

THE ELECTROPHORETIC PATTERN OF SERUM PROTEINS IN SHEEP WITH NATURALLY ACQUIRED GASTROINTESTINAL NEMATODE INFECTIONS

NAGY Oskar, TÓTHOVÁ Csilla*, KLEIN Róbert, CHOVANOVÁ Frederika

Clinic of Ruminants, University of Veterinary Medicine and Pharmacy, Košice, Slovak Republic

(Received 24 October 2019, Accepted 11 June 2020)

The objective of this study was to describe the serum protein pattern in sheep with naturally acquired gastrointestinal parasitosis and to compare the distribution of protein fractions with the results obtained in animals with negative fecal examination results. Fecal and blood samples were taken from twenty-nine sheep positive for nematode eggs and twenty-four animals with negative results of fecal examination. Between the evaluated groups of sheep significant differences were noted in the relative mean values for all protein fractions and for albumin/globulin ratio ($p < 0.01$ and $p < 0.001$). The concentrations of total proteins showed no significant differences between both groups of sheep. The absolute mean values of albumin, α_2 -globulins and A/G ratio were significantly lower, the mean concentrations of α_1 -, β -, γ_1 - and γ_2 -globulins significantly higher in the nematode positive group of sheep ($p < 0.01$ and $p < 0.001$). In the nematode positive group the protein electrophoretic pattern showed a double α_2 -zone in three sheep and the γ -globulin zones were characterized by a diffuse higher broad and wide peaks. The presented results indicate that the gastrointestinal parasitic infections in sheep alter the distribution of serum proteins, and suggest their usefulness in animals with unchanged serum protein concentrations. The study brings new findings and extends the knowledge about the metabolic responses and consequences of gastrointestinal parasitic infections in sheep, particularly with regard to alterations in protein metabolism.

Key words: electrophoresis, gastrointestinal parasites, nematodes, serum proteins, sheep

INTRODUCTION

Gastrointestinal diseases caused by parasitic infections have been reported as one of the major challenges for small ruminant industry worldwide causing health disorders, as well as great production losses through decreased live-weight gain, wool and milk production and even mortality in severe cases [1-3]. The infestation is clinically manifested by enteritis, changes in the absorption of some vitamins and

*Corresponding author: e-mail: csilla.tothova@uvlf.sk

minerals, loss of nutrients, anemia, dehydration, reduced wool growth and feed intake resulting in decreased body weight, emaciation or even death [4,5]. Furthermore, the permanent irritation and damage caused by parasites to the gastrointestinal mucosa may be responsible also for the leakage of blood proteins and their consequent loss, which in cases of severe damage may result in lower concentrations of proteins in the blood [6,7]. Although several studies have been conducted to describe the impact of gastrointestinal parasitosis on some hematological and biochemical parameters [8,9], regarding the protein profile, these studies were oriented predominantly to the evaluation of total serum protein and albumin concentrations, while globulins were simply calculated subtracting the albumin values from total proteins. On the other hand, the abnormalities found in the biochemical profile are related to the type of parasite, degree of damage caused by the parasite, as well as to the severity of the parasitic infection [10,11]. Gastrointestinal parasitoses must not always be associated with abnormal total serum protein concentrations (according to the degree of damage, stage of the disease), but alterations may be observed in the distribution of serum protein fractions or some individual specific serum proteins. Protein electrophoresis is recommended to accurately determine abnormalities in the distribution of protein fractions and quantify several globulin fractions [12]. Even in cases with unchanged total serum protein concentrations, this laboratory technique may be useful to detect possible abnormalities in the serum protein pattern [13]. The effect of gastrointestinal parasitic infections on the protein profile was determined by Fernandez et al. [14] in goats. In sheep, the alterations in the serum protein profile were presented only in experimental *Haemonchus contortus* infections [15,16], while in other helminthic infections including natural gastrointestinal nematode infections are poorly documented. Under field conditions, the most of gastrointestinal nematode infections are mixed caused by several different nematode species [17]. Thus, the aim of the present study was to evaluate how gastrointestinal disorders due to naturally acquired infections by various endoparasites in sheep affect the electrophoretic pattern of serum proteins, especially the distribution of globulin fractions under these conditions, as well as to compare the possible alterations with values obtained in animals with negative results of fecal examination.

MATERIAL AND METHODS

Ethical approval

This study was based on fecal and blood sample collection. The samples were collected as per standard sampling procedure without any harm to the animals. All procedures with the animals in the study were conducted in accordance with national/ or institutional guidelines for the care and use of animals and the ethical standards of the institution or practice. The approval from Institutional Animal Ethics Committee was not required, the study did not affect the normal animal physiology.

