Animal Health Research Journal Vol. 7, No. 2, June 2019 pp. 181-190

ISSN: 2356-7767

Pathological, hematobiochemical and blood serum protein electrophoretogram changes in calves infected with rumen flukes (Paramphestomiasis) at Assiut governorate

***Mohammed, H. Rateb; **Lamiaa, Abouel–Hassan and ***Osman M. Mahran**

Biochemistry Unit, Assist. Laboratory, Animal Health Research Institute, ARC.*

Histopathological Unit, New-Valley Laboratory, Animal Health Research Institute, ARC.**

Parasitology Dept. Animal Health Research Institute, ARC, Dokki.***

Received in 11/6/2019
Accepted in 6/7/2019

Abstract

The present work aimed to investigate some parasitological affection, pathological and hematobiochemical changes in calves naturally infected with Paramphestomum. Blood samples were collected from suspected calves during the anti-mortem examination, which was further confirmed during post-mortem examination. Slices of ruminal and intestinal tissue containing flukes were obtained for histopathological studies. The average total number of bright pink-color mature paramphistomes recovered from the rumen, abomasum and duodenum of each the eleven affected calves ranged from 250-400 flukes of 11 affected calves. Microscopic pictures of the parasite used in identification defined the similarity in the morphology and histology of the anterior sucker, pharynx, esophagus, genitalia and posterior sucker (acetabulum) to the *P. cervi*. Migration of young flukes from the small intestine to the rumen caused significant damage in the rumen and intestine such as cornification, destruction and desquamation of the rumenal mucosa leading to loss of cells into the lumen and inflammatory cell reactions. Flukes were present in the intestinal lumen and penetrate the level of the muscularis mucosa. Haematological investigations showed significant ($P<0.05$) decrease in the values of haemoglobin (Hb), red blood cell count (RBCs) and packed cell volume (PCV) in affected calves when compared with control group. Biochemical studies showed significant decrease ($P<0.05$) in the concentrations of Ca, Ph, Na, K and Cl in affected calves when compared with control group. Affected calves showed also significant decrease ($P<0.05$) in the concentrations of total protein, albumin and a significant increase ($P<0.05$) in α_1-globulin, α_2-globulin and γ-globulin in affected calves when compared with control group. This study demonstrated that paramphestomiasis can affect health of calves through histopathological consequences accompanied by clinicohaematological changes.

Keywords: *Paramphestomiasis, calves, pathological, hematobiochemical*

Introduction

Paramphistomum spp. (Plathyhelminthes, phylum Trematoda, Digenea subclass class, family Paramphistomidae) are trematodias of the rumen and reticulum of ruminants. *P. cervi* is the species most commonly found **(Grist 2008).** The paramfistomíase is distributed worldwide, but the highest prevalence has been reported in tropical and subtropical regions that possess the climatic characteristics required for development of the parasite (**Ozdal *et al.* 2010).** *P. cervi* are plug feeders **(Melaku and Addis, 2012)** and cause serious disease by burying themselves into the sub mucosa of the duodenum and feeding on the epithelial cells of the Brunner's glands resulting in anorexia, profuse fetid diarrhea, drop in plasma protein concentration and anemia, which weaken the host **(Maule, 2000).** Mature Paramphistomes are also responsible for rumenitis, irregular rumination, un-thriftiness, lower nutrition conversion and loss of body condition, resulting in considerable economic losses **(Melaku and Addis, 2012).** The affected animals exhibited

normal to loose faeces, decreased appetite, and rough hair coat, normal conjunctivae and reduced ruminal motility **(Thakur, *et al.,* 2007).** The pathogenic effects of *Paramphistomum* spp. are associated with the larval stage in the intestine, which can cause enteritis, diarrhea, anorexia, intense thirst, tenesmus and rectal bleeding, with mortality in acute outbreaks reaching 90% (**Taylor *et al.* 2007**).In Egypt, the fresh water snails including Bulinus truncates and Bulinus forskalii are prevalent and play role in the distribution of Amphistomes **(Elsokkary *et al.,* 2009)** .The intermediate hosts (fresh water snails) living under some particular conditions including the presence of vegetation, humidity and mild temperatures **(Pinedo *et al.,* 2010).** Amphistomes are prevalent among ruminants in Egypt (**Elshahawy *et al.* 2014; Halium, *et al.* 2014; and El-Bahy *et al.* 2017).** Further, amphistomosis has been described worldwide in sheep and goats **(Rolfe *et al.,* 1994),** in buffaloes **(Panda and Misra, 1980)** and in cattle **(Mavenyengwa *et al,* 2010)** and the prevalence reached up to 39% **(Al-Gaabary *et al.,* 2009).** The disease may be an impact of high economic losses **(Sargison *et al.,* 2016).** The histopathology and clinical pathology associated with the pathogenicity of experimental amphistome infections has been studied in ruminants **(Boray, 1985 and Misra *et al.*, 1996),** with a trend of protein loss in ruminants infected with juvenile amphistomes **(Pillai and Alikutty, 1995).**

In spite of the mentioned prevailing situation and the presence of a number of problems due to gastrointestinal parasites, there is paucity of well-documented information on the effect of paramphestomiasis on health of the naturally affected host. Therefore, the present work aimed to evaluate some parasitological events and the pathological changes resulted from parasitic invasion in the rumen and intestine due to natural infection by paramphestomiasis in calves. Further, to investigate the effect of natural infection by paramphestomiasis on some biochemical constituents in blood including some mineral status and protein electrophoresis of blood serum of infected calves.

