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RESEARCH ARTICLE

Combined Effect of Monieziosis and Hypomicroelementosis on Some Hematological, Biochemical and Hormonal Parameters in Merino Sheep

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A B S T R A C T

This study was done to determine the impact of monieziosis infection combined with hypomicroelementosis on some hematological, biochemical and hormonal parameters of Soviet Merino sheep in the Astrakhan region. 20 sheep, aging 3 years old and average 43±1.6 kg body weight were used. Sheep were divided into two groups. First group contained 10 sheep and these sheep were naturally infected with monieziosis and clinically were suffered from hypomicroelementosis, emaciation, reduced growth rate, anemia, diarrhea and pale mucosa. Second group contained 10 sheep were apparently healthy and free from internal parasites and they were used as a control group. The first group was given Praziver (praziquantel and ivermectin) for treatment monieziosis, while they intramuscularly injected with Sedimin (selenium, iodine and iron) and were introduced daily into the feed with $CoCl_2$.
Faecal and blood samples from both groups were collected, before and 30 days after treatment, and analyzed for some hematological, biochemical and hormonal parameters. Our results revealed that there was a significant decrease in Hb and RBCs values, while total WBCs and eosinophils were significantly increased in the diseased group than in healthy one. Biochemical analysis showed a significant decrease in serum antioxidant enzymes (CAT, SOD and GSH-Px) and a significant increase in serum DC and MDA in the diseased group as compared with the control group. Hormonal analysis showed a significant increase in ACTH, TSH, Cortisol and a significant decrease in serum T4 and T3. After treatment with Praziver, Sedemin and $CoCl_2$, there was a significant effectiveness to maintain blood parameters within normal levels in the experimental group and increase reproductive outcome from these sheep.

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INTRODUCTION

Sheep raising in the Russian Federation is a specialized branch of animal husbandry with a rich gene pool and has about 40 breeds and breed groups. The Soviet Merino is a fine-wooled sheep breed with a high yield of high-quality fleece and meat. Merino sheep breeding is a priority development branch of agriculture in the south of Russia, especially in the Astrakhan region (Gorlov *et al.,* 2016). The main objective of sheep industry is to obtain high-quality products, but various animal diseases cause a lot of damage. Moniezia is one of the most important sheep parasites that causes an important problem in sheep breeding, which has been considered as the most common helminth parasite infecting sheep after post-mortem studies (Efremov, 2016; Kaiaty *et al.,* 2019; Sadrutdin *et al.,* 2019). Lambs are more susceptible than adult to *Moniezia expansa* infection and massive infection causes diarrhea and reduced weight gain (Jalajakshi *et al.,* 2016).

Long-term biogeochemical monitoring of the main components of terrestrial ecosystems of the Astrakhan region (soil, water, various plant species, plant feed, organs and tissues of various animal species) showed low levels of selenium, iodine and cobalt (Khismetov and Vorobev, 2015; Vorobiov *et al.,* 2017) relative to similar

indicators of the chernozem region and in a number of other regions of Russia, where microelements in the environment and animal organism are in optimal quantities (Minkina *et al.,* 2015; Polkovnichenko *et al.,* 2020). Minerals play role in defense mechanism against oxidative damage to the tissues, reduce inflammatory reactions and association with antioxidant enzymes. Trace elements act as cofactor for certain enzymes (Hussein *et al.,* 2012). Limited information is available which shows connection between microelements deficiency and gastrointestinal parasitic infections in livestock.

So the present study was undertaken to obtain the clinical appraisal and haemato-biochemical and hormonal alterations in Soviet Merino sheep diseased with monieziosis and hypomicroelementois under the biogeochemical conditions of the Astrakhan region, as well as therapeutic effectiveness evaluation of anthelmintic activity of Praziver (praziquantel and ivermectin) for treatment of sheep infected with monieziosis in combination with the organic drug Sedimin (selenium, iodine and iron) and Cobalt chloride ($CoCl_2$) to correct hypomicroelementosis in the diseased sheep. Sedimin ® with intramuscular administration is absorbed and deposited in the liver and blood-forming organs, filling the deficiency of iron, iodine and selenium in the body.