Animals and sample collection

Sheep of the crossbreed Merino and Improved Valachian breed at the age of 2 – 4 years from a commercial sheep farm with a history of natural gastrointestinal parasitic infections were included into the study. Based on the results of coprological examination of fecal samples for the presence of gastrointestinal nematode eggs the animals were divided into two experimental groups. Fecal samples from twenty-nine evaluated sheep in the first group (group P+) were positive for several types of nematode eggs. Their average body weight was 41.3 ± 3.5 kg and the mean body condition score was 2.1 ± 0.2 . Fecal samples from twenty-four sheep in the second group (group P-) were free from gastrointestinal nematode eggs. Their average body weight was 48.3 ± 4.7 kg and the mean body condition score was 3.0 ± 0.2 . Body condition score (BCS) evaluation was used according to description of BCS technique by Kenyon et al. [18]. All animals were kept under the traditional extensive management system and had the same rearing conditions. On the farm 250 sheep were kept and irregularly dewormed using an ivermectin-based injection product. Examinations and collections of feces and blood were carried out in the spring before the sheep were driven to pasture, where they are then grazed until autumn on native grasses and shrubs. The animals were housed during the winter time in the stable on the farm on deep bedding and the feeding was uniform. They had free access to meadow hay and drinking water, concentrate was provided once a day. At the time of sampling, lambs were already weaned from the sheep and the ewes were milked twice a day.

Fecal samples were collected directly from the rectum using plastic gloves and put into plastic fecal containers. The samples were labelled and kept at 4°C until taken to the laboratory. Examination of the samples was done within a day. Blood samples for biochemical analyses were obtained from the *v. jugularis* into serum gel blood collection tubes with clotting activator (Meus, Piove di Sacco, Italy). The blood samples were allowed to coagulate at room temperature, and then were centrifuged at 3000 g for 20 minutes to separate the serum fraction from the clot. The harvested serum was aliquoted into plastic tubes, which were stored at -20 °C until the assay.

Laboratory analyses

Fecal samples were examined for the detection of the presence of nematode helminth eggs and the evaluation of the incidence of nematode infection by standard direct flotation technique using flotation solution based on saturated sodium chloride with specific gravity 1.20 according to the method as described by Taylor et al. [19]. Using microscopic analysis, the eggs of parasites present were identified from their morphological features using standard parasitological criteria [20] and a mixed infection with gastrointestinal nematodes, as well as coccidia was detected. The nematodes detected included the following type of eggs: *Strongylida*, *Strongyloides*, *Nematodirus*, *Trichuris* and *Eimeria*. Sedimentation for the examination of trematode eggs was also used with negative results. To evaluate the changes in the protein

profile, serum samples were analysed for the concentrations of total proteins and main protein fractions. The total serum protein concentrations were determined by an automated chemistry analyser Alizé (Lisabio, Pouilly en Auxois, France) according to the biuret method using commercially available diagnostic kits (Randox, Crumlin, United Kingdom). The electrophoretic pattern of serum proteins was analysed by zone electrophoresis on an agarose gel using an automated electrophoresis system Hydrasys (Sebia Corporate, Lisses, Evry Cedex, France) with commercial diagnostic kits Hydragel 7 Proteine (Sebia Corporate, Lisses, Evry Cedex, France) according to the procedure described by the manufacturer. After electrophoresis, the stained gels were scanned using the densitometry optical scanning system Epson Perfection V700 (Epson America Inc., USA) and evaluated according to the principles of light transmission through the stained gel and conversion into an optical density curve. The computer software programme Phoresis 5.50 (Sebia Corporate, France) was used to visualize the bands as peaks (electrophoretogram). The protein fractions identified on the electrophoretograms were: albumin, α_1 - and α_2 -globulins, β -globulins, and γ_1 - and γ_2 -globulins. According to the obtained optical density, the area under each peak was evaluated and the relative concentrations (%) of individual zones were calculated as percent of the total serum proteins. Consequently, the absolute concentrations (g/l) of each band were derived from percent and quantified from the total serum protein concentrations. The ratios of albumin to globulins (A/G) were also calculated.

Statistical analysis

The analysis of the data was performed using a statistical software programme GraphPad Prism V5.02 (GraphPad Software Inc., California, USA). Arithmetic means (\bar{x}) and standard deviations (SD) were determined by descriptive statistical methods for each evaluated variable and group of sheep. The distribution of data was evaluated by Kolmogorov-Smirnov test for normality, which showed a non-parametric distribution of the most of the obtained results. The Mann-Whitney test was applied to statistically analyse the differences between the results obtained in the two groups of sheep, with the level of statistical significance set at $p < 0.05$.

