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Research Article

Histopathological and haematological changes in haemonchosis caused by *Haemonchus contortus* in small ruminants of Bangladesh

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ABSTRACT

Haemonchosis is an endo-parasitic infection of ruminants caused by Haemonchus parasites and causes economic losses of production system in developing countries. Proper diagnosis is important for prevention and control strategies. The present study was conducted to observe the histopathological and haematological changes in Haemonchus infection in small ruminants (sheep and goat). A total of 50 gastrointestinal tracts and livers were collected from 50 slaughtered animals while 17 samples were found as positive to haemonchosis. These parasites were detected by morphometric features. In the histopathological section, all abomasa of the infected host (17) disclosed hemorrhages, edema, congestion of the blood vessels and desquamation of abomasal villi. The lymphocytic and huge amount of eosinophilic infiltration (52.94%) was found in mucous and gastric glands of the abomasum. Liver showed (17.64%) congestion, hepatocyte degeneration, bile duct hyperplasia and mononuclear cell infiltration. Uniform basophilic calcified mass and lined by thick fibrous capsule were also observed in the liver. In the haematological study, the packed cell volume (%) was significantly (P=0.005) declined (24.53±4.69) compared to the normal value (25-40%). The RBC (6.15±1.85 million/mm3) and Hb (8.40±0.92 g/dl) concentration were apparently lower than the normal values. Eosinophil (5.59±2.37%) and lymphocyte (78.11±9.71%) count were apparently increased compared to their normal values (1-6% and 50-70%, respectively) in Differential cell count. These findings of the present study may upgrade the diagnostic tools of haemonchosis in small ruminants in Bangladesh.

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1. INTRODUCTION

The gastrointestinal nematode, *Haemonchus contortus* (Barber's pole worm) is one of the major pathogens of the small ruminants (sheep and goat) throughout the temperate and tropical regions of the world (O' Connor et al. 2006) including Bangladesh (Khatun et al. 2013). Female worm is 18-30 mm long and is easily recognized by the 'barber's pole' appearance and male is 10-20 mm long (Soulsby, 1982; Mannan, 2017). The worm sucks blood and causes hemorrhage in the abomasal wall of the host (Mir et al. 2007). This infection causes large economic losses of production and showed anaemia, edema, ill thrift and lethargy due to blood loss (Gharamah et al. 2012). Thus,

Haemonchosis leads to reduced appetite, haematological changes and reduced digestive capability of the abomasum (Love and Hutchinson, 2003). Ahmed et al. (2007) showed that histopathological changes of the abomasum were mucosal and submucosal haemorrhages. In the same study, Gastric glands revealed some changes and mononuclear cells dominated with eosinophils.

Anaemia due to the blood loss is defined as an absolute decrease in the packed cell volume (PCV), red blood cell (RBC) and haemoglobin (HB) concentration below the normal values (Radostits et al. 1994). Haematological changes include eosinophilia, and terminal death is possible due to the weakness (Ameen et al. 2010). The diagnosis of haemonchosis based on morphometric findings is not always reliable. Accurate diagnosis is important to take effective treatment and control measures. Therefore, the present work was designed for the study on histopathological and haematological changes in haemonchosis caused by *H. contortus* in sheep and goats.

2. Materials and Methods

2.1. Study area and population

The research work was conducted on sheep and goats slaughtered in local abattoirs of Chittagong city (The Chittagong city in Bangladesh; geographical coordinates: 22°21'94'' North, 91°48'12'' East). The total of 50 gastrointestinal tracts and livers from 50 small ruminants were collected from the period of July to December, 2016. A Cross sectional study design and multi-stage simple random sampling method were followed during sample collection.

2.2. Parasite collection and identification

The adult Haemonchus worms were collected from the abomasum according to the protocol described by Hansen and Perry (1994) with some modifications. The collected parasites were washed with normal saline and placed in sterile petridishes. The parasites were examined with naked eye and under light microscope using low power of magnification (10x and 40x). Other endo and exo-parasitic infections were checked and avoided. Any infectious (bacterial/viral) hosts found during animal examination were also avoided during sample collection.