MATERIALS AND METHODS

Animal and experimental design: The experiment was conducted at the faculty of Veterinary Medicine of Astrakhan State University. Sheep, Soviet Merino, aging 3 years old with a live weight of 43±1.6 kg, were taken in the Astrakhan region, Russia, Limansky district. Sheep were divided into two equal groups (10 in each). In the first group, monieziosis and hypomicroelementosis were diagnosed. The second group of sheep was healthy and was established as a control group. The experiment, including therapy, hematological and biochemical analysis was carried out in accordance with the standards of humane treatment of animals and is based on the EU directive (86/609/EEC) and the Helsinki Declaration.

Sampling and measurements: Faecal samples were taken rectally, from all animals, before treatment and 30 days after treatment for the diagnosis of monieziosis. In the laboratory, the faeces in the containers was stirred with a glass rod, 1 g each, were weighed, and then analysed using the Fülleborn's flotation technique (Gałęcki *et al.,* 2015). Blood was taken from all animals before and 30 days after treatment. Two blood samples were collected from each animal in both groups, before feeding, by vein puncture in a clean test tube as following: The first blood sample collected on anticoagulant (EDTA, 1 mg/5ml blood) for estimation of different hematological parameters, including the hemoglobin concentration (Hb), red blood cells (RBCs), erythrocyte sedimentation rate (ESR), white blood cells (WBCs) and the differential number of leukocytes. The second blood sample were collected without anticoagulant to obtain non hemolysed sera which stored in deep freeze (-20°C) until used for serum biochemical analysis of total calcium, inorganic phosphorus, total protein, total lipids, glucose. Microelements, vitamins and enzymes in the collected samples were determined by atomic absorption method on a CHITAHI 180-50 spectrophotometer (Japan), including selenium (Se), iodine (I_2), cobalt (Co), antioxidant vitamins (A, C, E and B_{12}), the level of peroxidation products (diene conjugates (DC) and malondialdehyde (MDA)) and antioxidant enzymes [(catalase (CAT, E.C. 1.11.1.6), superoxide dismutase (SOD, EC 1.15. 1.1) and glutathione peroxidase (GSH-Px, EC 1.11.1.9)]. Adrenocorticotropic (ACTH) and total thyroid stimulating hormone (TSH) hormone, total triiodothyronine (T3), total thyroxine (T4) and Cortisol in blood serum were determined by the enzyme-linked immunosorbent assay using the Biomerica CT test systems A "(Biomerica ATCH ELISA, USA), CORTIZOL-IFA K210, XEMA Co, Ltd, Russia on ELISIS Quattro ELISA (Germany). Infected group of sheep with monieziosis was given orally, anthelmintic, Praziver in a dose 0.4 ml/10 kg of animal weight. The therapeutic effect of the organic drug Sedimin and $CoCl_2$ on hypomicroelementosis (Se, I_2 and Co) in sheep was studied. Sheep was intramuscularly injected with Sedimin, at a dose of 5 ml per head, into the upper third of the thigh. In 1 ml of Sedimin, it was found: 7.5 mg/ml of iodine and 0.09 mg/ml of stabilized selenium (corresponding to 0.2 mg/ml of Sodium selenite). In addition, sheep from the experimental group were introduced daily into the feed Cobalt chloride ($CoCl_2$) at a dose of 5 mg/kg per head. The diets of sheep feeding in the experimental and control groups were the same.

Statistical analysis: Research data were processed statistically using the mathematical analysis package Microsoft Excel 2007. The significance of the differences was determined by Student's t-test with a significance level of $P<0.01$-0.05.