RESULTS

Tables 1 and 2 display the mean values, standard deviations and the statistical analysis of differences between the evaluated groups of sheep. Representative examples of the serum protein electrophoretic pattern from both groups of sheep are shown in Figure 1a-c.

Sheep infected with gastrointestinal parasites had significantly and about 10% lower relative concentrations of albumin than negative animals (Table 1, $p < 0.001$). Significantly higher α_1 -globulin was observed in infected sheep ($p < 0.01$), while the relative concentrations of α_2 -globulins were significantly lower compared to

parasitologically negative animals ($p < 0.001$). Sheep with endoparasite eggs were found to have significantly higher relative mean values of β -, γ_1 - and γ_2 -globulins ($p < 0.001$), when compared to those obtained in nematode eggs free animals. The γ -globulin pattern were in the eggs positive group of sheep characterized by higher values observable as diffuse broad peaks on the electrophoretogram (Figure 1a-b). A double α_2 -zone was observable in three sheep with gastrointestinal parasites (Figure 1c). The A/G ratio was significantly lower in the group of infected animals than in the negative group of sheep ($p < 0.001$).

Table 1. Differences in the relative concentrations of serum protein fractions (%) and albumin/globulin ratio (A/G) between the evaluated groups of sheep (mean \pm SD)

Variables	Groups of sheep		p value
	p+ (n=29)	p- (n=24)	
Albumin	39.4 \pm 5.8	49.3 \pm 5.3	< 0.001
α_1 -globulins	7.1 \pm 1.3	6.1 \pm 0.9	< 0.01
α_2 -globulins	11.7 \pm 1.9	13.5 \pm 1.5	< 0.001
β -globulins	6.2 \pm 1.5	4.3 \pm 1.8	< 0.001
γ_1 -globulins	28.1 \pm 5.7	22.2 \pm 3.8	< 0.001
γ_2 -globulins	7.5 \pm 3.3	5.7 \pm 5.7	< 0.001
A/G	0.66 \pm 0.17	0.99 \pm 0.20	< 0.001

p value – significance of the mean differences, A/G – albumin/globulin ratio

Table 2. Differences in the concentrations of total serum proteins (TP, g/l) and absolute values of protein fractions (g/l) between the evaluated groups of sheep (mean \pm SD)

Variables	Groups of sheep		p value
	p+ (n=29)	p- (n=24)	
TP	66.7 \pm 6.4	64.1 \pm 4.0	0.5027
Albumin	26.2 \pm 3.6	32.3 \pm 2.9	< 0.001
α_1 -globulins	4.7 \pm 0.9	4.0 \pm 0.7	< 0.01
α_2 -globulins	7.8 \pm 1.3	8.8 \pm 1.1	< 0.01
β -globulins	4.1 \pm 1.1	2.8 \pm 1.2	< 0.001
γ_1 -globulins	18.9 \pm 4.7	14.7 \pm 3.3	< 0.001
γ_2 -globulins	5.0 \pm 2.4	3.0 \pm 1.7	< 0.001

p value – significance of the mean differences, TP – total proteins

No significant differences were found in the mean concentrations of total serum proteins between both groups of sheep. The analyses of the absolute concentrations of protein fractions showed in the parasitologically positive sheep significantly lower

mean values of albumin and α_2 -globulins ($p < 0.001$ and $p < 0.01$, respectively). On the other hand, significantly higher mean concentrations of α_1 - ($p < 0.01$), β - ($p < 0.001$), γ_1 - ($p < 0.001$) and γ_2 -globulins ($p < 0.001$) were found in sheep with gastrointestinal parasites than in eggs negative animals.

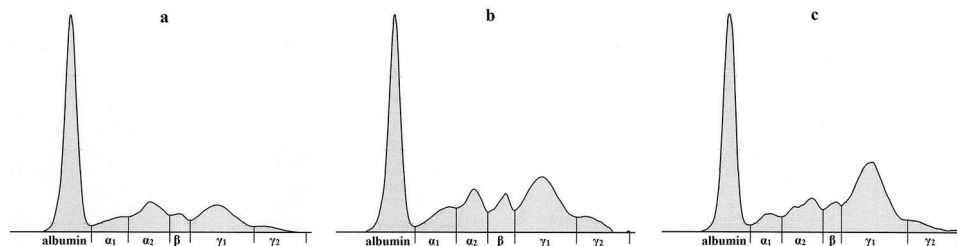


Figure 1. Representative electrophoretograms in the evaluated sheep: **a)** negative for nematode eggs, **b)** positive for nematode eggs, **c)** positive sheep with double α_2 zone