2.3. Histopathological examination

The suspected tissue samples (abomasa and livers) were collected maintaining proper techniques for histopathological study at the Clinical Pathology laboratory of Chittagong Veterinary and Animal Sciences University (CVASU). The collected tissue samples were preserved in Bouin's solution for 7 days. The histopathological slides were prepared according to the established protocol developed by pathology laboratory of this university and this protocol was some modified based on the procedure described by Lee G. Luna (1968). After that the samples were made smaller (5mm thickness) size and washed over night in running tape water. Then the tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule. The tissues were dehydrated in 50%, 70%, 70%, 80%, 90%, 95%, 100%, 100%, 100% alcohol, one hour in each; one hour in absolute alcohol and absolute xyline mixture; two hours in 100% xyline and two hours in 100% xyline, Impregnation was done in melted paraffin (60°C) for 3 changes and two hour for each change. After this step, sample was kept at room temperature for overnight to dry. Then a block of sample was made using melted paraffin. These blocks of tissue sample were dried at room temperature. Then these blocks were sectioned with a microtome (MicroTec®, D-69190 walldorf, Type- cut 4050, Germany) at 5-cm thickness. A small amount of citric acid (or gelatin) was added to the water bath (60°C) for better adhesion of the section to the slide. The tissue sections were allowed to spread on warm water bath at 60°C. Then the sections were taken on grease free clear slides. The slides containing section were dried room temperature and kept in cool place. Finally, the slide was prepared for routine hematoxylin and eosin staining.

The sectioned tissues were deparaffinized in two changes of xyline (two min in each). Then the sectioned tissues were rehydrated through descending grades of alcohol (100%, 100%, 95%, 80% and 70%; two minutes in each) followed by washing in running tape water for five min. Then the tissues were stained with Harris hematoxylin for ten min and then washed in running tap water for 15 min. Then the tissues were differentiated in 1% acid alcohol (1 part HCL and 99 parts 70% alcohol) by 2 to 4 quick dips. Again the slide washed in running tap water for five min and followed by 2- 4 dips in ammonium. Again, the tissue sample was washed in running tape water for ten min. After washing, the sections were stained with 1% eosin for two min. Again, the tissue were dehydrated in ascending grades of alcohol (70%, 80%, 95%, 100% and 100% alcohol; 30 sec, 45 sec, 2 min, 2 min and 2 min in each step; respectively). Then the tissue sample was treated in absolute alcohol and

xyline mixture, 100% xyline for 2 min in each step. Finally the slide was kept in 100% xyline for until mounting. Then stained slide were mounted with cover slip and using DPX mourtant. Then slides were dried at room temperature and examined under a light microscope at low (10 x) and high (40 x, 100 x) power of magnification.

2.4. Haematological test

The blood samples were collected from the jugular vein of the study population (5ml from each animal) prior to slaughter and were taken into collecting vials (vacutainer; Golden Vac™, Ref: GD020EK3). The vials were passed to the clinical pathology laboratory of CVASU and kept in room temperature for few minute until work. The blood sample was analyzed for total erythrocyte count (TEC/RBC), total leucocyte count (TLC/WBC), differential leucocytes counts (DLC), packed cell volumes (PCV), haemoglobin concentration (Hb) and erythrocyte sedimentation rate (ESR) according to the procedure described by Jain, (1986) and Sharma and Singh (2000).

2.5. Data analysis

All data were collected and recorded in MS excel sheet and analyzed using STATA version 11.0 (Stata Corporation, 2009). The average value and standard deviation (SD) of the data were calculated.

3. Results

3.1. Identification of Haemonchus parasite

Male worm measured 10.65 mm in average body length and female worm was 21.33 mm. Microscopically, the tail ends of male possessed a bursa. Vulvar flap was situated in the posterior third of the body in female worm (Fig 1). The "barber-pole appearance" of female worms was observed.

3.2. Histopathological observation

In the histopathological observation, the abomasum showed hemorrhages, edema and congestion in the blood vessels of the lamina propria, desquamation in the apical border of abomasal villi, inflammation and mucus secretions around the lesions. Thickening (64.7%) of abomasal mucosa due to hyperplasia of mucus glands was also found. (Fig 2 a and b; Fig 3). The mononuclear cells infiltration (especially lymphocytes) and a large amount of eosinophilic infiltration in mucosa and gastric glands of the abomasum were observed. In some cases, this even was also penetrated to the sub-mucosa. The perivascular hyperemia with lymphocytic infiltration was reported (Fig 2c). In case of liver, it showed (17.64%; 3/17) congestion, degeneration of hepatocyte, bile duct hyperplasia with narrowing of the bile duct-lumen and mononuclear cell infiltration (Fig 2d). Homogenous basophilic calcified mass, lined by thick fibrous capsule were also found in the fresh sample of liver tissue.

3.3. Haematological observation

The haematological values revealed (Table 1) reduction in the PCV (Mean±SD= 24.53±4.69%) compared to the normal value (25-40%) (P=0.005). The total erythrocyte count (6.15 million/mm3) and Hb (8.4 g/dl) concentration was apparently lower than the normal values (8-18 million/mm3 and 8-12 g/dl, respectively). In the differential leukocyte count, percentage of the lymphocyte (78%) was apparently higher compared to the normal (50-70%).