RESULTS

The results revealed that there was a significant decrease ($P<0.05$) in Hb and RBCs in sheep group diseased with monieziosis and hypomicroelementosis compared with healthy group. Elevated levels of total WBCs count and eosinophils were observed in diseased sheep (Table 1). Regarding the results of the biochemical analysis of blood in Table 2, serum levels of Se, I_2, Co, as well as antioxidant vitamins (A, C and E) were decreased in the diseased group of sheep than in the healthy group. As shown in Table 2, we recorded a low level of antioxidant enzymes (CAT, SOD and GSH-Px) in the blood of the diseased sheep with moniziosis and hypomicroelementosis compared with the healthy group of sheep. On the contrary, we noted a higher level of DC and MDA in the blood of diseased group than in healthy group, which are representative in lipid peroxidation products and is an indicator of oxidative stress in animals. From Table 3, we noted a significant increase ($P<0.05$) of adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH) and Cortisol in the blood of diseased sheep with moniziosis hypomicroelementosis compared to healthy sheep. In addition, we recorded a lower level of thyroid hormones (T4 and T3) in diseased sheep.

Table 1: Some hematological parameters of Merino sheep diseased with monieziosis and hypomicroelmentosis in the Astrakhan region

Hematological parameter	Animals group	
	Diseased group of sheep (n=10)	Control group of sheep (n=10)
Hb (*g/L*)	81.02±2.14	92.50±0.41*
RBCs (10^{12}/L)	6.74±0.49	9.77±0.09*
ESR (mm/*h*)	4.11±0.69*	2.29±0.04
WBCs (10^9/L)	6.51±0.72*	4.11±0.02
Eosinophils%	4.83±0.66*	2.55±0.21
Basophils%	0.54±0.05*	0.34±0.05
Segmented Neutrophils%	36.94±0.82*	20.94±1.25
Band cells%	5.16±0.59*	2.28±0.06
Lymphocytes%	36.42±0.92	52.65±0.67*
Monocytes%	2.16±0.19*	1.53±0.33

* P<0.05 relative to similar data for control group of sheep

Table 2: Serum biochemical parameters of Merino sheep diseased with monieziosis and hypomicroelmentosis in the Astrakhan region

Biochemical parameter	Animal group	
	Diseased group of sheep (n=10)	Control group of sheep (n=10)
Total calcium (mmol/L)	1.41±1.34	2.84±0.77*
Inorganic phosphorus (mmol/L)	0.95±0.77	1.73±0.69*
Total protein (g/L)	57.22±5.57	67.62±2.43*
Total lipids (g/L)	2.56±1.89	4.24±0.06*
Glucose (mmol/L)	1.06±0.51	2.66±0.36*
Se (µmol/L)	0.025±0.003	0.25±0.02*
I_2 (µmol/L)	0.12±0,007	0.23±0.01*
Co (mg/kg)	1.19±0.07	3.85±0.13*
Vitamin A (µmol/L)	0.63±0.002	0.66±0.06*
Vitamin C (µmol/L)	0.021±0.001	0.08±0.007*
Vitamin E (µmol/L)	4.48±0.18	5.72±0.18*
Vitamin B_{12} (pmol/L)	0.16±1.34	0.25±1.39*
DC (µmol/mL)	4.00±0.71*	1.38±0.45
MDA (µmol/mL)	4.06±0.32*	1.12±0.09
CAT (µmol mL)	1.98±0.68	4.15±0.64*
SOD (U/min)	129±11.3	164±8.12*
GSH-Px (nmol/mg)	4.25±00.3	8.15±1.01*

* P<0.05 relative to similar data for healthy group (control) of sheep.

Table 3: Hormonal status of diseased sheep in the Astrakhan region

Hormonal parameter	Animal group	
	Diseased Group of sheep (n=10)	Control group of sheep (n=10)
ACTH (pmol/L)	127±4.1*	65±5.14
Cortisol (nmol/L)	198±3.7*	143±4.21
TSH (U/mL)	0.32±0.01*	0.28±0.02
Total T4 (nmol/L)	130.1±7.3	147±9.2*
Total T3 (nmol/L)	1.61±0.07	2.11±0.05*

* P<0.05 relative to similar data for control group of sheep.