DISCUSSION

Natural infections with a range of gastrointestinal nematodes belong to common disorders in small ruminants that may cause important metabolic changes and alterations from normal physiology also in the serum biochemistry of the infected animals, including the protein profile [11]. Earlier studies dealing with the evaluation of the effect of gastrointestinal parasitosis on the biochemical profile reported a significant decrease of total serum protein concentrations in infected sheep [3,21,22]. According to Coop and Holmes [23], the main cause of this is the increased endogenous loss of proteins into the gastrointestinal tract due to the damaged intestinal mucosa, as well as the movement of proteins from productive processes into the repair of the gastrointestinal tract and mucoprotein production. Furthermore, the protein deficiency observed in sheep with gastrointestinal parasitic infections may be related to the increased demand for amino acids in the alimentary tract, while their supply is reduced through the depression of appetite and chronic reduction in feed intake [24,25]. On the other hand, the results of the present study showed no marked differences in the total serum protein concentrations between both groups of sheep. Some researchers stated that the impact of nematode infections on the metabolic reactions of the animals is dependent on the intensity and severity of infection, as well as the physiological, nutritional and immunological status of the infected animal [17,26]. Similarly, Coop and Kyriazakis [27] and Hoste et al. [28] reported that the nutritional status of the animal influences the ability of the host to respond against parasite development, and despite the reduced absorptive area due to the loss of mature villi, the host is able to compensate to some extent for impaired metabolic processes, digestion and absorption. Despite the non significant differences in total protein concentrations,

further analyses of the blood serum protein profile showed marked alterations and differences in the distribution of protein fractions.

In the sheep positive for gastrointestinal nematode eggs, significantly lower albumin concentrations were found compared to the eggs negative group, most probably caused by its leakage into the intestine. Similar findings have been presented by Pandey et al. [6] and Tiwari et al. [8] in sheep affected by gastrointestinal parasites. Albumin has a lower molecular weight than the most of globulins and smaller osmotic sensitivity to fluid movement, thus can be selectively lost through the damaged intestinal mucosa [29,30]. Furthermore, Tanwar and Mishra [31] stated that hypoalbuminemia in intestinal parasitism may be aggravated by the consequently increased fractional catabolic rate of albumin. On the other hand, albumin is the major negative acute phase protein with markedly reduced synthesis during the acute phase response, seeing that the majority of amino acids are used mainly for the synthesis of positive acute phase proteins [32,33]. Gastrointestinal parasites usually cause not only local inflammation of the intestine and damage to the mucosal structures, but also induce general metabolic disturbances and inflammatory reactions, including the acute phase response. The markedly increased synthesis of positive acute phase proteins belongs to the most important reactions occurring during the acute phase response [34]. Many important acute phase proteins can be found in the α -globulin fraction [35], thus the increased production of some of them (e.g. α_1 -antitrypsin, α_1 -acid glycoprotein, serum amyloid A) caused by the damage, tissue injury and inflammation due to gastrointestinal parasites might result in increased concentrations of α_1 -globulins observed in our study in infected sheep. Markedly increased α -globulin values have been determined by Diogenes et al. [36] in goats infected with *Haemonchus contortus*, which was related to the the inflammatory reactions caused by the infection with endoparasites. The alterations in the distribution of α -globulins in sheep affected by gastrointestinal parasites are not well described. In the present study, the concentrations of α_2 -globulins in sheep with endoparasites were comparable to those presented by Nagy et al. [37] in healthy sheep. This fraction includes further acute phase proteins such as haptoglobin, α_2 -macroglobulin, as well as ceruloplasmin [38]. It seems that natural infections with gastrointestinal nematodes in sheep did not evoke a sufficient inflammatory response to give a more marked increase in the concentrations of proteins from this fraction, and thus the whole α_2 -globulin zone. It was reported also by Ganheim et al. [39] that the serum concentrations of haptoglobin did not increase during common gastrointestinal nematode infections in sheep. On the other hand, Zhong et al. [40] found a 10 – 30 fold increase of haptoglobin values in sheep experimentally infected with *Haemonchus contortus*, while this increase exhibited two peaks, the first occurred on day 3 and the second on day 28 after the infection. Furthermore, a double α_2 -zone was observable in our study in three sheep with gastrointestinal parasites. A split α_2 -peak was found by Moore and Avery [41] in an 8-year old spayed female Scottish Terrier with megakaryocytic leukemia manifesting as anemia, thrombocytopenia, and atypical circulating leukocytes. In veterinary medicine, this pattern was usually presented as of unknown significance, but in human medicine

the split of α_2 -zone may be interpreted as the evidence of variant haptoglobin genes [42]. However, in the light of the above mentioned contradictory data, further studies are needed to correctly explain these alterations.