Table 1. Histopathological changes in *Haemonchus contortus* infection in sheep and goats

Haematological tests	Infected (X) (Mean±SD)	Non-infected (Mean±SD)	Min value in X	Max value in X	Normal value*	P-value (t-test)
TEC(million/mm^3)	6.15±1.85	10.30±1.45	3.42	8.8	08-18	0.061
TLC(thousand/mm^3)	7.85±2.73	6.09±1.34	4	13.3	04-13	0.004
PCV (%)	24.53±4.69	28.44±3.30	18	32	25-40	0.005
Hb (g/dl)	8.40±0.92	9.07±1.04	7.2	11	08-12	0.160
ESR (mm in 1^{st} hour)	00	00	00	00	00	
DLC						
Eosinophil (%)	5.59±2.37	4±2.26	2	9	1-6	0.324
Basophil (%)	00	00	00	00	0-1	
Neutrophil (%)	19.65±9.47	33±1.121	5	40	30-48	0.268
Monocyte (%)	2.94±2.54	4.21±3.220	0	7	0-4	0.164
Lymphocyte (%)	78.11±9.71	65±6.032	49	92	50-70	0.632

*Sharma and Singh (2000)

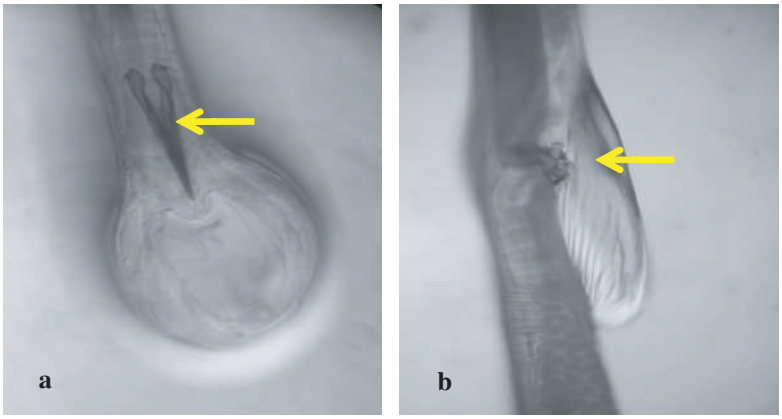

Fig 1: Posterior part of *Hemonchus contortus.* (a) Two lateral spicules in the bursa situated at the tail end of the male worm (Light microscope, 10x) while **(b)** The vulvar flap at the posterior third of the body in the female worm (Light microscope, 10x)

