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Study of amphistomes and its clinical pathology in slaughtered buffaloes of malwa region of Madhya Pradesh

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Abstract
Total 165 rumen of slaughtered buffaloes were examined to get 50 positive rumen infected with amphistomes from slaughter house of Mhow situated in Malwa region of Madhya Pradesh where buffaloes were brought for slaughter from different district of Malwa region. Two species of amphistomes were documented i.e. *Paramphistomum cervi* and *Cotylophoron cotylophorum*. No major gross and histopathological changes reported from rumen except whitish area at the site of attachment and infiltration of various inflammatory cells. Value of Hb, PCV and TEC were decreased significantly (<0.01%) whereas value of eosinophils and neutrophils increase significantly.

Keywords: Amphistomes, histopathology, haematology, buffaloes

Introduction
The growth rate of buffalo population from 2007-2012 was 3.19% whereas growth rate of cattle population from 2007-2012 was in negative i.e. - 4.10% [1]. It clearly indicate farmers are more interested to rare buffaloes than cattle probably due to various religious issue. Milk production of cattle and buffaloes in the world is first and second rank, respectively. [1] Total milk production in India was 137.69 million metric tonnes in 2009-10 out of which cow produced 52.20 million metric tonnes whereas buffaloes produced 59.758 million metric tonnes [2]. Average yield of milk per day per buffalo was 5.76 liter for indigenous buffaloes in 2015-16 Total contribution of buffaloes in milk production was 49%. Total meat production of buffaloes was 1611 metric ton which share 23% of total meat production in 2015-16. Indian buffaloes produced three times milk than cow. [3].
Indian buffaloes are two types i.e. river and swamp and both are scientifically known as *Bubalus bubalis.* Although most of the buffaloes in India are river type. Buffaloes are more susceptible to amphistomes due to their fond of water because the intermediate host for amphistomes is fresh water snail. Amphistomosis is a disease caused by paramphistomatidae family which are cosmopolitan in distribution and cause economic losses due to lower feed conversion which leads to poor growth and lower milk production [4]. Report on Economic losses caused by amphistomes generally do not quote the species of amphistomes due to difficulty to identify [5]. Since scanty information is available on the percent frequency of amphistomes and its clinical pathology in slaughtered buffaloes of Malwa region of Madhya Pradesh. Therefore, the present work has been designed for developing worm management strategies for control of parasitic diseases.

Materials and Methods
Madhya Pradesh is the central state of India which is located between 17° to 25° latitude and 72° to 85° longitude. The Malwa region located between 21˚10' N 73˚45'E and 25˚10'N 79˚14'E and geographically it occupies a plateau in Western Madhya Pradesh and South Eastern Rajasthan with Gujarat in the west [6]. Agar, Dewas, Dhar, Indore, Jhabua, Mandsaur, Neemuch, Rajgarh, Ratlam, Shajapur, Ujjain, and part of Guna and Sehore and the Rajasthan districts of Jhalawar and parts of Kota, Banswara and Pratapgarh come under the region of Malwa.

Morphological identification of amphistomes
Identification of amphistomes was done on the basis of morphological character from 50 amphistomes infected slaughtered buffaloes [7].

Haematological study
EDTA (Ethylene Diamine Tetra acetic Acid) containing vacutainer tubes were used for collection of 2 ml blood samples from jugular vein of buffaloes during the anti-mortem examination brought for slaughter and then divided into two groups of amphistomes infected and non-infected buffaloes each having number of buffaloes was 50 for hematological analysis.

Histopathological study
For histopathological study, a total of 30 positive amphistomes infected organs were collected from slaughter house. 10% neutral buffered formal saline solution were used for preservation of collected tissue pieces and parasites, and processed by paraffin embedding and stained with Ehrlich's Hematoxylin and Eosin as per [8]. The prepared sections were examined by microscopy.

Results and Discussions
Identification of different amphistomes species in slaughtered buffaloes
Two genera of amphistomes found in buffaloes in descending order of their prevalence were *Paramphistomum cervi* (53.24%) and *Cotylophoron* (46.67%). Swanker *et al*. (2014) [9] also reported highest percentage of *Paramphistomum cervi* (74.71%) while Shanila and Hafeez (2005) [10] reported highest percentage of *Gastrothylax crumenifer* (62.5%) in cattle. The variation in predominant type of amphistomes might be due to type and densitiy of snail intermediate host in particular geographical area. Sometime difficult to differentiate different species of family paramphistomatidae based on morphological differentiation of stained specimen [11]. Likewise identification of different type of amphistome eggs based on morphology was not possible [12].