In sheep affected by gastrointestinal parasites, a trend of higher values was observed also for β -globulins, but the reasons for these alterations in sheep are poorly understood. Some diagnostically important proteins were identified in the β -globulin region, including transferrin, complement, as well as ferritin [43]. Higher concentrations of transferrin are usually associated with iron deficiency anemia, resulting in a negative correlation between hemoglobin and transferrin [44]. In trichostrongyloides-infected cattle and sheep, severe normocytic anemia and symptoms of the depression of erythropoiesis have been reported previously [45]. Thus, the increase in the concentrations of β -globulins might be caused by elevated production of transferrin associated with anaemia in sheep infected with gastrointestinal nematodes. The elevated production of complement belongs to another possible cause of hyper- β -globulinaemia, which may be related to the inflammatory processes induced by the infection with parasites and consequent tissue damage. The elimination of nematodes in sheep requires several events, including the activation of nonspecific defense mechanisms [46]. The activation of the complement pathway is one of the first innate responses to infections with parasitic helminths. This response is developed by the synthesis of chemotactic peptides C3a and C5a which mobilize eosinophils to the area of infection [47,48]. C-reactive protein (CRP) is another protein that belongs to the β -globulin fraction. In ruminants, it is a constitutively synthesised protein, with only a minor increase during disease processes [49]. Higher serum CRP concentrations were found in sheep in the course of *Mannheimia haemolytica* infection as part of the acute phase response [50]. The behavior of CRP in sheep infected with gastrointestinal nematodes was not yet described. Therefore, further analyses using high resolution electrophoresis or evaluations of individual serum proteins are needed to establish which proteins are responsible for a markedly higher β -globulin fraction in sheep affected by gastrointestinal parasites.

Sheep are a distinct ruminant species, in which γ -globulins do not migrate as one overall fraction, but are separated in two subfractions (γ_1 and γ_2), while the γ_1 -globulin fraction is higher compared to γ_2 -globulins. In our study, markedly higher concentrations of γ_1 , as well as γ_2 -globulins were found in sheep with gastrointestinal parasitic infections compared to negative animals, which was seen as an increased broad peak on the electrophoretogram. Diogenes *et al.* [36] recorded in goats infected with *Haemonchus contortus* significantly higher values of γ_2 -globulins than in controls. On the other hand, Tiwari *et al.* [8] observed in diarrhoeic sheep with parasitic infections a decrease of total serum globulin values. Both innate and adaptive immunities protect the host from parasitic infections. Thus, the increase in the concentrations of γ -globulins could be related to the appropriate humoral immune response to stimulation manifested by the animals against nematode infections [15]. According to Kaneko [29], the increase of γ -globulins may be attributed predominantly to the production of immunoglobulins

(Ig) directed against the invading agents, including the so called fast and slow immunoglobulins contributing to the two subfractions. Furthermore, the response of the infected animals to gastrointestinal nematodes involves also the production of parasite-specific immunoglobulins, especially IgA, IgG1 and IgE [51,52], which may be found in the γ_1 - globulins zone, as well as partially in the β - zone of globulins. These alterations presented in the concentrations of albumin and globulin fractions resulted also in changes of the A/G ratio. In those sheep which were affected by gastrointestinal parasites and in poor nutritional state we obtained significantly lower A/G ratio compared to eggs negative animals. These lower A/G values reflect the alterations associated with the infection with gastrointestinal parasites, and are caused by the selective loss of albumin through the damaged intestinal mucosa or by the overproduction of globulins due to the stimulation of the host's immune system.

