Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 27 (3-4)
Sofia ● 2020

Serum Trace Elements and Enzymes in Lambs with Introduced Haemonchosis

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Haemonchosis is an important parasitic infection in sheep and goats. The aim of our study was to evaluate the levels of trace zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), cobalt (Co) and ultra-trace molybdenum (Mo), selenium (Se) elements and the activity of enzymes in serum (alkaline phosphatase, alanine aminotranspherase, aspartate aminotranspherase and lactate dehydrogenase). Levels of Zn, Cu, Fe, Se, Mo and Co were decreased in the serum from infected lambs compared to control. Mn level was non-significantly higher in the serum of infected animals than non-infected ones. Serum ALP, ALT and AST activity was increased in the infected lamb compared to the controls. LDH activity was not significantly changed in the infected lambs compared with healthy lambs. In this study increased pathological marker enzymes and an imbalance in the trace elements profile was observed. *Haemonchus contortus* plays an imperative task as anemia and oxidative stressors on lambs.

Key words: haemonchosis, lamb, trace elements, enzymes, serum

Introduction

Parasitic diseases are a serious problem for livestock economy worldwide. The important nematode species of small ruminants are related to genera *Haemonchus, Ostertagia* and *Trichostrongylus*. Haemonchosis is a prevalent infection in sheep and goats [3], (**Fig.1**) Clinical signs of the infection include anemia, digestion-absorption syndromes and, in many cases the death of young animals [8]. Several factors are involved in the pathogenesis of haemonchosis and the most important of them are parasite virulence and host

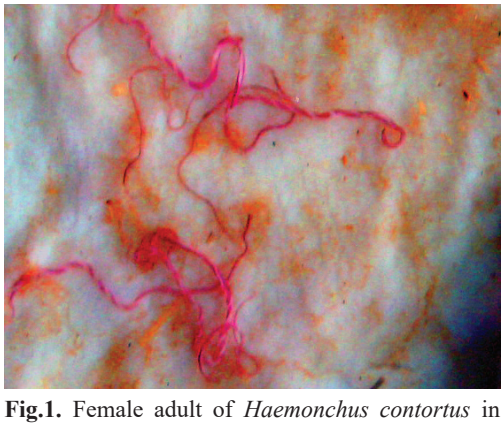

Fig.1. Female adult of *Haemonchus contortus* in abomasum of experimentally infected lamb (16×).

response [11]. Minerals are involved in various fundamental physiological processes in organism. Trace (cobalt, zinc, manganese, iron, copper) and ultra-trace (molybdenum, selenium) elements participate in different responses of infected hosts [9]. Studies have shown that improved mineral status of the animals may reduce parasite burden and improve immunity of the hosts [4,7]. Trace elements e. g. Zn, Se, Mn and Cu are necessary for the immune system and increase the resistance against parasite [8]. Copper has various functions in the organism. It acts as more than 20 metaloenzymes, metallo-proteins in organism. [13] documented that plasma Cu level decreased up to 50% in lambs with haemonchosis compared to non-infected lambs. Zinc is important to build up a successful immune response having capabilities to affect parasites [8]. Investigations indicated a positive impact of dietary Zn in infected hosts to improve immune response against gastrointestinal nematode infections [8]. Iron has presumably no direct effects on gastrointestinal parasites. However, Fe supplementation improves host performance because it restores Fe status in the organism which is lost through blood during the infection [4]. Investigations in animals infected with helminths have shown the positive effect of cobalt salt on their immune system. [7] reported that Co deficiency reduced the resistance of small ruminants against nematode infections. Cobalt is an essential component of vitamin B12. The role of molybdenum in immunity against endoparasites is well established. [10] noted decreased *H. contortus* burden in Mo- supplemented lambs. The authors observed that Mo-supplementation reduced *H. contortus* burden by 78%. Small number of data concerning selenium deficiency and infections in ruminants are noted. [12] reported a reduced burden of *H. contortus* in lambs supplemented with Se. The use of Se may provide better antioxidant defense system in lambs infected with *H. contortus* [9].

Levels of pathological marker enzymes and their interaction with trace elements are not study enough. [16] reported non-significant changes in the serum enzymes ALT, ALP and AST in the infected sheep with *H. contortus*. According [2] the levels of the same enzymes were elevated in the infected lambs and goats.

