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Dynamics of Cellular Factors of the Immune System of Arctic Foxes (*Vulpes lagopus*) on the Background of Mixtinvasion

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Abstract

The article presents data on the dynamics of cellular immune system factors of the arctic foxes (*Vulpes lagopus*) while being affected by mixtinvasion of protozoa (*Isospora vulpina*) and helminth (*Toxascaris leonina*). The changes in the blood cell composition, in particular those responsible for the body's immunity and immune status, reflect the pathological effect of the endoparasites on the animal's body. The development of the invasive process during mixtinvasions causes and is accompanied by a decrease in the level of nonspecific resistance of the host organism. In addition, a significant process of inhibition of the lysozyme complementary activity in the blood serum and the development of secondary immunodeficiencies is observed. The aim of our work was to study the development of the pathological process in arctic foxes, caused by previously detected mixtinvasion by eimeriidoses and helminthiases, as well as to identify dynamical patterns of the immune system cellular factors. Intravital diagnostics of parasitoses was carried out by means of coproscopic studies with the help of conventional flotation methods. 127 arctic foxes participated in the experiment. Among them individuals with double invasion, an invasion with both protozoa (*placeI. vulpina*) and helminth (*T. leonina),* were selected for the experiment. Healthy animals served as the control group. Arctic foxes which took part in the experiment were divided into three groups of 10 animals each. Groups were formed by the method of balanced analogous groups. Evaluation of the T-system of immunity was carried out by the method of spontaneous rosetting according to Jondal (1972). Isolation of lymphocytes by the method of A. Boyum (1968), B-lymphocytes were determined by the method of E. Mendes (1973), theophylline-resistant and theophylline-sensitive T-lymphocytes were determined using the method of S. Limatibul et.al. (1978). The immunoregulation index was calculated by the ratio of T-helpers to T-suppressors. During the experiment, it was found that in infected animals the total number of lymphocytes was significantly 10.2 % higher than in animals in the control group, however, there was a tendency to a sharp decrease in the total number of lymphocytes in patients with *I. vulpina + T. leonina.* The T-helper dynamics in infected foxes was 18.9 ± 0.9 ($P \leq 0.05$), which is 21.9 % less than in the control -- 24.2 ± 0.6 ($P \leq 0, 05$). The dynamics of T-suppressors turned out to be directly opposite to the dynamics of the T-helpers. The dynamics of B-lymphocytes in the 2nd group turned out to be 1.76 times higher than in animals from the control. In the 3rd group same indicator was comparable with the control values of 2.9 ± 0.3,

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against 2.5 ± 0.4 in the control group. The study showed that mixtinvasion with placel. vulpina + T. leonina leads to immunosuppression in the arctic foxes. Interestingly the specific therapy did not straighten the immune state of the animals, but on the contrary it became more pronounced, which suggests that antiparasitic drugs have an immunosuppressive nature on the body of arctic foxes as evidenced by violations of the parameters of the body's natural resistance, the content of T-E-ROCK lymphocytes.

Keywords: immunity, endoparasites, arctic foxes, nematode Toxascaris leonina, protozoa Isospora vulpina, mixtinvasion.

1. Introduction

The changes in the blood cells, in particular those responsible for the body's immunity and immune status, reflect the pathological effect of endoparasites on the animal's body as well as indicating the severity of the disease [1--4]. Previously, we found that invasive diseases of fur animal often occur in combination with each other, as well as with other organisms that inhabit the gastrointestinal tract of the animals [5, 6]. Even though they have similar pathological effect on the body, there is a number of significant features that determine the development of pathogenesis caused by helminthiases [7, 8]. Thus, a mixed invasion of an animal has a more pathogenic effect than a helminthic invasion would have on its own [9, 10].

It was previously established that mixtinvasion cause suppression of the immune system (T- and B-lymphocytes) [11-14]. However, the role of immune suppression in the host organism is very ambiguous [15]. On the one hand, suppression of T-lymphocytes controls the stability of the host-parasite relationship. On the other hand, a to development of hyperreaction or excessive inflammatory reaction can occur causing the damage of its own tissues [16]. An increase of T-suppressors is one of the mechanisms of immunoregulation that limits the production of antibodies and the excessive formation of circulating immune complexes, which play a large role in the formation of immunopathological processes in mixtinvasions [5, 7]. The development of the invasive process during mixtinvasions causes and is accompanied by a decrease in the level of nonspecific resistance of the host organism. The lysozyme complementary activity of the blood serum is inhibited and the development of secondary immunodeficiencies is observed.

