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INCIDENCE AND HAEMATOBIOCHEMICAL STUDY OF AMPHISTOMOSIS IN BUFFALOES (*Bubalus bubalis*) IN MHOW, MADHYA PRADESH

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ABSTRACT

In the present study, samples from 100 buffaloes of either sex were collected from slaughterhouse located at Cantonment Board Mhow for the detection of ruminal amphistomosis. Blood samples with or without anticoagulant were collected to study the haematobiochemical changes in buffaloes suffered from amphistomosis. Amphistomes were also collected for morphological identification of amphistome species in slaughtered animals. The prevalence of ruminal amphistomes was found to be 8%. The infected buffaloes in this study showed a reduction in the mean values of Hb, TEC and PCV. There was an increase in TLC and neutrophil count. Lymphocytes showed a slight decrease in their mean values and no significant changes were seen in values of basophil and eosinophil count. The infected buffaloes in this study showed a reduction in the mean values of total protein. The mean values of SGOT, SGPT and alkaline phosphatase showed an increment in the infected buffalo.

Keywords: *Bubalus bubalis*, buffaloes, amphistomosis, haematobiochemical, incidence

INTRODUCTION

India has the largest livestock population in the world, which contributes substantially to the national economy, but these ruminants are infested by multifarious parasites such as nematodes, trematodes and cestodes (Sykes*,* 2008*)*. The milk production in buffaloes suffering from gastrointestinal nematodosis can be increased to the tune of 0.43 litre/buffalo per day when the animals were treated with anthelminthic as compared to the non-treated animals (Sahil *et al*., 2012). Amongst all the ailments of buffalo, parasitic diseases have been recognized as one of the most important diseases which have reduced the development of the livestock industry in the country like India. Worldwide, gastrointestinal parasitism is considered as one of the most harmful conditions limiting the buffalo production. Amphistomosis is one of the most important parasitic ailments in domesticated animals like cattle, buffalo, sheep, and goat responsible for colossal monetary losses annually to the livestock industry (Khan *et al*., 2008). This ailment is widely prevalent in India causing heavy losses due to heavy infectivity of the diseases, death of infected animals and reduced meat and milk production. Juyal *et al*. (2003) recorded

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a very high morbidity up to 80-90 % in domestic ruminants. As per Anuracpreeda *et al*. (2008), in different regions, this disease is caused by specific species of the amphistome. Considering the above facts, it was decided to study the changes in the pathology and hematobiochemical parameters in affected buffaloes in order to correlate with the infection and its adverse effects on the animal health for initiating the appropriate preventive and control measures.

MATERIALS AND METHODS

Collection of samples

Blood samples of buffaloes were collected before slaughter aseptically from the jugular vein in the vial containing EDTA 2 mg/ ml of blood (anticoagulant) for haematology and without anticoagulant for separation of serum. For separation of serum, samples were centrifuged in centrifuge machine for 10 minutes at 3000 rpm. Serum was collected and stored at -20^{o}C for further use.

Collection and Identification of ruminal amphistomes

The amphistomes collected from rumen (Figure 1) were pressed between two slides and tied with thread. The slides were kept in formalin overnight and the following procedure was performed as given by Puttalakshmamma (2010): The processed specimens were washed under running water for 45 to 60 minutes to remove the formalin so that the formalin does not interfere in the staining reaction. After washing the specimens were stained with borax carmine stain for 12 to 18 h. To ensure proper uptake of the stain by internal organs, the specimens were stained excessively and subsequently subjected to destaining. After overstaining the specimens were subjected to destaining by transfering them into petridish containing 1% acid water (99 ml distilled water +1 ml concentrated HCl). After destaining in 1% acid water the specimens were transferred to cavity block containing distilled water for 20 to 30 minutes during which the water was changed 8 times. The specimens were subjected to dehydration by placing them in ascending grades of alcohol starting from 30%, 50%, 70%, 90% and absolute alcohol. The specimens were kept for 20 minutes in each grade of alcohol to ensure complete dehydration. After dehydration the specimens were transferred to petridish containing clove oil which acted as a clearing agent. Specimens were kept for 20 minutes in clove oil for clearing the cuticle. After clearing the cuticle, the specimens were mounted on glass slide using DPX as a mountant. Then mountant specimens were observed under dissecting microscope for identification of amphistome species.

Parameters Studied

- Total Erythrocyte Count (TEC) (Million / cu.mm)
- Total Leucocyte Count (TLC) (Thousand/cu.mm)
- Haemoglobin (Hb) Concentration (gm/dl)
- Packed Cell Volume (PCV) (%)
- Differential Leukocyte Count (DLC) (%).
- Total protein(g/dl)
- Alkaline phosphatase (IU/L)
- Serum glutamic pyruvic transaminase (IU/L)
- Serum glutamic oxaloacetic transaminase (IU/L)

RESULT AND DISCUSSION

The present study was conducted on the slaughtered buffaloes of slaughterhouse Mhow.

In infected animals, several amphistomes were found attached on ruminal papillae (Figure 1). On the basis of morphological characters, collected amphistomes were identified as *Paramphistomum epiclitum* (Figure 2). Out of 100 samples, 8 (8%) samples were found to be positive for ruminal amphistomosis and negative for intestinal and biliary amphistomosis. The incidence of amphistomosis is shown in Table 1.