The aim of the study was to evaluate the level of trace (Zn, Mn, Cu, Fe, Co) and ultra-trace (Mo, Se) elements as well as the activity of enzymes in serum of lambs experimentally infected with *Haemonchus contortus.*

Material and Methods

For this study were used 16 male 6-month-old lambs of the Black head Pleven breed with a middle weight 26-27 kg. The lambs were divided into two groups (eight lambs in a group): Group 2 – lambs infected with *Haemonchus contortus* larvae and Group 1 – controls. Before experimental infection animals were kept in collective pens located in the vivarium of the Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS, for a month for adaptation to the diet and to the experimental environment. During this period the lambs were treated with antihelmintics – combination of closantel and albendazole. The first group was infected two times every two days with 1 800 *H. contortus* larvae (L3) per a lamb. The Baermann technique was used to extract the L3 larval stages of *H. contortus* intestinal nematodes and counted under a dissecting microscope to determine the larval counts by [17]. The samples

were taken 58 days after infection. Blood samples were collected from the jugular vein in vacutainers. Serum was studied for trace element levels. The study was conducted in compliance with the requirements of the European Convention for the protection of Vertebrate animals used for experimental and other specific purposes and current Bulgarian laws and regulations. All procedures for animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences. (Permit number: 96, 22.05.2014).

The levels of Zn, Cu, Mn, Mo, Co and Fe were determined with an atomic absorption spectrophotometer (PYE UNICAM sp. 2900, PYE UNICAM). Selenium level was determined fluorimetrically.

The statistical analysis was carried out on the Prism 6 program. The distribution of data was determined – a Gaussian one (normal for all values). The determination of the distribution was performed using the test of Kolmogorov-Smirnov and D`Agostino-Pearson. In the Grubb test application no extreme value have been found (they are strongly differing from the mean one, usually neglible). Variation analysis was used for determining the mean values, the standard deviation (SD) and the significance criterion (P). The comparison of the mean values of parameters was carried out using the one-way analysis of variance, Dunnett's Multiple Comparison Test. The results from these comparisons were also statistically significant: * ($p \leq 0.05$), ** ($p \leq 0.001$), *** ($p \leq 0.0001$).

Serum levels of alkaline phosphatase (ALP), alanine aminotranspherase (ALT), aspartate aminotranspherase (AST) and lactate dehydrogenase (LDH) were determined using a semi-auto chemistry analyzer BA-88 A (Mindray, China).

Results and Discussion

Serum enzyme activities: Serum ALP activity was increased in the infected lamb compared to the healthy ones by 40 %. Activity of ALT and AST was higher in the infected host than controls by 21-22 %. LDH activity was not significantly changed in the infected lambs compared with healthy ones (**Table 1**).

Table 1. Enzymes activity in serum of lambs IU/L

	ALP	ALT	AST	LDH
Control	155,40 ±10,16	28,70 ±2,18	120,49 ±3,50	271,40 ±19,50
Infected	216,79 ±18,44	34,80 ±9,62	146,93 ±28,11	265,33 ±29,40

Trace element levels: Zinc serum level was significantly reduced in the infected animals compared with controls. Copper level was lower in the infected lambs compared with non-infected ones too. Higher Manganese level was observed in the infected lambs than the controls but non-significantly. Serum cobalt level was reduced in the infected animals compared to the normal range. Iron serum level was significantly decreased in the infected animals than in the controls (**Table 2**).

Ultra trace elements: Mo and Se were significantly reduced in the infected lambs compared to controls (**Table 2**).

Table 2. Serum trace elements level mg/L

	Zn	Cu	Mn	Co	Mo	Se	Fe
Control lambs	0,97 ±0,04	0,83 ±0,05	0,22 ±0,06	0,09 ±0,0	0,011 ± 0,00	0,22 ±0,01	1,94 ±0,09
Infected lambs	0,46 ±0,01	0,70 ±0,06	0,28 ±0,01	0,02 ±0,0	0,002 ±0,00	0,13 ±0,07	0,97 ± 0,11

In this study increased pathological marker enzymes and an imbalance in the trace elements profile was observed. The elevation of serum ALP, ALT and AST level indicated some disruptive activities in organs of the origin or of altered membrane permeability. Their level could also rise due to lack of excretion or decrease due to impaired synthesis [16]. Ahmat and Ansari [2] reported a significant rise in serum ALP in sheep and goats with haemonchosis. The rise in enzyme activity might be attributed to the damage to abomasal mucosa by the parasites similar to that described by Alam et al. [3]. Specific hepatic function is affected by a wide variety of the pathological conditions of extrahepatic origin especially gastrointestinal origin. Similar findings were reported by Ahmat and Ansari [2] that the increase in enzymatic activities reflect the cellular abnormalities directly related to damage occurred in the hepatocytes and pathological lesions of intestines. As ALP is widely distributed in the body and found in high concentrations in intestinal mucosa, liver, increase of the ALP value is suggestive to damage to mucosal cells of intestine due to parasite pathogenesis in gastrointestinal disorders. Our results for serum enzymes in the infected with *H. contortus* lambs are in good agreements with those of Ahmat and Ansari [2]. Similar data for elevated levels of the enzymes ALP, AST and ALT are observed by Sharma et al. [16] in goats. Analysis of serum is an effective method to analyze trace elements profile of animals.