CONCLUSION

In conclusion, the results of the present study suggest in sheep marked alterations in the serum protein profile and changes in the distribution of protein fraction induced by the infection with gastrointestinal parasites. These changes were characterized by lower concentrations of albumin and α_2 -globulins in the eggs positive group of sheep and higher values of α_1 -, β -, and both γ -globulin fractions compared to the parasitologically negative animals. The study brings new findings and extends the knowledge about the metabolic responses and consequences of gastrointestinal parasitic infections in sheep, particularly with regard to alterations in protein metabolism. Serum protein electrophoresis can be a useful analytical method to standard basic biochemical techniques, which analyze total protein and albumin concentrations and then only calculate the globulin values. In veterinary medicine, the description of the serum protein electrophoretic pattern is a difficult and complex issue, and the interpretation of the protein electrophoretograms requires some experience in the field. The correct interpretation of the results of protein electrophoresis is possible only in the context of the clinical picture and current health state of the evaluated animal. However, further studies are needed to establish its diagnostic significance in sheep affected also by gastrointestinal parasitic infections.

Acknowledgements

This work was supported by Scientific Grant Agency of Ministry of Education SR No. 1/0398/18 and 1/0314/20.

Authors' contributions

NO conceived and designed the study, revised the manuscript critically, has given final approval of the version to be published. TC performed the laboratory analyses, performed the statistical analyses, contributed to interpretation of the data, drafted

the manuscript. KR and CHF collected samples and contributed to data collection and analysis. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Ahmad A, Anwar-ul-Hassan C, Anwar-ul-Haq A, Majeed MA: Serum proteinogram of lambs experimentally induced *Haemonchus contortus* infection. *Vet Arhiv* 1990, 60:195-200.
2. Aldred AR, Schreiber G 1993: The negative acute phase protein. In: Mackiewicz I, Kushner I, Baumann H: *Acute phase proteins. Molecular biology, biochemistry, and clinical applications*. Boca Raton, Florida, CRC Press, 1993, 21-37.
3. Ayaz E, Ertekin A, Ozdal N, Tas Z: Some biochemical parameters in sheep infected with endoparasites (*Fasciola* sp., *Dicrocoelium dendriticum*, hydatid cysts, *Trichostrongylidae* and *Protostrongylidae*). *Acta Parasitologica Turcica* 2007, 31:57-61.
4. Bordoloi G, Jas R, Ghosh JD: Changes in the haemato-biochemical pattern due to experimentally induced haemonchosis in Sahabadi sheep. *J Parasit Dis* 2012, 36:101-105.
5. Bossuyt X: Advances in serum protein electrophoresis. *Adv Clin Chem* 2006, 42:43-80.
6. Ceron JJ, Caldin M, Martinez-Subiela S: Electrophoresis and acute phase protein measurement. In: Douglas J, Weiss K, Wardrop J: *Schalm's Veterinary Hematology*. 6th ed. Ames: Blackwell Publishing Ltd., 2010, 1157-1161.
7. Ceron JJ, Caldin M, Martinez-Subiela S: Answers to some common questions on serum protein electrophoresis. *Vet Rec* 2011, 168:453-454.
8. Clemente M, Marín L, Iazbik MC, Couto CG: Serum concentrations of IgG, IgA, and IgM in retired racing Greyhound dogs. *Vet Clin Pathol* 2010, 39:436-439.
9. Coop RL, Holmes PH: Nutrition and parasite interaction. *Int J Parasitol* 1996, 26:951-962.
10. Coop RL, Kyriazakis I: Nutrition-parasite interaction. *Vet Parasitology* 1999, 84:187-204.
11. Diogenes PVA, Suassuna ACD, Ahid SMM, Soto-Blanco B: Serum protein electrophoretic profile of goats infected with *Haemonchus contortus*. *Journal of Animal and Veterinary Advances* 2010, 9:1603-1606.
12. Douglas J, Weiss K, Wardrop J: *Schalm's Veterinary Hematology*. 6th ed. Ames: Blackwell Publishing Ltd., 2010, 1232 p.
13. Eckersall PD: Proteins, proteomics, and the dysproteinemias. In: Kaneko JJ, Harvey JW, Bruss ML: *Clinical biochemistry of domestic animals*. 6th ed. California: Academic Press, 2008, 117-155.
14. Eckersall PD, Bell R: Acute phase proteins: biomarkers of infection and inflammation. *Vet J* 2010, 185:23-27.
15. Fausto GC, Pivoto FL, Costa MM, dos Anjos Lopes ST, França RT, Molento MB, Minervino AHH, da Rocha JBT, do Rêgo Leal ML: Protein profile of lambs experimentally infected