Materials and Methods

Cross-bred calves (20-30 months old) admitted for normal or emergency slaughters at Assiut abattoirs were clinically examined. Blood samples were collected from suspected calves (scouring, unthrifty calves) during the antimortem examination, which was further confirmed during post-mortem examination, in 10-ml heparinized vacutainer tubes (**Terumo Europe N.V, Interleuvenlaan 40, 3001Leuven, Belgium).** Fecal samples were also collected for parasitological examinations. Blood and fecal samples were stored in ice-box until further investigations at the laboratory within 6-8 hours. After slaughtering, carcasses were perfectly and thoroughly examined for abnormal lesions. Emphasis was given on the examination of the entire lumen of the rumen, small and large intestines for parasitic affections. Intestines and rumen with rumen pathological lesions were sampled and fixed in 10% neutral buffered formalin for histological investigations. According to parasitological examinations, a total of 26 calves were selected for this study and their blood was classified into 2 groups. Group one was considered as an infected group (N=11) and the second group was parasite free healthy calves (control group, N=15).

Parasitological examination

The small intestine of each animal was separated from the rest of the organs with minimal manipulation. Starting from the proximal end of the duodenum, 2 meter long of small intestinal loops were tied at both ends, then cut and placed in labeled plastic containers. The contents of the abomasum, omasum and the reticulum were washed into glass containers and sieved under pressure through 2400 mm and 850 mm sieves, respectively. Excess water was decanted and the recovered flukes preserved in 70% alcohol **(Mavenyengwa *et al.,* 2008)** for counting and identification by histological examinations.

Fecal samples were examined by direct smear and concentration sedimentation techniques and the eggs of amphistomes were stained by Lugol's iodine solution, and then identified by their morphology according to **Thienpont, *et al.* (1979) and Rolfe and Boray (1987)**. The flukes were classified depending on their anatomical morphology and their organs by median sagittal sections stained with H&E stain (pharynex, acetabulum, and genitalia) accord-

ing to **Eduardo (1982); Urquhart, *et al.* (1996) and Dube and Aisien (2010).**

Blood collection.
26 Blood samples were classified into 2 groups. Group one was considered as an infected group (N=11) and the second group was parasite free healthy calves (control group, N=15). Blood samples from infected calves were divided into two tubes, one with anticoagulant (NaEDTA) for immediate hematological investigations, and the other one was without anticoagulant used for separation of serum, which stored at -20˚C until biochemical analysis.

Haematological assay:
Hematological investigations were carried-out by standard methods of hematology after **Jain (1993).** The count of red blood cells (RBC) was determined using a hemocytometer, whereas packed cell volume (PCV) and hemoglobin concentration (Hb) were determined by microhematocrit and cyanomethemoglobin methods, respectively.

Biochemical assay
Blood sera were used for determination of concentrations of total proteins, albumin, calcium, phosphorous, sodium, potassium and chloride by using specific commercially available test kits (Salucea, The Netherlands). Protein electrophoretogram was carried out by using Titan III cellulose acetate plate at pH 8.8 at ionic strength of 0.067, stained with Ponceau S dye and scanned by auto densitometer **(Helena Laboratories, Cat. 1023)** at absorption peak of 525 nm according to manufacture instructions.

Histopathological examinations
For histopathological studies, three representative tissues specimens of rumens and intestines containing flukes were fixed in 10% neutral buffered formalin and were processed by paraffin embedding technique, sectioned at 6 µm and stained with hematoxylin-eosin **(Bancroft and Steven, 1993).**

Statistical analysis:
Data were analyzed using the packaged SPSS program for windows version 20.0.1 (SPSS Inc., Chicago, IL.). Data were expressed as means±standard error (SE). Differences between groups were determined using an analysis of variance (ANOVA) followed by the Student t-test. Significance level was set at $P \leq 0.05$.

results
Parasitological finding
Large numbers of paramphistomes were found attached to the mucosa of the rumen and abomasum (Fig. 1a) the average total number of mature paramphistomes recovered from the rumen, abomasum and duodenum of each calf examined ranged from 250-400 flukes. Large numbers of paramphistome eggs (i.e. thin shelled, operculated with average measurements of 123 x 75 µ) were present (Fig. 1d) in the extremely fluid intestinal contents. Fresh flukes are bright pink color. The body is conical, curved, somewhat concave ventrally and convex dorsally and measures 6.2-14.3 mm X 2.2-4.3 mm. Acetabulum is subterminal, 1.26-2.68 mm in diameter. Testes are tandem, oval to spherical and lobed with post-testicular ovary. The adult paramphistomes (Fig. 2a) have an eosinophilic tegument, digestive tract (sucker, pharynx, oesophagus and posterior sucker (acetabulum) tests and ovary (hermaphrodites).

