishahor. Prevalence of spirocercosis in Pachlaish and West khulshi area was 36.36% and 40.00% respectively. The prevalence was considerably higher (50.00%) in summer than (31.00%) in winter (Table 1). In respect to the body condition the prevalence of spirocercosis observed highest (47.36%) in dogs with poor body condition, where only 30.00% dogs with fair health showed infection. Dogs with good health also showed infection in 42.82% dogs in this study (Table 1). In the present study no significant difference observed between spirocercosis with the gender variation, seasonality, locality and body condition of the stray dogs. As because dogs below 6-9 month age do not exhibit any typical oesophageal lesion and in this study the age of the dogs were not considered. Again, it was practically impossible to study the breed predilection of the disease as all captured dogs were of indiscriminate native types.

Table-2. The biometric values of the oesophageal nodules

Variables	Mean ± SE	Range	
		Minimum	Maximum
Nodule size (cm) N=24	2.10±.14	0.70	3.00
Parasite per Nodules (number) N=24	3.75±.69	1.00	12.00

Necropsy Findings: Minimum One to maximum three oesophageal nodules were found in each infected dog having a broad firm nipple like protuberance bulging from the oesophageal wall. Most of the nodular lesions had smooth surface with a window like orifice at the center and adult parasites were seen protruding out through that opening (Figure I). The nodules were also seen distorting the oesophageal lumen. The nodular lesions varied in size from less than 1 cm (0.7 cm) to more than 3 cm in diameter and usually situated at the distal portion (less than 4 cm from the cardia of stomach) of the thoracic oesophagus (Figure II). The average size of the oesophageal nodules was 2.104 ± 0.139 cm (Table 2). Red colored spirally coiled adult *S. lupi* were seen embedded within the nodular lesions. Several sinus pockets and parasitic migratory tracts were also seen in the incised nodules. The number of worms per nodule varied from one to twelve with an average of $3.750 \pm .686$ (Table 2).

The haematological tests exhibits reduction in Hb (g/dl) concentration and RBC counts in 66% spirocerca positive cases although no significant

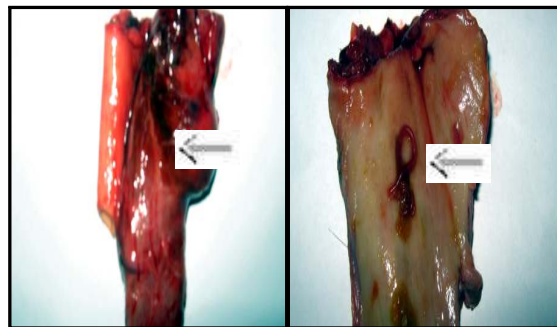


Figure -I. Large nodular lesion with smooth surface in the wall of esophagus protruding outwards from the serosal surface (Lt); broad base nodule with window like opening (aero head) for releasing eggs; note the red colored coiled adult parasite emerged from the opening (Rt)

Table-3. Haematological values of infected and non infected groups of stray dogs

Variables	Positive (N=24)	Negative (N=36)	Ref Value	P Value
Hb (g/dl)	9.60 ± 0.27(8.00-12.00)	10.16 ±0.41(6.00-16.00)	14.2-19.2	0.31
PCV (%)	34.50 ±1.04(27.00-44.00)	32.80 ± 1.99(10.00-46.00)	29-55	0.51
ESR (% in first hour)	1.83 ± 0.30 (1.00-5.00)	3.30 ± 0.51(0-10.00)	0-6	0.03
RBC (x106 cells/µl)	5.92 ±0 .66 (2.50-12.15)	6.24 ± 0.48(2.20-11.72)	5.5-8.5	0.69
WBC (x103)	13.71 ± 1.23 (3.34-22.10)	12.49 ±0.81(2.23-19.10)	5.9-16.6	0.39
Lymphocyte (%)	45.25 ± 2.15 (28.00-69.00)	50.02 ± 2.01(31.00-72.00)	8-38	0.11
Neutrophils (%)	45.41 ± 2.18 (25.00-62.00)	38.66 ± 2.22(6.00-62.00)	51-84	0.04
Eosinophil (%)	4.33 ±0.58 (1.00-11.00)	4.83 ±0.53 (1.00-13.00)	0-9	0.53
Monocyte (%)	5.16 ± .53 (1.00-12.00)	5.47 ±.36 (0.00-11.00)	1-9	0.62
Basophil (%)	0.33 ± 0.13 (0.00-1.00)	0.194±0 .07(0.00-1.00)	0-1	0.23
MCV (fl)	77.56 ± 9.59 (26.34-176.00)	62.04 ± 5.68 (12.58 -135.80)	65-80	0.14
MCH (pg)	20.12 ± 1.65 (7.57-33.60)	20.08 ±1.53 (6.31-34.60)	12.2-25.4	0.99
MCHC (%)	28.39 ± 1.08 (18.18 -36.36)	36.87 ±3.31(17.62 -100)	32-36	0.04

