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Histochemical distribution and physiological significance of acid phosphatase in the bovine amphistome *Paramphistomum cervi* (Paramphistomatidae: Digenea)

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ABSTRACT

The present study was carried out for histochemical localization of acid phosphatase and its functional significance in bovine amphistome Paramphistomum cervi. Paramphistomum cervi were collected from the rumen of cattle in local abattoirs. Frozen sections were cut and histochemical localization of acid phosphatase was done by using Lead Acetate method. Acid phosphatase activity was observed in musculature of oral sucker, acetabulum, tegument, digestive system, reproductive system and parenchymal cells. Muscles of pharynx, its tegument as well as tegument lining the mouth and acetabulam showed intense reaction for acid phosphatase. Similar reaction for acid phosphatase was recorded in the intestinal caecae and various muscle layers of acetabulam. Vitelline cells and their secretory products were also acid phosphatase positive. The possible functional activity of this enzyme in different tissues has been discussed. Acid phosphatase was detected histochemically in the sites where absorption, secretion and excretion occur and is postulated to be related with these functions.

Key words*: *Histochemistry, Acid phosphatase, bovine, Paramphistomum cervi,

I INTRODUCTION

Paramphistomes are parasitic in the alimentary canal of many ruminants. Mature parasites are especially prevalent in the reticulum and rumen. Mature paramphistomes rarely produce clinical symptoms [1,2], however immature migrating parasites have been reported causing serious disease and even the death of their hosts by burying themselves in the sub mucosa of duodenum and feeding on the epithelial cells of Brunner's glands which results in anorexia, polydypsia, profuse diarrhoea, a drop in plasma protein concentration and anemia [3,4,5]. The survival of the parasites is influenced by the general biotic factors associated with the micro and macro environments as well as by the intimate physiological and immunological interactions between the parasite and the host which forms the basis of host-parasite relationship. The histochemical studies help us to investigate qualitatively the biochemical pattern of different tissues in cellular architecture. Histochemical studies will increase the knowledge of worm physiology, which could further lead to deeper understanding of the well recognized host parasite interactions and such information would be valuable in designing control measures that are efficient and economical[6]. The purpose of the present study was to examine the histocemical

distribution of acid phosphatase and establish its physiological importance in *Paramphistomum cervi.* Such studies could increase our knowledge of the biochemistry of the parasites, which could further lead to deeper understanding of well recognized host parasite relationship.

II MATERIAL AND METHODS

The live amphistomes were collected from rumen of freshly slaughtered cattle in local abattoirs. Worms were carefully removed with the help of fine forceps, placed in normal saline (0.75%) and washed thoroughly. The worms were fixed in cold Neutral Formalin for 1- 4 hours in deep freezer and washed thoroughly with running water. The frozen sections 8 – 10 microns thick were cut at – 20 ^{0}C by using ***Leica CM 3050 S*** cryostat. The frozen sections were fixed on clean glass slides, dried in air at room temperature, incubated for 15-30 minutes at 37^{0}C in a solution containing Sodium-β-Glycerophosphate (an organic substrate for this enzyme) and lead acetate and processed according to the procedure of Lead Acetate method [7] for the detection of acid phosphatase. Control sections were incubated in a solution without substrate. Lead phosphate deposition occurred at the site of enzyme action which was made visible by subsequent treatment with ammonium sulphide, resulting in the formation of black or dark brown precipitate of lead sulphide at site of enzyme action.

III RESULTS

In the present study, acid phosphatase activity was observed in almost all tissues of *Paramphistomum cervi*. Intense enzyme activity was observed in the muscles of pharynx, its tegument as well as tegument lining the mouth and posterior sucker. Various muscle layers of posterior sucker showed moderate to high enzyme activity. Luminal surface of the gut was also intensely positive, where as its outer muscle layer exhibits activity of comparatively less intensity. Vitelline cells as well as their secretory products were found acid phosphatase positive in the present study. The marked histochemical reaction for acid phosphatase in well defined organs of *Paramphistomum cervi* is presented in Table 1. Fig. 1-6 depicts the histochemical distribution of acid phosphatase in different tissues of *Paramphistomum cervi*.