Gross examination
The abomasal mucosa was oedematous and showed shallow erosions and petechiae (Fig. 1b). The intestine was congested and oedematous (Fig. 1c).

Microscopic pathology
(A)Rumen showed increased certification of the stratum corneum of the rumenal papillae. There was some inflammatory reaction consisting of infiltration of lymphocytes, plasma cells, neutrophils and eosinophils (Fig. 3b). In areas where the papillae had fallen off due to parasite anchorage and feeding, microabcesses (Fig. 2b). Stratified epithelium showed shortening, broadening, atrophy, segmental rupture, erosion, degeneration and necrosis. These changes were characterized by thickening of the stratum corneum (Fig. 2c).Parasites that were detected are attached and adhered to ruminal papillae through their suction cups (Fig. 2c).
(B) Intestine

Flukes were present in the intestinal lumen and penetrate the muscularis mucosa (Fig. 3a). The duodenal glands were variable in size; glands were dilated and opened directly into the lumen (Fig. 3b). The intestine showed also villous atrophy, crypt hyperplasia, goblet cell hyperplasia, cystic dilatation, inflammation of the stroma, and focal loss of the mucosa and loss of the mucosal integrity erosion and ulcerations were observed (Fig. 3c, d).

Hematological findings:

Changes in haematological parameters in calves with paramphistomiasis are presented in Table I. There was significant ($P<0.05$) decrease in the values of haemoglobin (Hb), RBCs and PCV in affected calves when compared with the control group.

Biochemical results:

Table 2 shows the changes in Ca and Ph concentrations in calves with paramphistomiasis. There was significant decrease ($P<0.05$) in the values of Ca and Ph in affected calves when compared with the control group. Table 2 shows also the changes in electrolyte parameters in calves with paramphistomiasis. There was significant decrease ($P<0.05$) in the values of Na, K and Cl in affected calves when compared with the control group.

Table3 and table 4 shows the Changes in serum protein parameters in calves with paramphistomiasis There was significant decrease ($P<0.05$) in the values of total protein, albumin and a significant increase ($P<0.05$) in α1-globulin, α2-globulin and γ-globulin in affected calves when compared with the control group.

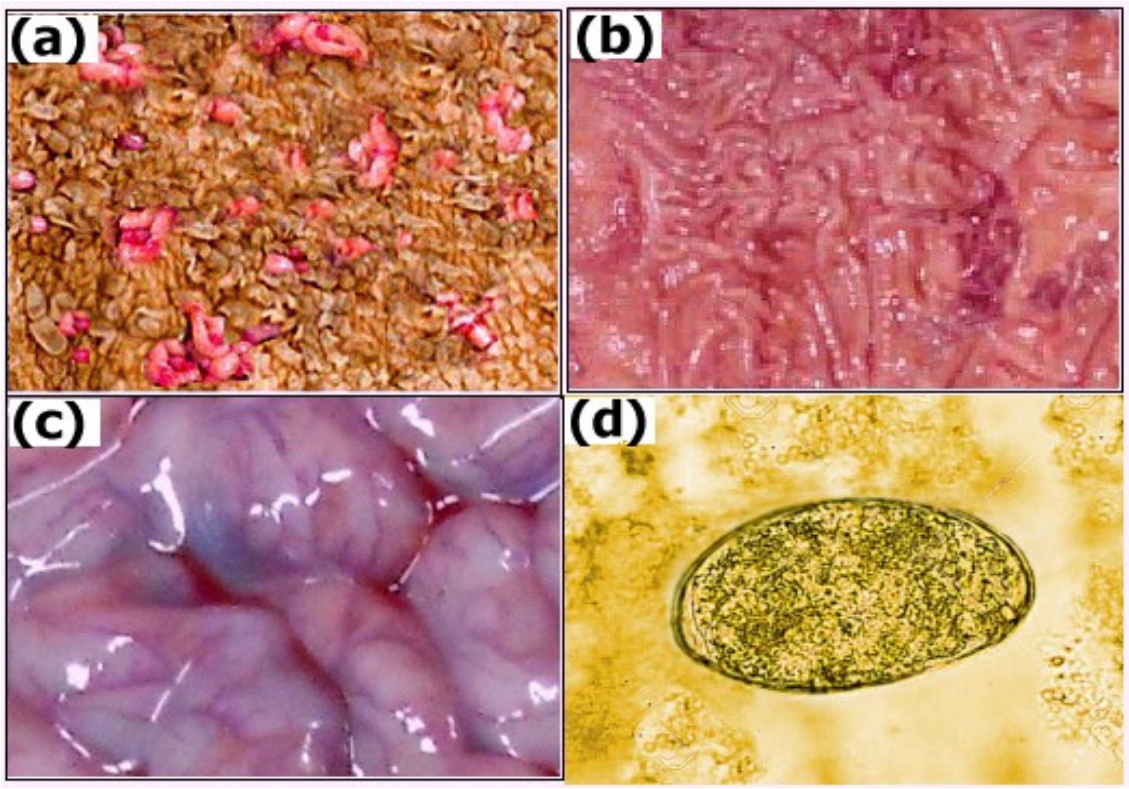

Fig. (1): a: Large numbers of paramphistomes attached to the mucosa of the rumen. b: abomasal mucosa showing oedema , shallow erosions and petechiae. C: The intestine is congested and oedematous. d: paramphistome egg.