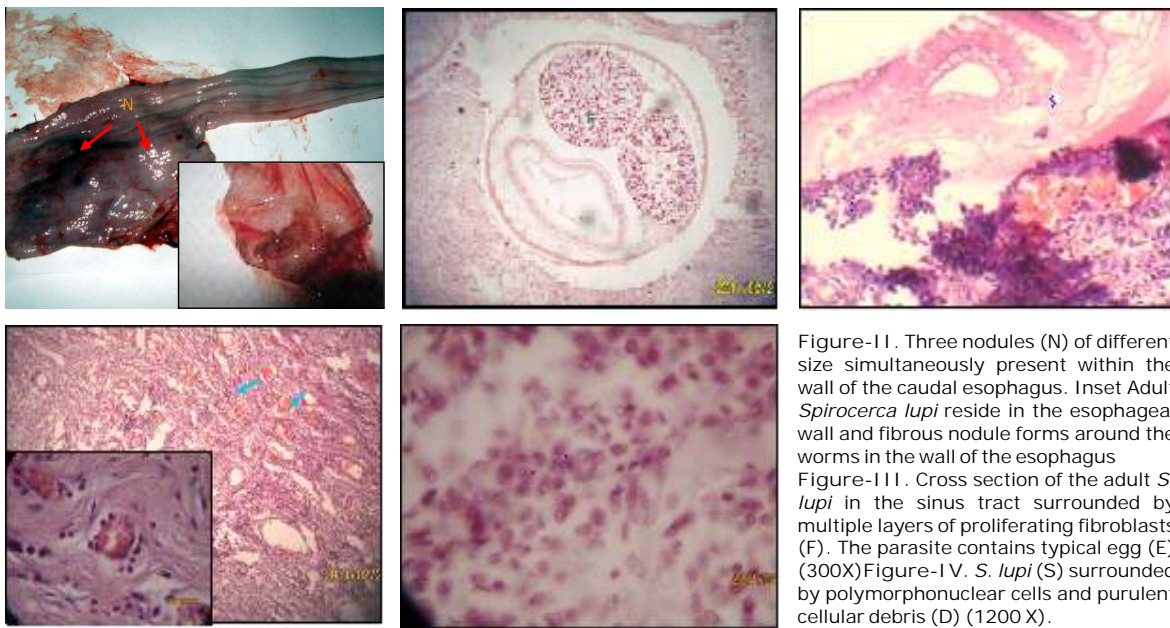


Figure-III. Three nodules (N) of different size simultaneously present within the wall of the caudal esophagus. Inset Adult *Spirocerca lupi* reside in the esophageal wall and fibrous nodule forms around the worms in the wall of the esophagus
Figure-III.1. Cross section of the adult *S. lupi* in the sinus tract surrounded by multiple layers of proliferating fibroblasts (F). The parasite contains typical egg (E) (300X) Figure-IV. *S. lupi* (S) surrounded by polymorphonuclear cells and purulent cellular debris (D) (1200 X).

Figure-V. Section from esophageal mass showing neovascularization (arrowhead) within dense fibrous bundles (80X). Accumulation of neutrophils, fewer lymphocytes, plasma cells and macrophages between the layers of fibroblasts (Inset 1200X).
Figure-VI. Fibrosarcoma indicated by plump, spindle-shaped (S) to undifferentiated polyhedral (P) fibroblasts with a large nucleus-cytoplasm ratio and increased mitotic figures. (2000X)

variation found between the infected and non infected groups. In 83.3% cases the mean corpuscular hematocrit by multiple layers of proliferating fibroblasts along concentration (MCHC %) was below the normal ranges whereas MCV remain almost normal. Statistically significant variation found in ESR values and MCHC% in infected dogs compared to non-infected. Differential leukocyte count showed increased WBC count in 37.5% of the infected cases. A significant degree of Neutrophilia also observed in the spirocerca infected dogs (Table 3).

