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Serum Protein Electrophoretic Profile of Goats Infected with *Haemonchus contortus*

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Abstract: Internal parasites represent an important cause of loss to goat production. Although, numerous gastrointestinal nematodes are present, *Haemonchus contortus* is one of the greatest concern in goats from tropical and sub-tropical countries. The main clinical effects are anemia, diarrhea, dehydration, edema, hydrothorax, hydroperitoneum and reduced growth rates. The present research aimed to determine the changes in the serum proteins of goats infected with *Haemonchus contortus*. Blood samples were collected from 15 goats infected with *H. contortus*. All the infected goats presented with submandibular edema and weakness and infection was confirmed by fecal analysis. Blood samples were also collected from 20 non-infected goats that served as controls. Sera were used for determination of total protein by colorimetric assay and protein fractions were separated by electrophoresis. The results were compared using an unpaired two-tailed t-test at the 5% level. The serum levels (g dL^{-1}) of Haemonchus infected and control goats were, respectively: total proteins 5.22±1.15 and 7.07±1.10, albumin 2.06±1.04 and 4.80±0.99, α-globulins 0.54±0.23 and 0.39±0.11, β-1 globulins 0.53±0.22 and 0.48±0.10, β-2 globulins 0.28±0.18 and 0.26±0.10, γ-1 globulins 0.91±0.37 and 0.92±0.43 and γ-2 globulins 0.57±0.25 and 0.23±0.10. There were statistically significant differences for total protein, albumin, α-globulins and γ-2 globulins. Infection with *H. contortus* in goats promotes intense hypoproteinemia, hypoalbuminemia increased α and γ-2 globulins and a decreased Albumin/Globulin (A/G) ratio.

Key words: Serum proteins, albumin, globulins, goats, *Haemonchus contortus*, Brazil

INTRODUCTION

Internal parasites represent an important cause of disease and loss of production in small ruminants. Although, numerous gastrointestinal nematodes are present, *Haemonchus contortus* is of the greatest concern in goats from tropical and sub-tropical regions (Zajac, 2006; Nematollahi *et al*., 2007). Haemonchus is one of the most prolific nematodes and has great pathogenic potential. The main clinical effects are anemia, diarrhea, dehydration, edema, hydrothorax, hydroperitoneum and reduced growth rates. Heavy infections cause death (Zajac, 2006). The main laboratorial finding in *H. contortus* infections is anemia (Osman *et al*., 2004; Ogundola *et al*., 2006; Okaiyeto *et al*., 2010).

Serum electrophoresis is a technique of laboratory diagnosis that provides useful information, though often underused about the protein fractions of the serum. The technique is used in differentiating acute from chronic disease states and in the diagnosis of inflammation (Thomas, 2006; Eckersall, 2008). The infections by parasites in ruminants is responsible by interference on serum proteinogram including decrease in total proteins and albumin levels (Oliveira and Penha, 1978; Fernandez *et al*., 2006; Lawal *et al*., 2007). Sheep experimentally infected by *H. contortus* presented decrease in total proteins, albumin, α and β-globulin levels and albumin/globulins ratio and increase in γ-globulin levels (Ahmad *et al*., 1990). However, the serum protein electrophoresis patterns of Haemonchus infected goats are not well understood. Thus, the present research aimed to determine the changes in serum proteins in goats infected with *H. contortus*. It was expected that inflammatory response to Haemonchus promote alterations in serum proteins profile of goats.

MATERIALS AND METHODS

It were used 35 female mixed-breed goats, 1-3 years old that were not pregnant or lactating, from two farms at municipality of Mossoro, RN, Brazil. They were fed with unlimited quantities of native hay on pasture. Fecal

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samples were collected from lambs for nematode fecal egg counts and coproculture for identification of third-stage Larvae (L3) which were performed according to the method of Ueno and Goncalves (1998). Fifteen of these goats were naturally infected with *Haemonchus contortus* and the infection was confirmed by fecal analysis (counts were >500 eggs g^{-1} of feces). They presented submandibular edema (bottle jaw), weakness, mild dehydration and diarrhea. The other 20 goats were non-infected as judged by fecal egg counts were clinically healthy and served as controls.

Blood samples were collected by jugular venipuncture into plain tubes and transferred to the laboratory within 30 min where serum was separated at 5.000 rpm and kept frozen at -20°C for up to 15 days until analysis. No lipemic or hemolytic samples were processed. Sera were used for determination of total protein by colorimetric assay and protein fractions were separated by electrophoresis. Protein analysis was performed in an automated biochemistry analyzer (SBA 200, CELM, Barueri, SP, Brazil) using a commercially available test (KATAL, Belo Horizonte, MG, Brazil).