Trace elements are important for optimal immunity and disease resistance in infected animals [8]. Metabolism of trace elements was seriously affected by the haemonchosis [3]. Their role as cofactors of certain enzymes has been recognized by Kojouri et al. [8]. Most of them act as antioxidants [8] as like Zn, Fe, Cu, Se. Zinc is a part of several enzymes. The active sites of most anhydrases contain Zn. This biogenic element also is required for the regulation of genes that are required for signal transduction, response to stress, growth and energy utilization [9]. The enzyme superoxide dismutase contains zinc, which is necessary to protect cells from superoxide radicals and participates in the antioxidant defense system [3]. Zn serum level was reduced in the infected animals compared with controls. The present Zn level is similar to that established by Kojouri et al. [8]. Zn concentration falls in a variety of disease associated with the anorexia [9]. Reduced Zn content could be associated with increased adult worms using the host Zn ions for their own metabolism.

Copper is an essential element for the synthesis of various enzymes, including cytochrome-c-oxidase, superoxide dismutase, ceruloplasmin. Clinical signs related to Cu-deficiency are anemia, severe diarrhea, fragile bones and depressed growth. Cu level was lower in the infected lambs compared than the non-infected ones. Reduced hydrolysis in the abomasum and proximal regions of the intestine when pH is elevated

by nematodes may contribute to reduced Cu availability and absorption [9]. Due to the biologically significant homeostatic relationship between Zn and Cu, both elements have been used as an indicator of severity of disease [16].

Manganese participates in the metabolism of lipids and carbohydrates. Manganese is a cofactor of the superoxide dismutase (MnSOD). Higher Mn level in the infected lambs observed by us is parallel to the observations by Ahmad et al. [1]. It is in a good agreement with our data for increased Mn-content in the lamb.

Cobalt is an essential component of vitamin B12, which is a cofactor for the enzymes methionine synthase and methylmalonyl Coenzyme A mutase [1]. Similar reduced serum Co content in our study was noted by Kojouri et al. [8]. Cobalt deficiency leads to developed of anemia [12].

Molybdenum is an essential component of the enzyme xanthine oxidase catalyzing the oxidation of hypoxanthine to xanthine and of sulfite oxidase which is required for the metabolism of sulfur-containing amino acids. Mo content in serum from infected animals was reduced compared to the control content. Molybdenum is an important co-factor in the integral complex that is important in weight gain [1].

Iron is an important element of hemoglobin and myoglobin, and is an essential component of cytochrome, catalase and peroxidase [4]. Deficiency of iron in ruminants leads to anemia, reduced food intake and chronic blood loss during parasitic infections [1]. The reduction of serum Fe level could be attributed to the expended erythropoiesis to compensate for blood loss leading to depression of Fe stores [4]. Serum deficiency of Fe observed by us confirms the data by Murad et al. [12]. Decreases in blood Fe were correlated to elevations in ALT observed by us now and by [4, 15].

Selenium is a component of glutathione peroxidase which plays an important role in the metabolism of lipid hydroperoxides. Selenium is a component of thioredoxin reductase which regulates transcription, recycles vitamin C and E, and absorbs calcium [8]. Serum Se level was reduced in the infected lambs compared to non-infected hosts. It is in a good agreement with the lowed activity of glutathione peroxidase [13]. Se deficiency leads to compromised immune function, weight loss and diarrhea.

H. contortus has serious effect on serum biochemistry and enzymatic assays like ALT, ALP and AST as well as on biogenic elements in serum. Interactions between reduced enzyme activity of ALT and a decrease in Zn serum level have been associated with a variety of liver diseases [16]. Zinc level is a significant predictor of ALT level [2]. Animals with high serum ALT were more likely to have lower Zn compared with normal level.

Data in the present study, on trace elements levels and enzymes activity changes, caused by haemonchosis in lambs carry importance as they may indicate the extent of damage to the abomasal mucosa and thereby help in better understanding of the pathogenesis of anemia especially in the absence of other possible factors which may influence those changes. *H. contortus* manipulates the lamb gastrointestinal microbiome, modifying the balance between host and gastric microbiota [5]. Our data evidence the role of trace- and ultra- trace minerals in these mechanisms. It can be concluded that *Haemonchus contortus* plays an imperative task as an anemia and oxidative stressors on lambs.

Conclusion

In generally accepted that trace elements have beneficial effect for controlling and prevention of gastrointestinal parasites in animals. There is a need to identify important trace elements needed for mucosal immunity function and whose imbalance and deficiency act as a predisposing factor for susceptibility to gastrointestinal parasitism in small ruminants.

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