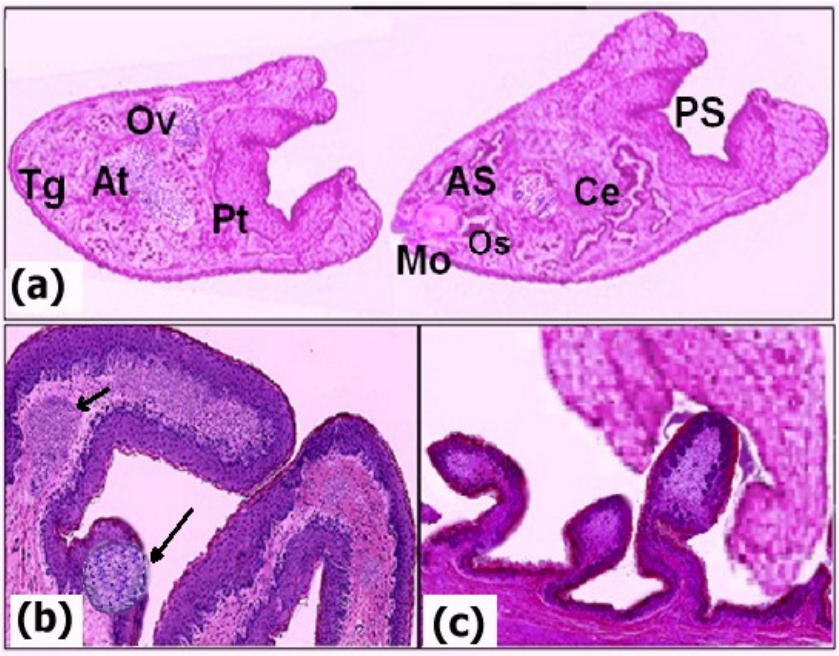

Fig. (2): a: adult paramphistomes with tegument (Tg), anterior and posterior testis (At, Pt) ovary (Ov), mouth (Mo), oesophagus (Os), anterior sucker (As), ceocum (Ce) and posterior sucker (acetabulum, Ps) x50. b: rumen showing increased cornification of the stratum corneum of the papillae, some inflammatory reactions. The arrow showing microabcesses in areas where the papillae fall off due to parasite anchorage and feeding, c) x50: Stratified epithelium showing shortening, broadening, atrophy, or segmental rupture, erosion, degeneration and. necrosis Parasite is attached and adhered to ruminal papillae through suction cups) x50.

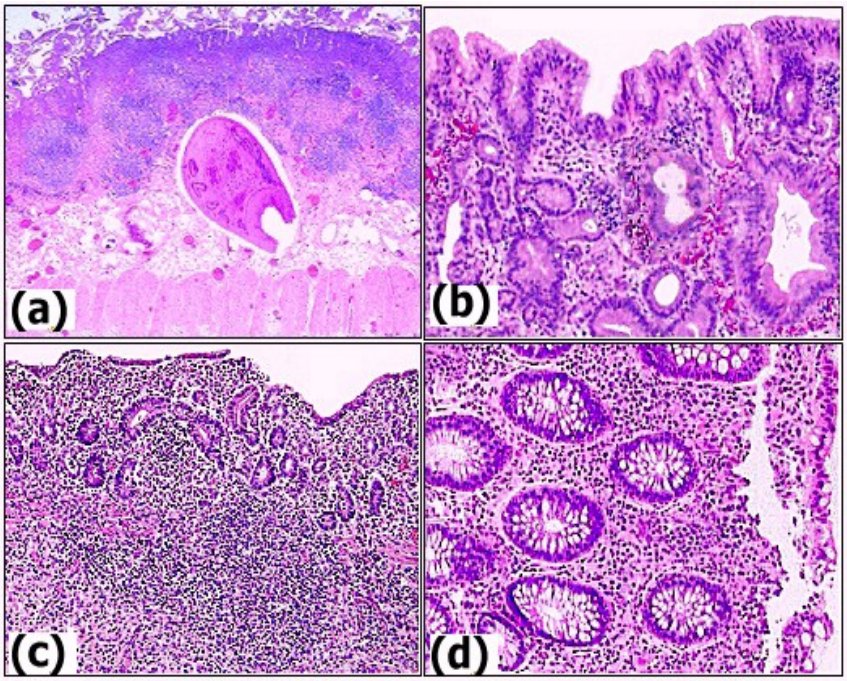

Fig. (3): a: Flukes penetrate the muscularis mucosa of the intestine x25. b: the duodenal glands were variable in size, some glands were dilated and opened directly into the lumen. c x150, d: the intestine showed villous atrophy, crypt hyperplasia, goblet cell hyperplasia, cystic dilatation, , inflammation of the stroma, and focal loss of the mucosal tissue, loss of the duodenal mucosal x200.