Agarose gel electrophoresis was performed with agarose kits (CELM, Barueri, SP, Brazil) and an electrophoresis system (SE-250, CELM, Barueri, SP, Brazil). After protein separation at pH 8.5, the gels were stained with Amido Black solution and scanned with a densitometer (DensitScan version 2.2, CELM, Barueri, SP, Brazil). The points of separation between fractions on the resultant Serum Protein Electrophoresis (SPE) profiles were marked manually using the midpoint between peaks on the electrophoretogram. The protein concentrations (g dL^{-1}) were determined by multiplying the percentage of each fraction by the total protein concentration.

Total globulins were calculated as the sum of the non-albumin protein concentrations. The following protein fractions were determined: albumin, α globulins, β-1 globulins, β-2 globulins, γ-1 globulins and γ-2 globulins (Santarosa *et al*., 2005). The Albumin/Globulin (A/G) ratio was calculated manually. Data analysis was performed using a statistical software package (GraphPad Prism v.4 for Mac, La Jolla, CA, USA). The homogeneity of variances was verified by Bartlett´s test. The results were compared using an unpaired t-test with the level of statistical significance set at $p<0.05$.

RESULTS AND DISCUSSION

Goats from the group naturally infected with *H. contortus* presented submandibular edema (bottle jaw), weakness, mild dehydration and diarrhea and their body condition score ranged from 1.5-2.5. Control goats were clinically healthy with body condition score 2.5-3.5. The serum protein levels (in g dL^{-1}) of Haemonchus infected and control goats are shown in Table 1. Goats infected with Haemonchus had significantly lower ($p<0.05$) total protein and albumin levels and albumin/globulin ratio and significantly higher ($p<0.05$) levels of α-globulins and γ-2 globulins than controls.

No siginificant differences were found in levels of total globulins, β-1, 2 and γ-1 globulins. Examples of electrophoresis pattern from a control goat and an infected goat are shown at Fig. 1. The infections by

Table 1: Serum levels of proteins in goats infected with *Haemonchus contortus* and non-infected (control) goats

Components	Goats: Haemonchus infected (n = 15)	Control (n = 20)
Total proteins	5.22±1.15*	7.07±1.10
Albumin (g dL^{-1})	2.25±0.93*	4.80±0.99
Globulins (g dL^{-1})	2.80±0.77	2.27±0.72
α globulins (g dL^{-1})	0.54±0.23*	0.39±0.11
β-1 globulins (g dL^{-1})	0.53±0.22	0.48±0.10
β-2 globulins (g dL^{-1})	0.28±0.18	0.26±0.10
γ-1 globulins (g dL^{-1})	0.91±0.37	0.92±0.43
γ-2 globulins (g dL^{-1})	0.57±0.25*	0.23±0.10
Albumin/globulin ratio	0.89±0.50*	2.34±0.86

*$p<0.05$ (unpaired t-test)

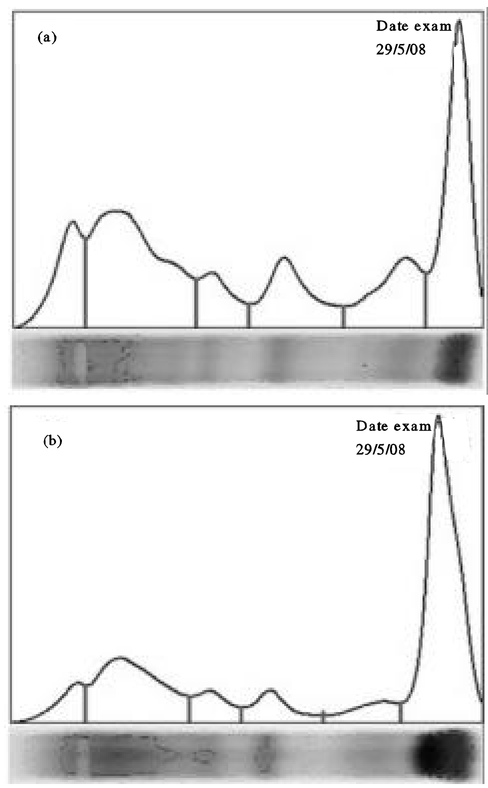

Fig. 1: Electrophoresis patterns from (a) an *Haemonchus contortus* infected goat and (b) a control goat

parasites in ruminants is responsible by interference on serum proteinogram. It was found that the infection in cattle by *Cooperia* sp., *Oesophagostomum* sp. and *Haemonchus* sp. was responsible by reduction of serum levels of total proteins and all fractions but without change in albumin/globulins ratio (Oliveira and Penha, 1978). Goats naturally infected by *Haemonchus* sp., *Trichostrongylus* sp., *Oesophagostomum* sp. and *Eimeria* sp., presented decrease in total proteins and albumin levels (Fernandez *et al*., 2006). Lambs experimentally infected by *H. contortus* presented decrease in total proteins, albumin, α and β-globulin levels and albumin/globulins ratio. At peak of the infection, sheep also presented increase in γ-globulin levels (Ahmad *et al*., 1990). It was observed in this study that goats infected with *H. contortus* present abnormalities in serum proteins, characterized by intense hypoproteinemia, hypoalbuminemia increased α and γ-2 globulins and a decreased A/G ratio.