At the end of the experiment, there was a significant increase (P<0.05) in the concentration of Hb, RBCs in the experimental group compared with a healthy one. Hb level increased significantly (P<0.05) on average by 11.97% relative to the control. RBCs count in sheep from the experimental group increased by 33.53% in Table 4. The level of serum Co and vitamin B_{12} in sheep from the experimental group was increased by 79.83% and 39.77%, respectively. By the end of the experiment, a decrease in DC by 67.25% and MDA by 59.85% was found in the blood of sheep of the experimental group. The level of antioxidant enzymes (CAT, SOD and GSH-Px) in the blood of the experimental group increased by 133, 14.73 and 98.82%, respectively, (Table 5). After treatment, the level of ACTH, TSH, and Cortisol in the blood of experimental group of sheep was decreased significantly (P <0.05) by 32.28, 15.62 and 14.65%, respectively compared with similar data that were recorded before treatment. On the contrary, the level of thyroid hormones (T4 and T3) was increased by 13.7 and 32.92% at the end of treatment in the experimental group, respectively (Table 6). The weight of newborn lambs in the experimental herd was, on average, 5.9% higher than in the control. After a month, the live weight of the lambs from the experimental herd exceeded the corresponding indicator of the control lambs by 14.46%. Milk yield in experimental group per day was increase significantly (P<0.05) by 9.27% more than in sheep from the control herd (Table 7).

DISCUSSION

Monieziosis infection leads to cause a significant decrease of hematological parameters, Hb and RBCs counts, which may cause an anaemia in diseased animal. This may be due to cobalt deficiency, because cobalt is part of hemoglobin, as well as in the molecule of antianemic vitamin B_{12} (cobalamin) (Denizhan *et al.* 2017). Cobalt deficiency leads to a decrease in resistance to parasitic infection (Abd El-Rahim, 2017). Infected group of Merino sheep showed a significant increase (P<0.05) in total WBCs count and eosinophils may be due to the immune response of the body against the parasites and resistance to infection as a means of self-defense (Huang and Appleton, 2016). From the results of biochemical analysis, serum levels of Se, I_2 and Co as well as antioxidant vitamins (A, C, E and B_{12}) were decreased in the diseased group of sheep than in the healthy, while significantly increase of DC and MDA. In the Lower Volga region, especially in the Astrakhan region, low levels of Se, I_2, and Co was found in the main components of terrestrial ecosystems (Vorobiov *et al.*, 2017). Vitamin B_{12} deficiency can be caused by prolonged use of adequate diets. Since ruminants are highly

Table 4: Hematological parameters of the studied Merino sheep after treatment

Hematological parameter	Animal group			
	Experimental group of sheep (n=10)		Control group of sheep (n=10)	
	Before treatment	After treatment	Before treatment	After treatment
Hb (g/L)	81.02±2.14	90.72±1.33*	92.50±0.41	94.04±0.60
RBCs (10^{12}/L)	6.74±0.49	9.00±0.38*	9.77±0.09	9.86±0.06
ESR (mm/h)	4.11±0.69*	2.68±0.34	2.29±0.04	2.34±0.07
WBCs (10^9/L)	6.51±0.72*	4.68±0.33	4.11±0.02	4.13±0.03
Eosinophils%	4.83±0.66*	2.62±0.29	2.55±0.21	2.60±0.10
Basophils%	0.54±0.05*	0.22±0.04	0.34±0.05	0.30±0.07
Segmented Neutrophils%	36.94±0.82*	20.79±1.25	20.94±1.25	20.18±0.86
Band cells%	5.16±0.59*	2.57±0.32	2.28±0.06	2.36±0.05
Lymphocytes%	36.42±0.92	51.86±2.23*	52.65±0.67	51.39±2.49
Monocytes%	2.16±0.19*	2.06±0.17	1.53±0.33	2.03±0.14

* P<0.05 relative to the beginning of the experiment.