Tab. (1). Hematology in healthy and paramphestomiasis infected calves

	RBCs x10 ^{6}/ul	Hbgm/dl	PCV%
Control n=15	10.2±0.27	98.2±0.26	29.6±0.64
Diseased n =11	8.54±0.32	86.1±0.34	26.9±0.53
P	0.038	0.041	0.034

Tab. (2). Proteinogram (g/dl) in healthy and paramphestomiasis infected calves

	T. P	Albumin	Globulin	α1	α2	β	γ
Control n=15	7.05±0.15	3.77±0.07	3.28±0.20	0.15±0.01	0.61±0.03	1.01±0.05	1.51±0.05
Diseased n =11	6.35±0.10	2.71±0.05	3.64±0.31	0.21±0.02	0.70±0.03	0.99±0.07	1.74±0.04
P	0.042	0.006	0.041	0.032	0.036	0.104	0.039

Tab. (3). Mineral concentrations in healthy and paramphestomiasis infected calves

	Ca (mM)	Ph (mM)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)
Control n=15	2.66±0.11	1.99±0.08	142.1±1.01	4.12±0.24	101.3±0.71
Diseased n =11	2.01±0.09	1.61±0.08	131.2±0.94	3.51±0.31	91.5±0.63
P	0.012	0.023	0.046	0.009	0.034

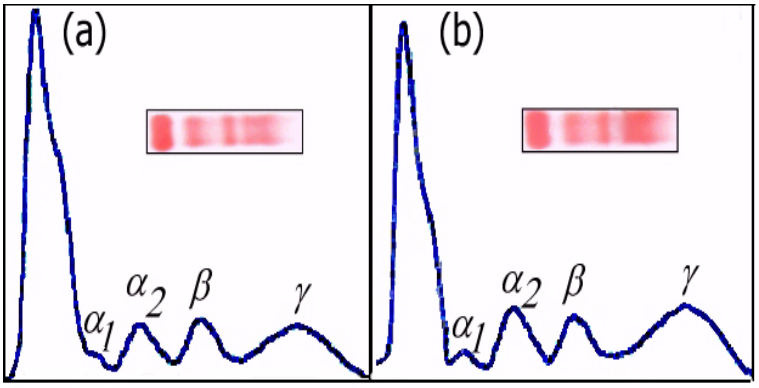

Fig (4). A model for the patterns of cellulose acetate electrophoretogram of calf serum protein. A Normal pattern from healthy calf, b- Abnormal pattern from paramphestomiasis infected calf

Discussion

Morphological and histological identification are important features used to diagnose Paramphistomes flukes **(Forbes, 2018 and Malek, 2018).** In the current work, microscopic pictures of the parasite used in identification pointed out the similarity in the morphology and histology of the anterior sucker, pharynx, esophagus, genitalia and posterior sucker (acetabulum) to the *P. cervi* (**Chaoudhary *et al.* 2015).**

Microscopical features showed large numbers of flukes migrate from small intestine to rumen cause significant damage in the rumen such as cornification, destruction and desquamation of the rumenal mucosa leading to the loss of cells into the lumen and accumulation of inflammatory cell reactions. These results are in agreement with those previously reported by **Toledo *et al.* (2006), Brown and Barker (2007) and Tariq *et al.* (2011).** The occurrence of clinical amphistomosis and subsequent pathology in ruminants is dependent on the dose, the virulence and the establishment levels of the infecting larvae in the small intestine of the host

(Urquhart, *et al.,* 1996). Mature amphistomes are considered non-pathogenic in the rumen but the finding of ruminal lesions in this study seem to suggest that when amphistomes present in large numbers they may cause disease directly or indirectly through systemic absorption of ruminal microflora via necrotic papillary ends **(Mavenyengwa, *et al.,* 2008).** In areas where the papillae had fallen off due to parasite feeding and anchorage, microabcesses were present confined to the stratum corneum associated with mild cell reaction confirm the virulence of the infecting amphistomes in this study.

Brunner's glands provide an ideal environment for fluke growth until they become mature enough to withstand the acid environment in the abomasum during migration to the rumen **(Boray 1985).** Brunner's glands were, cystic with an atrophied basal epithelium suggesting prior occupation. Some flukes, however, could be seen in the current study in the mucosal tissue probably migrating to the duodenal surface **(Deorani and Jain 1969).** There was glandular enlargement with severe villous atrophy characterized by destruction of absorptive enterocytes, fusion of villi and subsequent cryptal hyperplasia as reported in cattl**(Mavenyengwa, *et al.,* 2008).**