Several physiologic factors affect serum protein levels including age, hormones, gender, pregnancy, lactation (Thomas, 2006; Eckersall, 2008) and temperature season (Shaffer *et al*., 1981). In the resent study, these factors may not interfered in the results because all goats were from the same gender (females), age (1-3 years old) and ambient had the same diet were non-pregnant and non-lactating and blood samples were collected at same season without great variation of temperature and humidity. It is feasible to link hypoproteinemia and hypoalbuminemia with the accelerated protein loss that occurs as a result of abomasal hemorrhage caused by *H. contortus*. Initially, the fall in total serum protein that results from hemorrhage is due to hemodilution caused by compensatory reestablishment of volemia. Later, it is attributable to the loss of large amounts of serum proteins into the gut and the subsequent impaired synthesis of albumin (Sharma *et al*., 2001). An alternative explanation for hypoalbuminemia is decreased production of albumin as a result of acute phase response which is associated with elevated globulin production. Albumin is considered to be a negative acute phase protein because it decreases in concentration in response to inflammation (Murata *et al*., 2004).

The increased levels of alpha globulins could be explained by the production of acute phase proteins in response to the tissue injury and inflammation promoted by the parasite. Cytokines released from macrophages and other cell types during the acute phase response are transported to the liver where they induce hepatic synthesis of acute phase proteins such as serum amyloid A and haptoglobin (Petersen *et al*., 2004; Lomborg *et al*., 2008). The increase in the level of γ-2 globulins may be attributed to production of Immunoglobulins (Ig) directed against Haemonchus. In fact, the response of the infected animal to gastrointestinal nematodes involves production of parasite-specific IgA, IgG1 and IgE (Gill *et al*., 1993, 2000; Strain and Stear, 2001; Amarante *et al*., 2005; Bambou *et al*., 2008).

Disturbances in serum proteins in goats infected with *H. contortus* may be attributed to the host response to the parasite. The inflammatory response in goats infected with *H. contortus* has been described in detail in several studies (Al-Zubaidy *et al*., 1987; Perez *et al*., 2001, 2003, 2008). In infected goats, a rapid recruitment of lymphocytes to the abomasal mucosa (Al-Zubaidy *et al*., 1987) and the abomasal lymph nodes (Perez *et al*., 2008) was observed. Mast cells and eosinophils also increased rapidly in the abomasal mucosa (Perez *et al*., 2001, 2003). This cellular infiltrate may be responsible for the strong humoral response and the release of a number of pro-inflammatory cytokines.

CONCLUSION

In this study, the infection of goats by *H. contortus* promotes severe hypoproteinemia, hypoalbuminemia, increased alpha and gamma-2 globulins and decreased A/G ratio.

REFERENCES

Ahmad, A., C. Anwar-ul-Hassan, A. Anwar-ul-Haq and M.A. Majeed, 1990. Serum proteinogram of lambs experimentally induced *Haemonchus contortus* infection. Veterinarsk, 60: 195-200.

Al-Zubaidy, A.J., K.I. Altaif, H.H. Al-Qaisy and T.A. Makkawi, 1987. Gross pathology and histopathology of haemonchosis in sheep and goats in Iraq. Vet. Parasitol., 23: 249-256.

Amarante, A.F., P.A. Bricarello, J.F. Huntley, L.P. Mazzolin and J.C. Gomes, 2005. Relationship of abomasal histology and parasite-specific immunoglobulin A with the resistance to *Haemonchus contortus* infection in three breeds of sheep. Vet. Parasitol., 128: 99-107.

Bambou, J.C., C. Chevrotière, H. Varo, R. Arquet, F.N.J. Kooyman and N. Mandonnet, 2008. Serum antibody responses in Creole kids experimentally infected with *Haemonchus contortus*. Vet. Parasitol., 158: 311-318.

Eckersall, P.D., 2008. Proteins, Proteomics and the Dysproteinemias. In: Clinical Biochemistry of Domestic Animals, Kaneko J.J., J.W. Harvey and M.L. Bruss (Eds.). 6th Edn., Academic Press, San Diego, pp: 117-155.

Fernández, S.Y., E.E.V. Jesus, B.J.A. Paule, R.S. Uzeda, M.A.O. Almeida and J.E. Guimaraes, 2006. Proteinograma de caprinos da raça Pardo-Alpina infectados naturalmente por parasitos gastrintestinais. Arq. Bras. Med. Vet. Zootec., 58: 279-282.