Histopathological study
During gross examination of rumen, papillae were atrophied and affected area were looked whitish in colour. Histopathology of rumen showed infiltration of various inflammatory cells (fig 1 and 2). No major gross and histopathological changes were observed. According to Soulsby, 1992 [6] adult amphistomes in rumen do not cause any serious damage to host and once infected then host carry infection throughout their life. While Basak and Sinha (1978) [13] reported some knob infiltered with polymorph nuclear leucocytes and in such knob blood vessels were highly congested.

Comparison of various blood parameters between amphistomes non infected and infected buffaloes
The mean values of Hb (8.6±0.15 VS 10.6±0.14), TEC (4.4±0.04vs 5.2±0.06) and PCV (26.6±0.25vs 31.6±0.34) were reduced significantly ($p<0.01$) whereas values of the neutrophils count (30.7±0.20 VS 30±0.20) and eosinophil count (2±0.09vs 1.4±0.09) increased significantly ($p<0.05$) and highly significantly ($p<0.01$), respectively in infected buffaloes as compared to the non-infected buffaloes. The total leukocyte (8.3±0.08vs 8.1±0.08), monocyte (5.8±0.24vs 5.7±0.23) count and basophil counts (0.3±0.07vs 0.3±0.06) increased non-significantly in infected buffaloes (Table-1). Lymphocyte count (61.3±0.36vs 62.7±0.35) decreased none significantly in infected buffaloes. There was no significant change in the values of basophil counts (0.3±0.07vs 0.3±0.06) of control (apparently healthy) and infected buffaloes.
Highly significant different was observed between non infected and infected buffaloes in values of Hb, TEC and PCV might be due to blood sucking habit or hemorrahge during migration from submucosa [14]. Value of eosinophils increased might be due to penetration of amphistomes in gut wall as amphis tome antigen stimulate eoisnopoiesis in bone marrow [15].

Table 1: Comparison of various blood values between amphistomes non infected and infected buffaloes

Values	Non Infected Buffaloes	Infected Buffaloes	t value	Significance
Hb g/dl	10.6±0.14	8.6±0.15	8.86	**
TEC 10^6/µl	5.2±0.06	4.4±0.04	11.31	**
PCV %	31.6±0.34	26.6±0.25	11.5	**
TLC 10^3/µl	8.1±0.08	8.3±0.08	1.74	NS
Neutrophils (%)	30±0.20	30.7±0.20	2.61	*
Lymphocytes (%)	62.7±0.35	61.3±0.36	2.79	*
Monocytes (%)	5.7±0.23	5.8±0.24	0.24	NS
Eosinophils (%)	1.4±0.09	2±0.09	3.96	**
Basophils (%)	0.3±0.06	0.3±0.07	0.66	NS

Plat 1: Photograph showing mature amphistomes attached in the mucosae of the rumen

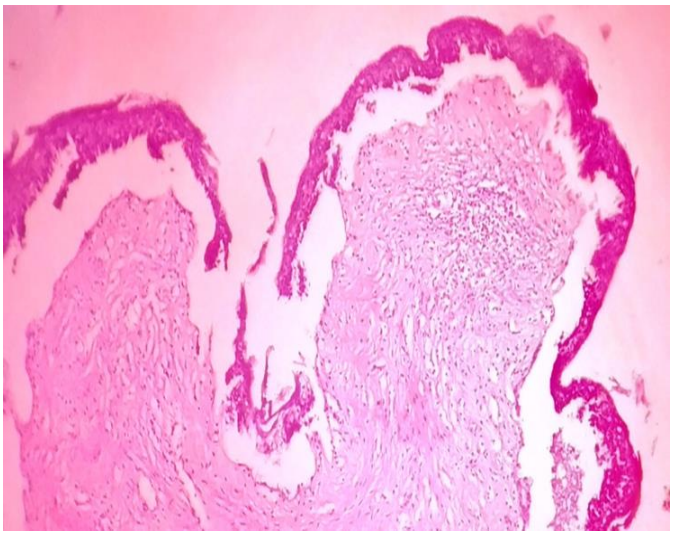

Plate 2: Photomicrograph showing infiltration of inflammatory cells in the villi of ruminal mucosae

Conclusion

Only two species of amphistomes reported namely *Paramphistome cervi* and *Cotylophoron* from rumen of slaughtered buffaloes. Significant decrease in value of Hb, PCV and TEC in amphistome infected buffaloes is indicative of anemia which leads to economic losses to farmers.

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