Villous atrophy characterized by villous and cryptal epithelial differentiation, hyperplasia and loss of function is a known feature of helminth infections and its mechanisms are obscure but could be related to immunological mechanisms against gastrointestinal parasites **(Brown, and Barker, 2007)** and probably responsible for the duodenal thickening and corrugation of the mucosa observed grossly in this study. In this study, the migration of immature flukes into the duodenal submucosa induced an acute inflammatory reaction characterized by an infiltration of inflammatory leukocytes, and lymphocytes into the mucosal parenchyma which are similar to those reported early by **Toledo, *et al.* (2006)**

The present study reported anemia, which is in agreement with the earlier reports that documented significant decrease in the hematological values of RBCs, PCV and Hb in ruminant amphistomiasis **Mavenyengwa *et al.,* 2008; Biswas, *et al.,* 2013).**

The lower values of RBCs, Hb and PCV in infected calves might be due to the loss of blood from severe hemorrhage and ulceration in rumenal mucosa and intestinal tissue due to migration of young immature flukes via the intestine to their predilection site rumen **(Chauhan, *et al.,* 2015).** It may be also due to extensive migration of the young fluke through the hepatic parenchyma and from blood sucking activity of adult fluke (**Chaoudhary, *et al.,* 2015).** In addition, **Varma and Swamy (2006**) studied mineral profile in serum of buffaloes infected with paramphistomosis and showed a significant reduction in iron level that apparently affects the value of Hb causing anemia.

The decrease in the mean calcium and phosphorus values in the current work is similar to those obtained in Paramphestomiasis infected sheep **(Horak and Clark, 1963)** and cattle **(Mavenyengwa *et al.,* 2008).** The loss of calcium would be expected as 30–50% of total blood calcium in domestic ruminants is bound to proteins, including **albumin (Kaneko, 1997),** and thus a loss of albumin automatically leads to a loss of calcium and a subsequent physiological phosphorus loss.

The loss of sodium, potassium and chloride ions in the present study is in agreement with the earlier reports that documented significant decrease these ions in ruminant amphistomiasis **(Biswas *et al.,* 2013).** A characteristic and persistent fetid diarrhea accompanied by anorexia and dehydration might be the cause of low level of serum sodium, potassium and chloride **(Radostits, *et al.,* 2009)** and **Anderson (1980)** also reported that anorexia and malabsorption in chronic parasitism results in fluid and ion loss.

The loss of proteins in the current work in is agreement with the earlier reports that documented lower levels of total plasma protein (6.87+0.32 and 6.43+0.29 g/dl) in parasitic animals than in healthy control animals **(Thakur, *et al.,* 2007).** A significant decrease in serum albumin and globulin was reported in ruminant amphistomiasis **(Biswas *et al.,* 2013).** Lower

level of total serum protein and albumin in the infected animals might be due to the severe enteritis associated with enormous numbers of immature migrating flukes in the duodenum, which might reduces the absorption of degraded proteins **(Radostits, *et al.,* 2009).**

The prominent decrease in total plasma protein concentration, mainly attributable to a significant decline in plasma albumin level, is observed in affected calves. Leakage of plasma protein into the small intestine in substantial amounts. This phenomenon is probably related to the pathogenic mechanism of amphistomes, which cause tissue destruction during the migratory phase through the tunica muscularis to the submucosa. During the migratory phase, the immature flukes attach to the mucosa using acetabula and cause strangulation of the engulfed mucosa. The strangulated tissues become necrotic and eventually slough-off leaving erosions and petechiae through which protein molecules can be lost **(Horak, 1966; Kumar (1998).** Submandibular oedema has been reported on various occasions in animals with the acute disease and is related to the marked hypoalbuminaemia **(Kumar, 1998).**

The increased total serum globulin concentration of the affected calves is a normal consequence to the increase in β and γ-globulin. The increases in β and γ globulin fractions in the current study indicate the occurrence of tissue damage **(Thomas, 2000a). Thomas (2000b)** reported that the increase of β-globulin often occurs in association with elevations in γ-globulin as a part of response to chronic inflammation or infection.

The α-globulin band consists mainly of α1-antitrypsin, α-2-macroglobulin, haptoglobulin, amyloid A and ceruloplasmin **(Gruys *et al.,* 1994).** These proteins are considered as positive acute phase reactant and increase rapidly in response to antigenic, traumatic and septic stimuli in cattle **(Murata *et al.,* 2004).** The increase in α-globulin in the current work reflects the inflammatory reaction which occurred in the affected calves. **Diogenes *et al. (*2010)** found also an increase in α-globulin in goats Infected with Haemonchus contortus. The varying increase of this fraction between groups reflects the intensity of the inflammatory response and progression of the disease. The increased γ-globulin in the current work may be attributed to the response of the animal against the paramphistomiasis flukes, which involved the production of specific immunoglobulins **(Anuracpreeda, *et al.,* 2017).**

Conclusion

In this study, the disease amphistomosis is characterized by severe anemia of normocytic normochromic type. Presence of anemia along with the involvement of parasite can be taken as an indicator to correlate it with subclinical infection of amphistomosis. Anemia of severe intensity accompanied by pathological and biochemical consequences can be responsible for mortality in animal's especially young ones.