Table 5: Biochemical parameters of the studied Merino sheep after treatment

Biochemical parameter	Animals group			
	Experimental group of sheep (n=10)		Control group of sheep (n=10)	
	Before treatment	After treatment	Before treatment	After treatment
Total calcium (mmol/L)	1.41±1.34	3.06±0.41*	2.84±0.77	2.74±0.79
Inorganic phosphorus (mmol/L)	0.95±0.77	1.98±0.23*	1.73±0.69	1.67±0.52
Total protein (g/L)	57.22±5.57	69.45±3.04*	67.62±2.43	70.84±3.78
Total lipids (g/L)	4.24±0.06*	2.45±0,04	2.56±1.89	2.71±1.11
Glucose (mmol/L)	1.06±0.51	2.88±0.26*	2.66±0.36	2.52±0.52
Se (µmol/L)	0.025±0.003	0.21±0.007*	0.25±0.01	0.21±0.03
I_2 (µmol/L)	0.12±0,007	0.19±0.002*	0.23±0.01	0.22±0.03
Co (mg/kg)	1.19±0.07	2.14±0.21*	3.85±0.04	2.87±0.06
Vitamin A (µmol/L)	0.63±0.002	0,65±0,004*	0,66±0,06	0.64±0.03
Vitamin C (µmol/L)	0.021±0.001	0,028±0,005*	0.08±0.007	0.09±0.004
Vitamin E (µmol/L)	4.5±0.02	5,8±0,22*	5.7±0.20	5.3±0.23
Vitamin B_{12} (pmol/L)	0.16±1.3	0.23±1.69*	0.25±1.4	0.22±1.28
DC (µmol/mL)	4.00±0.71*	1.31±0.21	1.38±0.45	1.58±0.87
MDA (µmol/mL)	4.06±0.32*	1.63±0.25	1.12±0.09	1.43±0.41
CAT (µmol mL)	1.98±0.68	4.62±0.55*	4.15±0.64	4.88±0.70
SOD (U/min)	129±11.3	148±10.3*	164±8.12	157±9.14
GSH-Px (nmol/mg)	4.25±00.3	8.45±1.04*	8.15±1.01	9.14±1.02

* P<0.05 relative to the beginning of the experiment.

Table 6: Hormonal status of the studied Merino sheep after treatment

Hormonal parameter	Animals group			
	Experimental group of sheep (n=10)		Control group of sheep (n=10)	
	Before treatment	Before treatment	Before treatment	Before treatment
ACTH (pmol/L)	127±4.1*	86±3.2	65±5.14	91±4.16
Cortisol (nmol/L)	198±3.7*	169±4.06	143±4.21	156±3.17
TSH (U/mL)	0.32±0.01*	0.27±0.03	0.28±0.02	0.25±0.05
Total T4 (nmol/L)	130.1±7.3	148±8.2*	147±9.2	149.7±7.5
Total T3 (nmol/L)	1.61±0.07	2.14±0.04*	2.11±0.05	1.95±0.08

* P<0.05 relative to the beginning of the experiment.

Table 7: Productivity and reproduction in a sheep herd as an indicator of treatment effectiveness

Mass of newborn lambs, kg	Mass of lambs in age 1 month, kg	Milk yield per day, kg
Control flock (n=10)		
3.4±0.06	8.3±1.23	5.5±0.04
Experimental flock (n=10)		
3.6±0.04*	9.5±1.36*	6.01±0.03*

* P<0.05 relative to control.