Microscopic features: Histopathological examination in all formalin-fixed oesophageal lesions revealed

extensive fibrous tissue proliferation. Adult *S. lupi* was seen contained within the sinus tracts surrounded by multiple layers of proliferating fibroblasts along with cellular infiltration (Figure III). The worm comprises a distinct cuticular outline and two muscular layers. The uterus found filled with typical flattened to ovoid shape eggs. Cross section of adult worms found suspended in cellular debris with accumulation of neutrophils and tissue debris and thus indicative of purulent inflammation (Figure IV). The nodular lesions showed different histological pictures according to their constituents and their stage of development.

Nodules showed diffuse thickening in the oesophageal wall with fibrous tissue accumulation having abundant collagen fiber, mature fibrocytes and intervening lymphoplasmacytic cell. Large numbers of newly formed blood vessel was seen in the loosely arranged fibrous layer giving the appearance of granulation tissue (Figure V). Large numbers of neutrophils, fewer lymphocytes, plasma cells and macrophages were commonly found in between the diffusely arranged connective tissue layers (Figure V).

A considerable degree of fibroplasia was also evident in some mature nodules with predominant fibroblasts having plump atypical nuclei and irregular collagen layers. Accumulation of multiple clumps of lymphocytes, plasma cells, macrophages and fewer neutrophils were observed in those lesions. Only one oesophageal lesion showed clear evidence of fibrosarcoma characterized by overgrowth of poorly differentiated spindle to polygonal fibroblasts and abnormal mitotic figures. The neoplastic cells residing in the intervening fibrillar collagenous matrix showed higher degrees of atypia having plump ovoid multiple nuclei which are often overlapping within the scanty cytoplasm (Figure VI).

Discussion

This study reports 40% prevalence of spirocercosis among the stray dog population of Chittagong Metropolitan area. Mosleuddin *et al.* (1992) and Rahman (1973) reported positive cases of spirocercosis in stray dogs from different districts of Bangladesh. Several other researchers throughout the world also reported heavy burden of infection among the stray dogs particularly in endemic regions with warmer climatic condition. From separately conducted studies 23.5% stray dogs in India, 85% in Kenya and 13% in South Africa was reported infected with spirocercosis (Minnaar *et al.* 2002, Ramachandran *et al.* 1984 and Brodley *et al.* 1977). The prevalence rate was slightly variable in different metropolitan than as which might be attributed to the degree of urbanization, density of market areas, proximity to intermediate and/or paratenic hosts and particularly the number of open tales (Dustbin) and slumps. High prevalence of spirocercosis suggests that an outbreak is going on and it could also be attributed to the large number of wandering stray dogs, easy contact with the poultry offal and insects/beetles (possible paratenic host) of the hilly terrain. The present study does not suggest any sex group variation or seasonal predilection on infection although greater numbers of dogs were found infected in summer season (50.00%) than winter (31.00%); but the correlation

was statistically insignificant. This result is comparable with the finding of other researchers who showed no sex predilection for this infection (Fox *et al.* 1988 and Wandera, 1976). Irrespective to the body condition the higher prevalence of spirocercosis was observed in dogs with both poor health (47.36%) and good health (42.82%) status. Thus the effect of spirocercosis in body condition remains inconclusive which supports the idea that *Spirocerca lupi* infection is mostly sub clinical and only shows clinical manifestation in severe oesophageal obstruction (Bailey, 1972, Hu and Hoeppli, 1936).