References

Al-Gaabary, M.H.; Osman, S.A. and El-Tonoby, A.G. (2009). studies on paramphistomiasis in ruminants. Kafrelsheikh, Vet. Med. J., 3rd Sci. Congress. 10-12, pp. (116-136).

Anderson, N.V. (1980). Veterinary Gastroenterology, Lea & Febiger, Philadelphia.

Anuracpreeda, P.; Watthanadirek, A.; Chawengkirttikul, R. and Sobhon, P. (2017). Production and characterization of a monoclonal antibody specific to 16ákDa antigen of Paramphistomum gracile. Parasitology research, 116(1), 167-175.

Bancroft, J.D. and Stevens, A. (1993). Theory and practice Of Histological Techniques, 3rd Ed. Long Man Group Limited.

Biswas, A.; Phukan, A.; Baruah, C.C.; Sarma, S.S. and Dutta, P.R. (2013). Haemato-biochemical changes in cattle with naturally acquired paramphistomiasis. Indian Vet. J., 90(10): 26-28.

Boray, J.C. (1985). Flukes of domestic animals: Paramphistomata. pp. 207–212 in Gafaar, S.M., Howard,W.E. & Marsh, R.E. (Eds) World animal science, disciplinary approach, Vol. B2: Parasites, pests and predators. Amsterdam, The Netherlands, Elsevier Science Publishers.

Brown, D.C., D.C. and Barker, I.K. (2007). Alimentary system, p.1-296.In: Maxie M.G. (ed.), Jub, Kennedy & Palmer's Pa-

thology of Domestic Animals. Vol. 2.4th ed. Academic Press, San Diego.

Chaoudhary, V.; Hasnani, J.J.; Khyalia, M.K.; Pandey, S.; Chauhan, V.D.; Pandya, S.S. and Patel, P.V. (2015). Morphological and histological identification of Paramphistomum cervi (Trematoda: Paramiphistoma) in the rumen of infected sheep. Veterinary world, 8(1), 125.

Deorani, S.V.P. and Jain, S.P. (1969). Reasons for the involvement of duodenum in parasitic part of the life cycle of 'rumen amphistomes' (Paramphistomidae: Trematoda). Indian Journal of Helminthology, 21: 177–182.

Diogenes, P.V.A.; Suassuna, A.C.D.; Ahid, S.M.M. and Soto-Blanco, B. (2010). Serum Protein Electrophoretie Profile of Goats Infected with Haemonchus contortus. Journal of Animal and Veterinary Advances, 9(11), 1603-1606.

Dube, S. and Aisien, M.S.O. (2010). Descriptive studies on paramphistomes of small domestic ruminants in Southern Nigeria. Zimb. J. Sci Technol.; 5: 12–21.

Eduardo, S.L. (1982). the taxonomy of the family Paramphistomidae Fischoeder, 1901 with special reference to the morphology of the species occurring in ruminants. I. General considerations. Systematic Parasitology 4, 7–57.

El-Bahy, N.M.; Bazh, E.K. and Elkhtam, A. (2017). New approach to molecular characterization of Paramphistomum cervi and Carmyerius gregarius and comparative analyses with selected trematodes. Parasitology research, 116(5), 1417-1422.

Elshahawy, I.; Metwally, A. and Ibrahim, D. (2014). An abattoir-based study on helminthes of slaughtered goats (Capra hircus L., 1758) in upper Egypt, Helminthologia, 51 (1): 67-72.

Elsokkary, D.; Heikal, Y. and Otify, F. (2009). Incidence of rumen fluke in Beheria province, Egypt. Alexandria .Journal of Veterinary Science 28 (1), 117-124.

Forbes, A. (2018). Rumen fluke: past, present and future. Livestock, 23(5), 227-231.

Grist A. (2008). Bovine Meat Inspection. 2nd ed. Nottingham University Press, Nottingham. 278p.

Gruys, E.; Obwolo, M.J. and Toussaint, M.J.M. (1994). Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. The Veterinary Bulletin, 64: 1009-1018.

Halium, M.M.A.; Salib, F.A.; Mousa, W.M. and Massieh, E.S.A. (2014). Prevalence and Classification of Amphistomes in Cattle and Buffaloes. Journal of Advanced Veterinary Research, 4(2), 60-66.

Horak, I.G. and Clark, R. (1963). Studies on Paramphistomiasis. V. The pathological physiology of the acute disease in sheep. Onderstepoort Journal of Veterinary Research, 30: 45–160.

Horak, I.G. (1966). Studies on Paramphistomiasis, VIII. The pathogenesis and symptoms of the disease in sheep. Journal of the South African Veterinary Association, 37: 428–430.

Jain, N.C. (1993). Essentials of veterinary hematology, Lea & Febiger, Philadelphia.

Kaneko, J.J., (1997): Serum Proteins and Dysproteinemias, In: Kaneko, J.J. Harvey, J.W., Bruss, M.L. (eds), Clinical Biochemistry of Domestic Animals. 5th ed. Academic Press, London, pp. 117-138.

Kumar, V. (1998). Trematode infections and diseases of man and animals. Springer Science & Business Media.