IV DISCUSSION

Acid phosphatase has been generally detected histochemically by many workers in the sites where absorption, secretion and excretion occur and is postulated to be related with these functions. In present study, acid phosphatase was observed to be present in higher intensity in anterior and posterior suckers. Similar results were reported in four different trematodes – *Gastrothylax crumenifer*, *Gigantocotyle explanatum*, *Echinostomum malayanum* and *Fasciola buski* [8]. Our results are also in agreement with those in *Ceylonocotyle scoliocoelium* (amphistome) [9] and in *Aspidogaster conchichola* [10].

Intense acid phosphatase activity was observed in the tegumental cells and moderate activity in subsyncytial zone (zone between surface syncytium and circular muscles). These results are in agreement with those of the other workers [8, 9, 11, 12, and 13]. Acid phosphatase in the tegument and in the caecal epithelium, their role in digestion and absorption has been speculated on from time to time [14, 15]. But Sharma and Hanna (1988)

while working on ultrastructure and histochemistry of tegument of *Orthocoelium scoliocoelium* and *Paramphistomum cervi* reported that acid phosphatase activity in subsyncytial zone are associated with the extensions of parenchymal cells and trabaculae from tegumental cells [12]. They also reported that mitochondria are absent from the tegument of amphistomes and indicated that high energy intermediates are not available in sufficient concentrations to power extensive active transport. Thus large quantities of amino acids and other metabolites are not taken up across the tegument of the rumen amphistomes. However in *Gastrothylax crumenifer* [11] and in Juvenile Paramphistomes [13] adequate ATP for tegumental function is procured from the parenchyma because there is apparently intimate association between the syncytium and parenchyma in adult amphistomes. Thus tegument plays a role in transportation and absorption of nutritive materials though to a limited extent.

Luminal surface (apical membrane of gastrodermis) of gut caeca was intensely positive for acid phosphatase, where as its outer muscle layer (basal membrane of gastrodermis) exhibited activity of comparatively less intensity. Similar results were reported in three paramphistome species – *Gigantocotyle explanatum*, *Gastrothylax crumenifer* and *Srivastavaia indica* [16]. Results of present study are also in agreement with other workers [8, 9, 15, and 17]. Acid phosphatases are involved in the hydrolytic and transport processes [18]. The more intense reaction for acid phosphatase in the anterior region of gastrodermis in the paramphistomes depicts a greater commitment to secretory and or absorptive process in this region [16]. Besides, the presence of enzyme at the basal lamina of gastrodermis indicates its involvement in transport of substances to the parenchyma [19]. The tight junctions between the gastrodermis and under lying parenchyma represent important sites of precursor transport between these two systems and the parenchyma beneath the gut, which is rich in mitochondria and lysosomes, plays a role in the processing of nutrients in transit between gut and deeper tissues which is also supported by Dunn *et al*. (1987a) [16].

In the present study moderate amount of acid phosphatase activity was observed in the parenchyma of *Paramphistomum cervi*. Similar results were observed in the parenchyma of paramphistomes – *Paramphistomum epiclitum* and *Fischoederius elongates* [20]. The presence of this enzyme in the parenchymal tissue itself indicates that parenchyma plays role of transport system in this animal and is not merely a packing or storage tissue. This is also supported by the fact that there are tight junctions between the basal lamina of gastrodermis and parenchyma on one side which are sites of transport of nutritive substances to parenchyma (Parshad and Guraya, 1978) [19] and on the other hand parenchyma establishes contact with the syncytium of tegument to meet the nutritive requirements of the tegument (Dunn *et al*., 1987b) [11].

Intense reaction for acid phosphatase was observed in the vitellaria in the present study. Presence of acid phosphatase in the vitelline cells has been reported by a number of workers [8, 9, 21, 22, and 23]. Roy (1980) reported intense reaction for acid phosphatase in vitellaria of bovine amphistome *Ceylonocotyle scoliocoelium* [9]. Haque and Siddiqi (1982) reported intense to moderate activity in four different trematodes - *Gigantocotyl explanatum*, *Gastrothylax crumenifer*, *Echinostomum malayanum* and *Fasciolopsis buski* [8]. Vitelline glands are important in the egg shell formation and in mature paramphistomes the vitellaria fill almost two third of the body and about 32 vitelline cells are incorporated into each egg [24, 25]. Role of acid phosphatase in the

vitelline glands is in transportation and formation and secretion of secretory granules in vitelline cells [9, 23, and 25].