- with *Haemonchus contortus* and supplemented with selenium and copper. *Parasit Vectors* 2014, 7:355.
16. Fernandez SY, Jesus EEV, Paule BJA, Uzeda RS, Almeida MAO, Guimaraes JE: Proteinogram of Alpine goat naturally infected by gastrointestinal parasites. *Arq Bras Med Vet Zootec* 2006, 58:279-282.
 17. Fox MT: Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. *Vet Parasitology* 1997, 72:285-308.
 18. Ganheim C, Höglund J, Waller KP: Acute phase proteins in response to *Dictyocaulus viviparus* infection in calves. *Acta Vet Scand* 2004, 45:79-86.
 19. Gill HS, Gray GD, Watson DL, Husband AJ: Isotypespecific antibody responses to *Haemonchus contortus* in genetically resistant sheep. *Parasite Immunol* 1993, 15:61-67.
 20. Hankins J: The role of albumin in fluid and electrolyte balance. *Journal of Infusion Nursing* 2006, 29:260-265.
 21. Hayat CS, Hussain SM, Iqbal Z, Hayat B, Akhtar M: Effect of parasitic nematodes on haematology and productivity of sheep. *Pak Vet J* 1996, 16, 2:81-83.
 22. Hoste H, Torres-Acosta JF, Paolini V, Aguilar-Caballero A, Etter E, Lefrileux Y, Chartier C, Broqua C: Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Res*, 2001, 60:141-151.
 23. Idris A, Moors E, Sohnrey B, Gauly M: Gastrointestinal nematode infections in German sheep. *Parasitol Res* 2012, 110:1453-1459.
 24. Jain S, Gautam V, Naseem S: Acute phase proteins: As diagnostic tool. *J Pharm Bioall Sci* 2011, 3:118-127.
 25. Jania B, Andrzejek K: Application of native agarose gel electrophoresis of serum proteins in veterinary diagnostics. *J Vet Res* 2016, 60:501-508.
 26. Jas R, Ghosh JD: Economic impact of gastrointestinal nematodosis in sheep: enhanced meat production by antihelminthic treatment. *Indian J Anim Sci* 2009, 79:3-5.
 27. Kaneko JJ: Serum proteins and the dysproteinemias. In: Kaneko JJ: *Clinical biochemistry of domestic animals*. 5th ed., Academic Press, London, United Kingdom, 1997, 117-138.
 28. Kenyon PR, Maloney SK, Blache D: Review of sheep body condition score in relation to production characteristics. *New Zeal J Agr Res* 2014, 57:38-64.
 29. Kumar S, Jakhar KK, Nehra V, Pal M: Pathomorphological and microbiological studies in sheep with special emphasis on gastrointestinal tract disorders. *Vet World* 2015, 8: 1015-1020.
 30. Kyriazakis I, Tolkamp BJ, Hutchings MR: Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Anim Behav* 1998, 56:265-274.
 31. Larsen JWA, Vizard AL, Anderson N: Production losses in Merino ewes and financial penalties caused by trichostrongylid infections during winter and spring. *Aust Vet J* 1995, 72:58-63.
 32. Maiti SK, Rao VN, Pal S, Ali SL: Clinico-haematological and therapeutic studies in parasitic gastroenteritis in sheep. *Indian Vet J* 1999, 76:435-437.
 33. Meeusen ENT, Balic A: Do eosinophils have a role in the killing of helminth parasites? *Parasitology Today* 2000, 16:95-101.
 34. Moore AR, Avery PR: Protein characterization using electrophoresis and immunofixation; a case-based review of dogs and cats. *Vet Clin Pathol* 2019, DOI:10.1111/vcp.12760.