The aim of our work was to study the development of the pathological process in arctic foxes, caused by previously detected mixtinvasion by eimeriidoses and helminthiases, as well as to identify patterns in the dynamic of cellular factors of the immune system.

2. Methods and Equipment

2.1. Methods

Intravital diagnosis of parasitoses was carried out by means of coproscopic studies in the generally accepted way. We also used the Darling method that was improved by us, using a universal flotation diagnostic fluid. The method is patented in the Russian Federation for invention No. 2472154 ``Liquid for the diagnosis of coccidia oocysts, balantidia and giardia cysts, helminth eggs of different classes, ticks, insects and their individual stages of development'' [17]. The studies were carried out in accordance with GOST R - 54627-2011 and guidelines of MUK 4.2.3145-13.

Blood was obtained from foxes before they were fed. The blood was sampled while the animal was fixed in a lateral position with the help of an assistant. A rubber tourniquet was applied to the pelvic limb, above the hock, and a puncture was made from a plantar vein, after wetting the hair with physiological saline, and the injection site was treated with alcohol.

The evaluation of the T-system of immunity was carried out by the method of spontaneous rosetting according to Jondal (1972). Isolation of lymphocytes by the method of A. Boyum (1968), B-lymphocytes were determined by the method of E. Mendes (1973), theophylline-resistant and theophylline-sensitive T-lymphocytes were determined using the method of S. Limatibul et.al. (1978). The immunoregulation index was calculated by the ratio of T-helpers to T-suppressors.

Altogether, 127 Arctic foxes were examined by a copro-occopic method in one of the farms located in Leningrad region. Only animals with mixtinvasion were selected for the experiment, in which an invasion was detected at the same time as protozoa (*Isospora vulpina*) and helminth (*Toxascaris leonina*). Clinically healthy were used as a control group. The experimental Arctic foxes were divided into three groups of 10 animals each, which were formed by the method of balanced analog groups.

The second group included Arctic foxes infected with *I. vulpina + T. leonina* mixtinvasion. The third group included arctic foxes infected with the parasites of the same type while being treated with Stop-Coccid + Febtal drug. In the 3rd group the blood analysis was performed on the next day after drug administration.

The data obtained was statistically analyzed with the help of variation statistics using a simple comparison of averages, the Student's t-test in Tippet's modification. In the statistical analysis, the differences were determined by p, which was assumed to be equal to 0.05 significance level. While the values were achieved in 3 levels of statistically significant differences: $p \leq 0.05$; $p \leq 0.01$; $p \leq 0.001$.

2.2. Equipment

For light microscopy of the obtained temporary samples using the bright field method, the material was examined on a Carl Zeiss Primo Star trinocular microscope with visualization at magnification (10x eyepiece, lens 10, 20, and 40) with the LOMO OMP Micrometer nozzle. Photoregistration was carried out using microscope cameras and a Mi MIX 2 smartphone (Xiaomi).

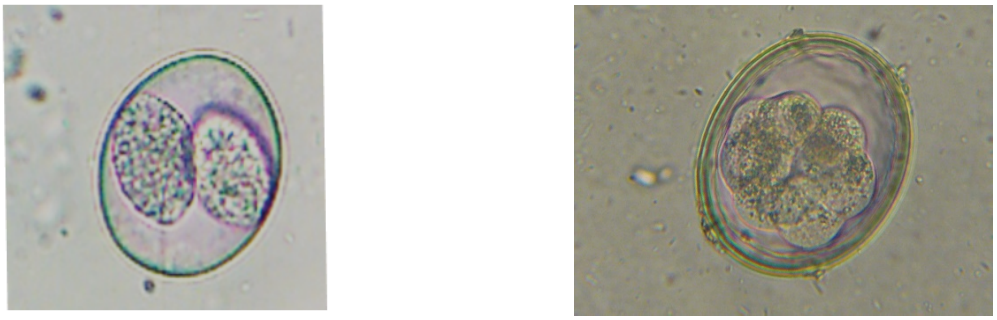
3. Results

According to the results of a survey of fecal masses of 127 arctic foxes by flotation methods, mixed animals were found to have been mixed in 30 animals. The oocysts *Isospora vulpina* Nieschulz & Bos, 1933 were found in the examined animals; they are elongated-oval in shape, light gray in color. Their shell is smooth, two-layer. Micropile and polar granule were absent. The sizes of oocysts on average were $27.48 \pm 0.36 \times 21.23 \pm 0.21$ µm. The form index is 1.29--1.32. In mature oocysts, two oval sporocysts of 13.6--17.4 × 10.2--12.6 µm in size were formed. In sporocysts, there were four sporozoites of spindle-shaped form, with a size of 14.2 × 3.2 µm. Between the sporozoites there was a coarse-grained residual body. These morphological characters allowed the parasite being attributed to *I. vulpina* (Figure 1).