From the present findings, slightly higher prevalence of amphistomosis in bovines was recorded by Gamit *et al*. (2017) who examined 170 liver specimens and 20 samples were found to be infected with *E. explanatum.* The findings by Sreedevi and Hafeez (2014) who examined 694 buffaloes and among them 279 (40.20%) buffaloes were found infected with one or more types of gastrointestinal parasites out of which amphistomes were predominant with 15.42% which were in contrast with the findings of the present study. Swarnakar *et al.* (2014) examined 435 rumen of buffaloes and found that 74.71% samples of buffaloes were infected with *P. cervi.* These findings were in contrast to the findings of the present study. The findings of Muhammad *et al.* (2015) and Murthy and D'souza (2016) were also in contrast to the present studies they recorded higher (21.24%) prevalence of *G. explanatum* and 24.4 and 23.1% prevalence in cattle & buffaloes, respectively. On the basis of morphological appearance, Choudhary *et al.* (2015) identified the parasite as *P. cervi* in sheep of Gujarat, which

Figure 1. Photograph showing colonies of amphistomes on ruminal papillae.

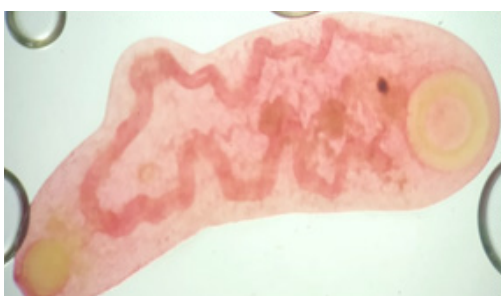

Figure 2. Photograph showing Stained species of *P. epiclitum*

Table 1. Incidence of amphistomosis in slaughtered buffaloes.

S. No	Type of parasite	Positive %	Negative%
1.	Ruminal amphistomes	08	92
2.	Intestinal amphistomes	00	100
3.	Biliary amphistomes	00	100

Table 2. Changes in haematological values (mean) in buffaloes infected with ruminal amphistomosis.

Parameters	Mean values
Haemoglobin (gm/dl)	6.18
Total erythrocyte count (TEC)	3.09
Packed cell volume (PCV %)	24.56
Total leucocyte count (TLC)	9737.5
Neutrophils (%)	86.3
Lymphocytes (%)	44
Basophils (%)	0.8
Monocytes (%)	00
Eosinophils (%)	00

Table 3. Biochemical changes in buffaloes infected with ruminal amphistomosis.

S. No.	Parameters	Mean Values
1	Total Serum Protein (TP)	7.4 g/dl
2	Serum glutamic oxaloacetic transaminase (SGOT)	31.75 IU/L
3	Serum glutamic pyruvic transaminase (SGPT)	10.075 IU/L
4	Alkaline phosphatase (AKP)	33.3 IU/L

is in line with the present investigation. Ruminal mucosa and tissue sections revealed knobs on which amphistomes were attached having congestion and infilteration of inflammatory cells (Figure 1).

Haematological changes

In the present study haematological values such as haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV) and differential leukocyte count (DLC) were studied in the amphistomes infected buffaloes. The findings in the changes of hematological values are shown in Table 2.

Similar findings were reported by Verma *et al.* (2006) which showed significant reduction in value of Haemoglobin which were in consonance with the findings of the present study. Nath (2007) found a significant decrease in Hb, PCV and TEC and increase in values of neutrophil and eosinophil were 6.42±1.43, 7.87±1.89, 23.87±1.76, 8.67±0.97,

34.52±0.65, 8.77±0.76 respectively. Contrasting values of lymphocytes were found (4.96±2.13). Eosinophila was evinced by Thakur *et al.* (2007) after studying the clinico-haematology of amphistomosis in cattle. After anlysing the mineral profile of buffaloes infected with amphistomosis, Varma *et al.* (2006) observed significant reduction in iron level which affected the values of haemoglobin, resulting in anemia. Mavenyengwa *et al.* (2010) studied the influence of *Calicophoron microbothrium* amphistomosis and found significant decrease in PCV, Hb and red blood cell (RBC) count, while increase in eosinophil count. The findings of the present study are having similarity with the findings of Biswas *et al.* (2013), who recorded a significant decrease in Hb, TEC & PCV values and increment in TLC, eosinophil and neutrophil values. Vandip *et al*. (2015) found similar findings with reduction in the mean Hb, TEC and PCV and increase in neutrophil and eosinophil counts.

Biochemical changes

In the present study a total number of 100 serum samples from buffaloes were examined for biochemical parameters such as total protein, alkaline Phosphatase (AKP), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) and findings of the present study are presented in Table 3.

Similar findings were reported by Hasnani (1992), who observed reduction in total protein and increase in SGPT and SGOT values. Findings of the current study are in consonance with the findings of Nath (2007) who reported significant decrease in the total serum protein (4.87±0.83) in infected cattle, while the significant increment in serum AST (36.78±1.87) and ALT (24.10±0.97) was recorded by them. The findings of Thakur *et al*. (2006), Hafeez and Usha (2007), Mavenyengwa *et al.* (2010); Dubey and Tizauone (2014) had similar findings with the present study wherein these authors also recorded the reduction in the values of total serum protein.

CONCLUSIONS

On the basis of gross lesions, the overall prevalence of ruminal amphistomosis in buffaloes was recorded as 8% which were slaughtered at slaughterhouse of Mhow. Current study recorded lower incidence of ruminal amphistomosis in buffaloes slaughtered at slaughterhouse Mhow. Reduction in Hb, TEC, PCV and total proteins, whereas increase in the TLC and neutrophil values was recorded in the present investigation. As per biochemical parameters, increment in SGPT, SGOT and alkaline phosphatase with reduction in total protein was in evidence. On the basis of morphological characters, the amphistome was identified as *Paramphistomum epiclitum.*

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