35. Moser M, Pfister H, Bruckmaier RM, Rehage J, Blum JW1994: Blood serum transferrin concentration in cattle in various physiological states, in veal calves fed different amounts of iron, and in cattle affected by infectious and non-infectious diseases. *Zentralbl Veterinärmed A* 1994, 41:413-420.
36. Nagy O, Tóthová Cs, Nagyová V, Kováč G: Comparison of serum protein electrophoretic pattern in cows and small ruminants. *Acta Vet* 2015, 84:187-195.
37. O'Connell TX, Horita TJ, Kasravi B: Understanding and interpreting serum protein electrophoresis. *Am Fam Physician* 2005, 71:105-112.
38. Otesile EB, Fagbemi BO, Adeyemo O: The effect of *T. brucei* infection on serum biochemical parameters in boars on different planes of dietary energy. *Vet Parasitol* 1991, 40:207-216.
39. Pandey V, Aditi Khajuriya JK, Soodan JS, Sharma N, Upadhyaya SR, Katoch R: Influence of gastrointestinal parasites on certain blood components of sheep. *Indian Journal of Small Ruminants*, 2010, 16:134-136.
40. Pandit S, Jas R, Ghosh JD, Moi S: Impact of naturally occurring gastrointestinal nematodosis on serum protein concentration in Garole sheep. *Environ Ecol* 2009, 27:1526-1529.
41. Rainbird MA, Macmillan D, Meeusen ENT: Eosinophil-mediated killing of *Haemonchus contortus* larvae: effect of eosinophil activation and role of antibody, complement and interleukin-5. *Parasite Immunol* 1998, 20:93-103.
42. Sancho FV: Atlas of Ovine Parasitology. Servet editorial – Grupo Asís Biomedica, S.L., Zaragoza, España, 2013, 137 p.
43. Sharma P, Sharma D, Dogra PK, Mandial RK: Comparative efficacy of fenbendazole and oxcylozanide-tetramisole combination against gastrointestinal nematodes in naturally infected Gaddi goats. *Vet Res Int* 2014, 2: 15-17.
44. Shao S, Sun X, Chen Y, Zhan B, Zhu X: Complement evasion: An effective strategy that parasites utilize to survive in the host. *Front Microbiol* 2019, 10:532.
45. Strain SAJ, Stear MJ: The influence of protein supplementation on the immune response to *Haemonchus contortus*. *Parasite Immunol* 2001, 23:527-531.
46. Sykes AR, Coop RL: Interactions between nutrition and gastrointestinal parasitism in sheep. *New Zeal Vet J* 2001, 49:222-226.
47. Tanwar RK, Mishra S: Clinico-haemato-biochemical studies on intestinal helminthiasis in poultry. *Vet Pract* 2001, 2:137-140.
48. Taylor MA, Coop RL, Wall RL: *Veterinary parasitology*. John Wiley & Sons, Ltd, UK, 2016, 1006 p.
49. Tiwari AN, Jakhar KK, Jangir BL, Sharma V: Haemato-biochemical alterations in gastrointestinal disorders of sheep with parasitic infection. *Indian J Anim Hlth* 2017, 56:233-238.
50. Ulutas PA, Ozpinar A: Effect of Mannheimia (Pasteurella) haemolytica infection on acute-phase proteins and some mineral levels in colostrum-breast milk-fec or colostrum-breast milk-deprived sheep. *Veterinary Research Communications* 2006, 30: 485-495.
51. Zeinab KE: Effect of gastrointestinal parasites infestation on some haematological and biochemical parameters in sheep. *Alexandria Journal of Veterinary Sciences* 2018, 59:44-47.
52. Zhong R, Yang LiH, Sun H, Zhou D: Effects of supplementation with dietary green teapolyphenols on parasite resistance and acute phase protein response to *Haemonchus contortus* infection in lambs. *Veterinary Parasitology* 2014, 205:199-207.

ELEKTROFOREZOGRAM SERUMSKIH PROTEINA OVACA PRIRODNO INFICIRANIH GASTROINTESTINALNIM NEMATODAMA

NAGY Oskar, TÓTHOVÁ Csilla, KLEIN Róbert, CHOVANOVÁ Frederika

Cilj ove studije je bio da se opišu serumski protein ovaca sa prirodno stečenom gastrointestinalnom parazitozom, kao i da se uporedi distribucija frakcija proteina sa rezultatima životinja sa negativnim koprološkim nalazom. Uzorci fecesa i krvi su uzeti od 29 ovaca pozitivnih na jaja nematoda i 24 ovce sa negativnim koprološkim nalazom. Između procenjenih grupa ovaca primećene su značajne razlike u relativnim srednjim vrednostima za sve frakcije proteina i za odnos albumina / globulina ($p < 0,01$ i $p < 0,001$). Koncentracije ukupnih proteina nisu pokazale značajne razlike između obe grupe ovaca. Apsolutne srednje vrednosti albumina, α 2-globulina i A / G odnosa su značajno niže, a srednje koncentracije α 1-, β -, γ 1- i γ 2-globulina značajno su veće u grupi pozitivnih ovaca ($p < 0,01$ i $p < 0,001$). U pozitivnoj grupi, elektroforetski obrazac proteina pokazao je dvostruku α 2 zonu kod tri ovce, a γ - globulinske zone bile su okarakterisane difuznim višim i širokim vrhovima. Predstavljeni rezultati ukazuju da gastrointestinalne parazitske infekcije kod ovaca menjaju distribuciju proteina u serumu i ukazuju na njihovu korisnost kod životinja sa nepromenjenim koncentracijama proteina u serumu. Studija donosi nova otkrića i proširuje saznanja o metaboličkim reakcijama i posledicama gastrointestinalnih parazitskih infekcija kod ovaca, posebno u vezi sa promenama u metabolizmu proteina.