In addition, protozoa toxopascaris eggs belonging to the Ascaridae family, the genus *Toxascaris*, and the species *T. leonina* were found in the same individuals during coproscopic studies (Figure 2).

In the process of development of pathological processes caused by mixtinvasion, the changes in cellular immunity took place. In addition, we managed to establish the effect of combined treatment on the immune response. The results obtained during the experiment are presented in Table 1.

During the experiment, it was found that in infected animals the total number of lymphocytes ($P \leq 0.01$) was 10.2 % higher than in animals in the control group, however, we found an interesting tendency to a sharp decrease in the total number of lymphocytes



a b

Figure 1: (a) oocyst *placel. vulpina*, (b) *T. leonina* egg.

TABLE 1: Dynamics of cellular factors of the immune system of arctic foxes against the background of mixtinvasion, %.

Groups of experimental animals	Lymphocyte populations					
	T-E-ROCK general lymph.	T-E-ROCK active lymph.	T-helpers (Tx)	T suppressors (Tf)	B lymph.	Immune regulatory index (Tx / Tc)
1st group -- control (clinically healthy animals) n = 10	42.9±1.3*	14.3±0.8	24.2±0.6*	19.8±2.1	2.5±04	1.22
2nd group -- Arctic foxes infected *I. vulpina* + *T. leonina*, n=10	47.3±0,6**	16.8±1.1	18.9±0.9*	27.5±2.2	4.4±0.3	0.68
3rd group -- infected Arctic foxes, then treated with Stop-Koktsid + Febtal gruff preparations, n = 10	38.8±0.8*	11.6±0.5*	26.7±1.7	11.2±0.6*	2.9±0.3	2.38

*P≤0.05; **P≤0.01

in patients with I. vulpina + T leonina of arctic foxes. Following indicator was 38.8 ± 0.8 (P≤0.05), which is 18 % lower than in the second experimental group and 9.5 % lower than in the animals from the control group. Approximately the same patterns were observed in dynamics of the active lymphocytes.

Dynamics of T-helpers: in infected foxes the indicator was 18.9 ± 0.9 (P≤0.05), which is 21.9 % less than in the control -- 24.2 ± 0.6 (P≤0.05). However, the day after the administration of antiparasitic drugs, this indicator was 10.3 % higher than in clinically healthy animals.

The dynamics of T-suppressors, on the contrary, was directly opposite to the dynamics of T-helpers. In infected animals from the second group it was 38.8% higher than in foxes from the control. While in combination with the specific therapy (3rd group), this indicator

significantly decreased to the level of 11.2 ± 0.6 (P≤0.05), which is 40.7 % lower than in animals from the 2nd group.

The dynamics of B-lymphocytes in the 2nd group was 1.76 times higher than in animals from the control. In the 3rd group, this indicator was comparable with the control values of 2.9 ± 0.3, against 2.5 ± 0.4 in the control group.

4. Discussion

The study showed that mixtinvasion with I. vulpina + T. leonina caused immunosuppression in arctic foxes. The specific therapy did not straighten, but on the contrary becomes more pronounced, which suggests that antiparasitic drugs have an immunosuppressive nature on the body of arctic foxes as evidenced by violations of the parameters of the body's natural resistance (the content of T-E-ROCK lymphocytes). These immune pathologies are complicated by the activation of suppressor reactions in the body. We found that the dynamics of T-suppressors, was on contrary the exact opposite of the dynamics of T-helpers. With the specific therapy, this indicator significantly decreased to the level of 11.2 ± 0.6 (P≤0.05), which is 40.7% lower than in animals from the 2nd group. The dynamics of B-lymphocytes in the 2nd group was 1.76 times higher than in animals from the control, in the 3rd group, this indicator was comparable with the control values of 2.9 ± 0.3, against 2.5 ± 0.4 in control.

5. Conclusion

With protozoa and helminthiases the immunity is formed, which develops under the influence of cellular and humoral factors. Its level depends on the type of the pathogen and the physiological state of the fur animals. All this affects the intensity of the epizootic process and its individual links, which must be taken into account when planning measures for the prevention and control of parasitosis.

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Conflict of Interest

The authors have no conflict of interest to declare.

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