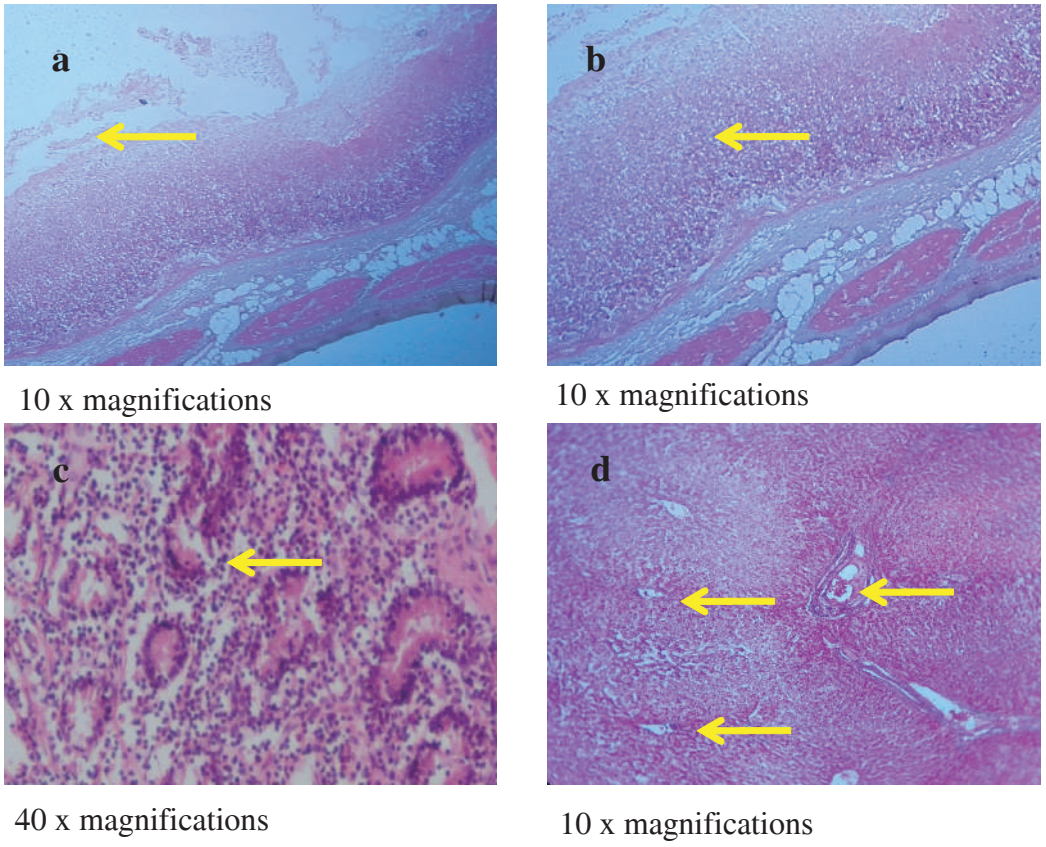

Fig 2: Histopathological changes in the abomasum and liver section. a: Desquamation of the apical border of abomasum, **b:** Thickening of the abomasal mucosa and gastric gland, **c:** Eosinophilic infiltration in the mucous and gastric glands of abomasum and **d:** Congested lesion in the liver with degenerated hepatocyte and bile duct hyperplasia

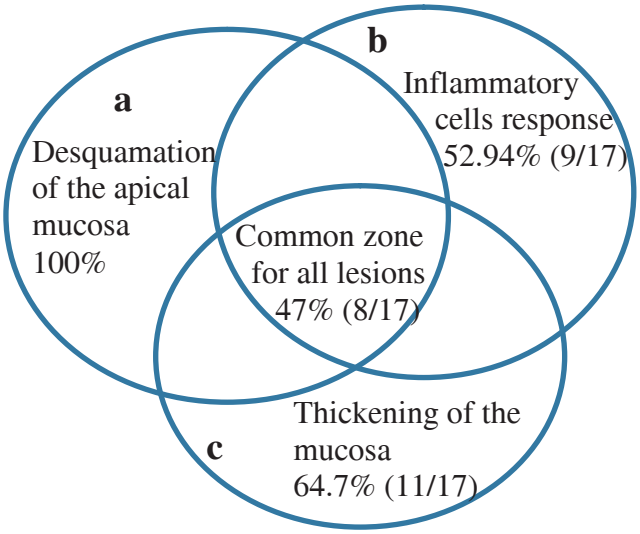

Fig 3: Histopathological lesions in infected samples while 47% (8/17) cases covered all positive lesions of haemonchosis

4. Discussion

4.1. Identification of Haemonchus parasite

The morphological findings (vulvar flap, lateral spicules and barber pole) in the present study were similar with Soulsby (1982).

4.2. Histopathological observation

In histopathological observation, eosinophilic infiltration has been observed that was supported with the observation of Tehrani et al. (2012). Eosinophils were considered as important elements in the response against Haemonchus infections according to Balic et al. (2000). Evidences from different studies suggest that eosinophils may contribute in pathogenesis during parasitic infection (Nickdel et al., 2001; Kelkele et al., 2012). Moreover, ovine gastrointestinal nematodes infections produce a lot of factors that promote eosinophil migration in the view of Wildblood et al. (2005). Again, focal accumulation of lymphocytes and tissue thickening were seen that was accorded to that reported by Scott et al. (1999). In the observation of Saminathan et al. (2015), proliferation of macrophages, fibroblasts, infiltration of lymphocytes and eosinophils have found around the adult worms in pyloric region. The severe liver congestion corresponded with the observation of McKenna (1998). Eosinophils responsible for pathogenesis in parasitic infection and are considered as first line of defence mechanism of the host body against parasitic infection, especially in haemonchosis. The apical border of the abomasal mucosa was desquamated due to continuous irritation and sucking of the blood by the adult parasites. The abomasal mucosa was also thickened due to hyperplasia of the mucous and gastric glands. These observations were in agreement with the observation of Saminathan et al. (2015).

4.3. Haematological observation

The haematological values revealed reduction in PCV and apparently decline in TEC and Hb concentration and differential leucocyte values were normal with the presence of reticulocytes. These findings were in agreement with the observation of Ameen et al. (2010). The results of their studies were in apparently decline of the erythrocytic values, PCV and Hb concentration in adult goat. Similar results were found in the observation of Oduye (1976) in kids between 1-6 months of age and adult goats. These results have also been confirmed with the findings, obtained by Radostits et al. (1994). The fall in PCV may due to continuous sucking of the blood by *H. contortus*. This study support that the helminthes particularly Haemonchus parasites has effect on blood profile and Ameen et al. (2010) also found similar hematologic changes in case of haemonchosis.

Our study area was based on slaughter house. We had limited number of information. However, we strongly believe that the results from this study will improve the methods for diagnosis of haemonchosis in Bangladesh.

Conclusion

The abomasal mucosa was thickened and desquamated. Eosinophilic infiltrations were found in the abomasal mucosa. The PCV was low in the blood test of the infected animal. A farm based extended study may be necessary to conduct in future to test more suspected factors.

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