Reproductive system, including gonads – testes and ovary, showed intense reaction for acid phosphatase in the amphistome under study. These findings are in agreement with those reported in many other trematodes including paramphistomes [9, 23, 15, 26, and 27]. The heavy reaction for acid phosphatase in these organs occurs because they are metabolically highly active.

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Table 1: Intensity of Acid phosphatase staining reaction in the tissues of *Paramphistomum cervi*

Tissue/ Organ	**Acid phosphatase Activity**
Tegument	++
Tegumental muscles	+++
Pharynx	+++
Oral sucker	+++
Acetabulam	+++
Intestinal caeca	++
Tunica of testes	+++
Testes	+++
Tunica of ovary	+++
Ovary	+++
Parenchyma	++
Vitellaria	+++
Excretory vesicles	++

+ Weak activity, ++ Moderate activity, +++ Intense.

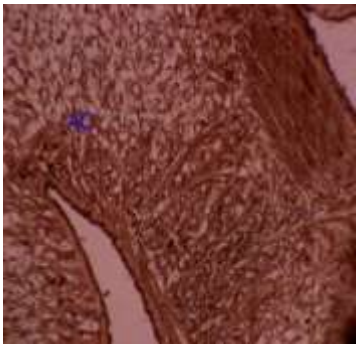

Fig.1

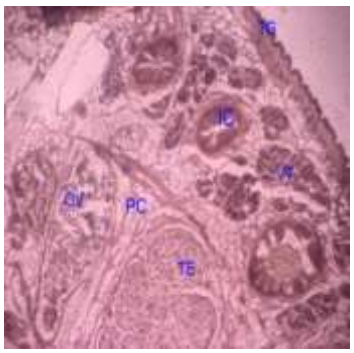

Fig. 2

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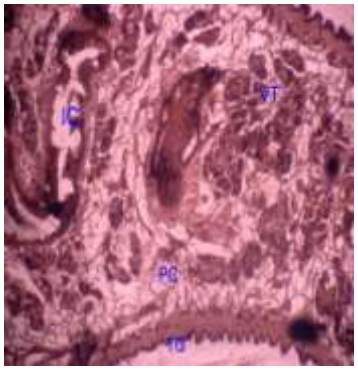

Fig. 3

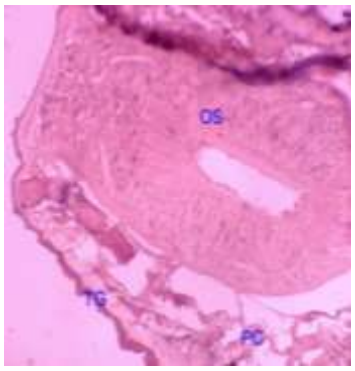

Fig. 4

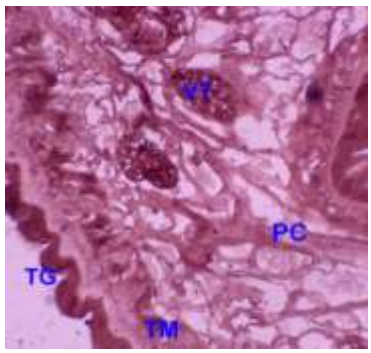

Fig. 5

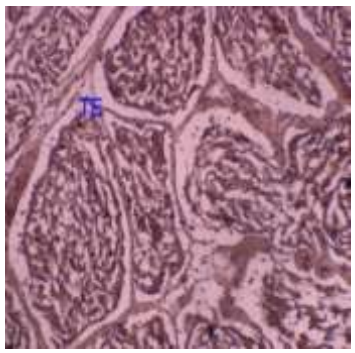

Fig. 6

Fig. 1-6 depict the histochemical distribution of acid phosphatase in different tissues of *Paramphistomum cervi*

Fig. 1 TS (transverse section) passing through AC (acetabulum).x400. **Fig. 2** LS (longitudinal section) passing through TE (testes) and OV (ovary).x200. **Fig. 3** L.S passing through IC (intestinal caeca), VT (vitellaria), PC (parenchyma) and TG (tegument) .x200. **Fig. 4** LS passing through OS (oral sucker).x200. **Fig. 5** LS through TG (tegument), TM (tegumental muscle), PC (parenchyma) and VT (vitellaria).x400. **Fig. 6** LS passing through TE (testes).x400