The Haematological picture of all infected dogs showed lower haemoglobin concentration than the reference value. Among the infected dogs a significant reduction in MCHC% and ESR values was observed but the MCV% remained within the normal range. Therefore, a variable degree of normocytic normochromic to normocytic hypochromic anaemia is evidently associated with spirocercosis. In previous studies mild anaemia was well described in *Spirocerca lupi* infected dogs (Ranen *et al.* 2004 and Mazaki-Tovi *et al.* 2000) and hence our finding is highly comparable with their reports. Mylonakis *et al.* (2006) and Mazaki-Tovi *et al.* (2002) also reported normocytic normochromic anaemia in patients with early diseases condition with the progressive advancement to leukocytosis. Mazaki-Tovi *et al.* (2002) and Ranen *et al.* (2004) showed that lymphocytosis was more common in cases of oesophageal sarcoma compared to early non neoplastic disease. In present study a sharp rise in total leukocyte counts particularly neutrophilia was observed in most of the cases. Therefore, it indicated that most of the infected dogs were in early disease stage having non neoplastic nodular lesions and secondary bacterial infection might have caused neutrophilia.

One to three nodular firm protuberances in the oesophageal wall was observed at necropsy and considered as positive for spirocercosis. The nodules were of variable size (2.104 ± 0.139 cm on average) causing distortion in the oesophageal lumen and containing maximum 12 red spirally coiled worms (3.750 ± 0.686 on average). These gross pathological observations are in close agreement with the findings of previous researchers who observed one to four worm-containing nodules varying <1 to >4 cm in diameter harboring few to more than 30 parasites (Ramachandran *et al.* 1984 and Bailey, 1963). Other than oesophagus, no aberrant migration to various organs by *Spirocerca* larvae was observed which were reported by Oryan *et al.* (2008); Dvir *et al.* (2001) and Singh *et al.* (1999).

Histopathological features of oesophageal nodules showed worm containing sinus tracts surrounded by highly vascularized fibrous connective tissue layers. Unlike many previously reported cases the term granuloma was not applicable for the lesions as there was no dense gathering of macrophages in any of these nodules. Van der Merwe *et al.* (2008) also noted that the nodules are usually incorrectly referred as granulomas. Because of the high degree of neovascularization, the lesions can rather be described as granulation tissue; and it has been previously observed by (Bailey, 1963).

The present study suggests most of the oesophageal lesions as non neoplastic nodules and they showed different histological features according to their stage of maturity. More than 60% of the nodules showed thickening in the oesophageal wall with diffuse fibrous tissue accumulation, predominant fibrocytes and intervening lymphoplasmacytic cells which can be described as the early inflammatory stage as per description of (Dvir *et al.* 2010.). These early lesions also attribute fewer neutrophils with predominant fibrocytes and mature collagen fibers. Some cases represent fibrinonecrotic cellular debris, large accumulation of neutrophils and macrophages around the worm tracts and diffuse connective tissue layers. This might be caused by necrosis, ulceration by irritating worms, eggs and secondary bacterial infections to the lesions. Dvir *et al.* (2010); Van der Merwe *et al.* (2008) and Oryan *et al.* (2008) also reported similar findings. Unlike the findings of Dvir *et al.* (2010) and Mosleuddin *et al.*, (1993) no predominant accumulation of eosinophils was observed. Some pre-neoplastic mature nodules with variable degree of fibroplasia, cellular atypia with accumulation of lymphoplasmacytic cells in between the unorganized fibrous tissues were observed. Dvir *et al.* (2010) described similar lesions and suggested it as a stage of pathological progression of *S. lupi* induced non neoplastic nodule. A typical case of fibrosarcoma was observed in one infected dog of which histopathological features are similar to the findings of Dvir *et al.* (2010) and Van der Merwe *et al.* (2008) having similar features of fibroblasts and cellular infiltrations. Several other reports demonstrated association of spirocercosis with fibrosarcoma and osteosarcoma in typical nodular lesions in caudal oesophagus (Dvir *et al.* 2010; Bailey, 1972 and Seibold *et al.* 1955). Histopathologically, osteosarcomas are more common than fibrosarcoma, (Ranen *et al.* 2004 and Bailey, 1963) but no case of such ossification was found but in this study.

Conclusion

High prevalence of spirocercosis among the stray dogs of Chittagong metropolitan describes it as an endemic region for this parasite that has capability to produce neoplasia. Further research should be attempted for detecting the probable paratenic host and to understanding the complex host-parasite relationship might be helpful for preventive measures. A new serological technique to confirm *S. lupi* infection prior developing incurable nodules will be helpful for treatment in such endemic areas.

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