Malek, E.A. (2018). Paramphistomatidiasis. In Snail Transmitted Parasitic Diseases. CRC Press. pp. 217-238.

Maule, A.G. (2000). Trematode Infections and Diseases of Man and Animals. Parasitology Today, 16(11), 502.

Mavenyengwa, M.; Mukaratirwa, S.; Obwolo, M.J. and Monrad, J. (2008). Bovine intestinal cellular responses following primary and challenge infections with Calicophoron microbothrium metacercariae. Onderstepoort Journal of Veterinary Research, 75: 109–120.

Mavenyengwa, M.; Mukaratirwa, S. and Monard, J. (2010). Influence of calicophoron microbothrium amphistomosis on the biochemical and blood cell counts of cattle. J. Helminthol, 84(4): 322-361.

Melaku, S. and Addis, M. (2012). Prevalence and intensity of Paramphistomum in ruminants slaughtered at Debre Zeit industrial abattoir, Ethiopia. Glob. Vet. 8: 315–319.

Misra, S.C.; Panda, D.N. and Parida, S. (1996). Haematological and histological alterations of immature paramphistomiasis in lambs. Indian Veterinary Journal,73: 1274–1276.

Murata, H.; Shimada, N. and Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. The Veterinary Journal 168, 28-40.

Ozdal, N.; Gul, A. and Deger, S. (2010). Prevalence of Paramphistomum infection in cattle and sheep in Vanprovince, Turkey. Helminthologia 47: 20-24.

Panda, B.K. and Misra, S.C. (1980). Observations on the etiology, clinical pathology and chemotherapy of immature amphistomiasis in buffalo calves. Indian Journal of Animal Health 19, 131–135.

Pillai, U.N. and Alikutty, K.M. (1995). Clinico-hematological observation in bovine amphistomiasis. Indian Journal of Veterinary Medicine 15, 38–39.

Pinedo, R.; Chávez, A.; Casas, A.; Suárez, F.; Sánchez, N. and Huamán, H. (2010). Prevalence of trematodes of the Paramphistomatidae family in cattle of Yurimaguas district, province of Alto Amazonas, Loreto. Rev. Investig. Vet. Perú 21, 161–167.

Radostitis, O.M.; Gay, C.C.; Hinchcliff, K.W. and Constable, P.D. (2009). Veterinary Medicine-A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th Edn. Saunders Elsevier publication.

Rolfe, P.F. and Boray, J.C. (1987). Mavenyengwa :Chemotherapy of paramphistomiasis in cattle. Aust. Vet. J., 64: 328-334.

Rolfe, F.; Boray, J.C. and Collins, G.H. (1994). Pathology of infection with Paramphistomum ichikawai in sheep. International Journal for Parasitology 24, 995–1004.

Sargison, N.; Francis, E.; Davison, C.; Barend, M.; Handel, I. and Mazeri, S. (2016). Observations on the biology, epidemiology and economic relevance of rumen flukes (Paramphistomidae) in cattle kept in a temperate environment. *Veterinary parasitology*, *219*, 7-16.

Tariq, A.; Chishti, M.Z.; Tanveer, S.; Baqui, A.; Darzi, M.M. and Mir, M.S. (2011). Histopathological study of the organs of sheep infected with Paramphistomum cervi in Kashmir valley. Nanobiotechnol. Univs, 2(1), 7-12.

Taylor, M.A.; Coop, R.L. and Wall, R.L. (2007). Veterinary Parasitology. 3 rd ed. Blacwell, Oxford. 874p.

Thakur, R.; Singh, R.; Mandial, R.K.; Bala, S. and Katoch, R. (2007). Clinico-haematological, biochemical, minerals and therapeutic studies on amphistomiasis in cattle of Himachal Pradesh. Indian J. Vet. Med., 26(1): 12-5.

Thienpont, D.; Rochete, F. and Vanprijs, O.F. (1979). Diagnosing helminthiasis through coprological examination. Janssen Research Foundation, 180 pp: 190-200.

Thomas, J.S. (2000a). Overview of plasma proteins. In: Feldman, B.F., Zinkl, J.G., Jain, N.C. (eds), Schalm's Veterinary Hematology, 5th ed. Lippincott Williams & Wilkins, Philadelphia and Baltimore, pp: 891-898.

Thomas, J.S. (2000b). Protein electrophoresis. In: Feldman, B.F., Zinkl, J.G., Jain, N.C. (eds), Schalm's Veterinary Hematology, 5th ed. Lippincott Williams & Wilkins, Philadelphia and Baltimore, pp: 899-903.

Toledo, R.; Esteban, J.G. and Fried, B. (2006). Immunology and pathology of intestinal trematodes in their definitive hosts. Advances in Parasitology, 63, 285-365.

Urquhart, G.M.; Armour, J.; Duncan, J.L.; Dunn, A.M. and Jennings F.W. (1996). Veterinary Parasitology. 2nd ed. Blackwell, Oxford. 307pp.

Varma, Y. and Swamy, M. (2006). Pathology of naturally occurring biliary amphistomiosis in buffaloes. Indian J. Vet. Pathol., 30(2): 62-63.