dependent on gluconeogenesis regarding their glucose intake. Co, as a component of vitamin B_{12}, is vital for these animals (Huwait *et al.,* 2015). Oxidative stress is a cellular phenomenon or condition that occurs as a result of a physiological imbalance between the levels of antioxidants and oxidizing agents (free radicals or active substances) in favor of oxidizing agents, this can activate the process of lipid peroxidation at the tissue, cellular or body levels. The role and effectiveness of the first-line protective antioxidants, which mainly include CAT, SOD and GSH-Px, are important and indispensable in the entire antioxidant protection strategy (Reckelhoff *et al.,* 2019). Recently, the biochemical role of Se as a component of the GSH-Px enzyme, which acts together with vitamin E in cells to control peroxidation, has been established (Biswal *et al.,* 2016). Sheep attracted attention as an animal model for studying the thyroid gland due to the close similarity of the metabolism of thyroid hormones in the ovine with that of the human fetus (Bianco *et al.,* 2014). Regarding the combined effect of monieziois and hypomicrelementois on hormonal levels, higher levels of ACTH, TSH and Cortisol in the blood of diseased sheep were recorded compared with healthy sheep. In addition, a lower level of thyroid hormones (T4 and T3) in diseased sheep. Selenium deficiency inhibits the activity of type I, 5'-iodothyronindeiodinase I in animal tissues and can increase iodine deficiency due to impaired conversion of T4 to T3. Cortisol, a steroid hormone in the class of glucocorticoid hormones, is usually secreted by the adrenal cortex and released into the bloodstream in response to stress and low blood glucose in most mammals. In acute stress, the level of cortisol in the blood rises rapidly (Young *et al.*, 2004).

By the end of the therapeutic experiment, there was a significant increase (P<0.05) in Hb concentration and total RBCs count in the experimental group of sheep diseased with monieziois and hypomicroelementosis compared to control group. The level of serum Co and vitamin B_{12} in sheep from the experimental group was increased. This may be an indicator of the good effectiveness of the organic drug $CoCl_2$ to increase the serum levels of Co. The level of antioxidant enzymes (CAT, SOD and GSH-Px) was increased, while a decrease in DC and MDA in the serum of the experimental group was found. Sedimin, containing Se and I, was effective in increasing the level of Se in the blood of sheep of the experimental group by the end of treatment. Selenium as a component of selenoproteins plays an important structural and enzymatic function. The main selenoproteins include glutathione peroxidase, which protects cellular lipids from the damaging effects of reactive oxygen species (Steinbrenner and Sies, 2009). The level of ACTH, TSH, and Cortisol in the serum of diseased sheep decreased significantly (P<0.05) compared with similar data that were recorded before treatment. On the contrary, the level of thyroid hormones (T4 and T3) increased at the end of treatment in the group of diseased sheep. Iodine is the only known currently a trace element involved in the biosynthesis of hormones (O'Kane *et al.*, 2018). This may be an indicator of the good effectiveness of Sedimin to

increase the serum levels of Iodine. At the end of the experiment, did not record any eggs for *Moniezia spp* in the faeces of experimental sheep. Praziver is a product containing Praziquantel and Ivermectin. They provide a wide range of antiparasitic actions against helminths. Praziquantel is a synthetic derivative of isoquinolinopyrazine, active against sexually mature and immature forms of tape helminths. Therapy and prophylaxis of combined (Se, I_2 and Co) hypomicroelementosis in experimental sheep by intramuscular injection of Sedimin using $CoCl_2$ into the feed allowed the sheep to produce more lambs with greater live weight, better growth and development of lambs, relative to their analogues obtained from sheep from control herd.

Conclusions: Thus, we have recorded the harmful effect of combined monieziosis and hypomicroelementosis on the hematological, biochemical and hormonal parameters of Soviet Merino sheep, which clearly shows the development of oxidative stress in the form of lower levels of antioxidant enzymes and higher levels of lipid peroxidation products and free radicals. In addition, the results of the pituitary-thyroid system (increased levels of ACTH, TSH and Cortisol in the blood, while decreased levels of the hormones T4 and T3 in the blood). For diseased sheep, hematological, biochemical and hormonal parameters were returned to normal levels after combined treatment of monieziosis with anthelmintic "Prazivera" and hypomicroelementosis with "Sedimin" and "$CoCl_2$". The effectiveness of the treatment was also recorded with an increase in the productivity and reproduction of the studied sheep after treatment.

Authors contribution: MA and VVI contributed to the conception of the research. MAA collected the samples and conducted experiments. MAA, LML and AKD analyzed data and wrote the manuscript. All the authors read the manuscript and approved the contents.

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