Gill, H.S., G.D. Gray, D.L. Watson and A.J. Husband, 1993. Isotypespecific antibody responses to *Haemonchus contortus* in genetically resistant sheep. Parasite Immunol., 15: 61-67.

Gill, H.S., K. Altmann, M.L. Cross and A.J. Husband, 2000. Induction of T helper 1- and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. Immunology, 99: 458-463.

Lawal, I.A., K.A.N. Esievo, M. Bisalla and N.D.G. Ibrahim, 2007. Serum proteins, thyroid hormones and alkaline phosphatase concentrationsin acute experimental *Trypanosoma congolense* infection in yankasa sheep immunomodulated with levamisole. J. Anim. Vet. Adv., 6: 586-590.

Lomborg, S.R., L.R. Nielsen, P.M.H. Heegaard and S. Jacobsen, 2008. Acute phase proteins in cattle after exposure to complex stress. Vet. Res. Commun., 32: 575-582.

Murata, H., N. Shimada and M. Yoshika, 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. Vet. J., 168: 28-40.

Nematollahi, A., A. Eslami and S.H. Hosseini, 2007. Experimental study on the causative agents of hypobios is of *Haemonchus contortus* in sheep in Iran. J. Anim. Vet. Adv., 6: 458-460.

Ogundola, A.F., A.O. Ige, B.O. Oyebanji, O.S. Adedeji, R.A. Joshua, S.A. Ameen and T.A. Rafiu, 2006. Experimental studies on gastro-intestinal nematode infection: Clinical observations and haematological changes following *Haemonchus contortus* infection in West African Dwarf (WAD) kids. J. Anim. Vet. Adv., 5: 511-514.

Okaiyeto, S.O., O.J. Ajanusi, A.K. Sackey and L.B. Tekdek, 2010. Changes in some haematological values associated with mixed *Trypanosoma congolense* and *Haemonchus contortus* infection in Yankassa sheep. Vet. Res., 3: 9-13.

Oliveira, A.R. and A.M. Penha, 1978. Alteracoes do proteinograma eletroforético de bezerros com helmintose experimental. Arq. Inst. Biol., 45: 191-196.

Osman, A.Y., A.D. Abakar and A.E. Alamin, 2004. Haematological and biochemical changes following concurrent infections with *Coccidia* and *Haemonchus contortus* in desert lambs. J. Anim. Vet. Adv., 3: 643-647.

Petersen, H.H., J.P. Nielsen and P.M.H. Heegard, 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res., 35: 163-187.

Pérez, J., P.M. Garcia, S. Hernández, A. Martinez-Moreno, J.M. de las Mulas and S. Cámara, 2001. Pathological and immunohistochemical study of the abomasum and abomasal lymph nodes in goats experimentally infected with *Haemonchus contortus*. Vet. Res., 32: 463-473.

Pérez, J., P.M. Garcíaa, S. Hernándezb, E. Mozosa, S. Cámarab and A. Martínez-Moreno, 2003. Experimental haemonchosis in goats: Effects of single and multiple infections in the host response. Vet. Parasitol., 111: 333-342.

Pérez, J., R. Zafra, L. Buffoni, S. Hernández, S. Cámara and A. Martínez-Moreno, 2008. Cellular phenotypes in the abomasal mucosa and abomasal lymph nodes of goats infected with *Haemonchus contortus*. J. Comp. Pathol., 138: 102-107.

Santarosa, K.T., R.C.R.E Silva, J.B.A. Silva and B. Soto-Blanco, 2005. Valores de referência para o perfil eletroforético de proteínas séricas em cabras. Arch. Vet. Sci., 10: 46-48.

Shaffer, L., J.D. Roussel and K.L. Koonce, 1981. Effects of age, temperature-season and breed on blood characteristics of dairy cattle. J. Dairy Sci., 64: 62-70.

Sharma, D.K., P.P.S. Chauhan and R.D. Agrawal, 2001. Changes in the levels of serum enzymens and total protein during experimental haemonchosis in Barbari goats. Small Rumin. Res., 42: 119-123.

Strain, S.A.J. and M.J. Stear, 2001. The influence of protein supplementation on the immune response to *Haemonchus contortus*. Parasite Immunol., 23: 527-531.

Thomas, J.S., 2006. Overview of Plasma Proteins. In: Schalm's Veterinary Hematology, Feldman, B.F., J.G. Zinkl and N.C. Jain (Eds.). 5th Edn., Blackwell Publishing, Ames, pp: 891-898.

Ueno, H. and P.C. Gonçalves, 1998. Manual Para Diagnóstico das Helmintoses de Ruminantes. JICA, Tokyo, Japan.

Zajac, A.M., 2006. Gastrointestinal nematodes of small ruminants: Life cycles, anthelmintics and diagnosis. Vet. Clin. N. Am. Food Anim. Practice, 22: 529-541.