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The state of the immune system of dogs in experimental toxocariasis

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Contents

Abstract

The aim of the study was to determine the state of the dogs' immune system in experimental toxocariasis. The work was performed during 2017-2020 at the Department of Parasitology and Ichthyopathology in Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. The 12 dogs aged 2 to 4 months were used for research studies. Two groups were formed of six animals in each: control and experimental. Puppies of the experimental group (E) were infected with the causative agent of toxocariasis at a dose of 5000 invasive eggs of *Toxocara canis* per kg of body weight. The control puppies were clinically healthy. At the clinical display of toxocariasis invasion at dogs the cellular, humoral and nonspecific links of the immune system are suppressed and the secondary immunodeficiency comes. Suppression of cellular immunity was accompanied by a decrease in the T- and B-lymphocytes count in the blood of dogs of the (E) group, which indicates the suppression of the lymphoid immune system and a reduction in the resistance of animals. Along with the fall in cellular immunity in infected puppies, suppression of the nonspecific immune system was established, which is manifested by a decline in the phagocytic activity of neutrophils and a decrease in the phagocytic index. Thus, on the 15th and 20th days of the test, the phagocytic activity of neutrophils reduced to 1.6 and 3.9 %, while the phagocytic index – to 5.4 and 6.9 %, respectively. In the study of antimicrobial activity of serum of infected dogs, *T. canis* found inhibition of bactericidal and lysozyme activity, which reflects the suppression of the physiological state of the humoral immune system of animals with the development of toxocariasis. On the 25th day of the research, a deccline in BABS to 24.0 ± 2.7 %, while in the (C) group of dogs this figure was 30.6 ± 1.9 %. The lowest LABS was on the 25th day of the (E) in animals that were experimentally infected with the pathogen toxocariasis, where compared with the (C) group, this figure decreased by 6.0 %, respectively. The high level of CIC in the serum of dogs infected with *T. canis* indicates the suppression of the immune system of their body due to the accession of specific antibodies to the products of metabolism of toxocara, which act as antigens

Key words: invasion, helminths, toxocariasis, dogs, immune system.

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1. Introduction

Among the invasive diseases of dogs, the most common in our country and abroad are gastrointestinal helminthiasis, among which the leading place is occupied by toxocariasis – nematode infestation of the order *Ascaridata* (Congdon & Lloyd, 2011; Svirzhevska, 2013; Ozlati et al., 2016).

Infection of puppies mainly occurs in utero, by transplacental transmission of larvae from mother to fetus in the second half of pregnancy, or in the first days of life with milk (Hlushko, 2013; Moisieieva et al., 2017).

The direct route of infection is by swallowing Toxocara eggs directly from the soil or contaminated soil (Uga et al., 2000; Macuhova et al., 2010; Durant et al., 2012). In the stomach, or in the small intestine of puppies and young dogs, larvae emerge from the eggs, which migrate similar to the migration of larvae of *Ascaris suum* in pigs. Through the intestinal mucosa, the larvae of the second stage penetrate the venous vessels of the intestine, the portal vein system and enter the liver, wherein the inferior vena cava, then in the right half of the heart, through the pulmonary artery to the pulmonary capillaries, where the second molting occurs. From capillary pulmonary vessels, larvae of the third stage actively get to bronchioles, bronchial tubes, and trachea from which together with mucus get to an oral cavity and are swallowed with saliva. In the small intestine there are two more molts and in 4–5 weeks after infection parasites reach sexual maturity (Usachova & Dralova, 2012; Said et al., 2018).

In adult dogs (older than one year) the larvae do not fully develop, it stops in the larvae of the second stage. Part of the it, reaching the lungs, penetrating the pulmonary vein, migrates through the heart into the great circle of blood circulation, where the arterial system enters the various organs and tissues (lungs, liver, kidneys, muscles), where it incises, maintaining its viability for many years. In the case of reduced resistance, or changes in hormone levels (pregnancy, lactation), the larvae leave their localization (Pryima, 2010; Svirzhevska, 2011; Dralo et al., 2017).

The available literature, which reflects the numerous results of studies on the effects of Toxocara and their metabolites on animals, does not fully reflect their mechanism of

action, especially on the immune system. Research have been performed in dogs to detect disorders of the cardiovascular and central nervous systems and the digestive tract with the development of toxocariasis. Some scientists have established the complexity of the pathogenesis of toxocariasis in animals: metabolic disorders, disturb of physiological functions of the body, and also proposed various methods of treatment of animals with this pathology (Stybel & Pryima, 2010; Noor et al., 2019).

After analyzing the above national literature data, we found that the effects of toxocariasis invasion on the immune system of sick dogs have not been completely studied, and need some clarification.

That is why the study aimed to determine the state of the dogs' immune system in experimental toxocariasis.

2. Materials and methods

The work was performed during 2017–2020 at the Department of Parasitology and Ichthyopathology in Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. The 12 dogs aged 2 to 4 months were used for research studies. Two groups were formed of six animals in each: control and experimental. Puppies of the experimental group (E) were infected with the causative agent of toxocariasis at a dose of 5000 invasive eggs of *Toxocara canis* per kg of body weight. The control puppies were clinically healthy.

All animal manipulations were performed in accordance with the "General Ethical Principles of Animal Experiments" (Ukraine, 2001), in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals" used for experimental and other scientific purposes (Strasbourg, 1985).

Lysozyme activity of blood serum (LABS) was determined using as a test microbe daily culture of Micrococcus lysodeicticus strain VKM-109 by nephelometric method, optical density was measured at a wavelength of 540 nm. Bactericidal activity in blood serum (BABS) samples was studied by the method of Yu. M. Markov (1968) using a daily culture of E. coli strain VKM-125. Photocolorimetry was performed before and after 3 hours of incubation. Determination of the content of circulating immune complexes (CIC)in the serum was performed using borate buffer. Selective precipitation of antigen-antibody complexes occurred under the influence of high molecular weight PEG, mass 6000 Da. The results were recorded by photocolorimetry of the precipitate density at a wavelength of 450 nm. The phagocytic response of blood neutrophils was evaluated by phagocytic activity (PhA) and phagocytic index (PhI) by the method of V. S. Gostev (1950) (Vlizlo, 2012).

The analysis of research results was performed using the software package Statistica 6.0. The probability of differences was assessed by Student's t-test. The results of the mean values were considered statistically significant at * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$ (ANOVA).

3. Results and discussion

Evaluating the activity of the immune system in dogs, it should be borne in mind significant fluctuations in immunological parameters in toxocariasis. An important role in immune responses are performe by lymphocytes that have cellular receptors that recognize antigens (Hariv et al., 2016; Borisenko et al., 2019; Martyshuk et al., 2020; Hunchak et al., 2020; Lavryshyn et al., 2020). The results of the study of cellular immunity in infected dogs are presented in table 1.

Table 1
The T-lymphocytes count in the blood of dogs in experimental toxocariasis (M ± m, n = 6)

Blood test time (days)	T-lymphocytes, %	
	Groups of animals	
	Control (C)	Experimental (E)
Before infection	37.6 ± 1.52	37.7 ± 1.45
5th day	37.5 ± 1.45	35.5 ± 1,23
10th day	37.7 ± 1.61	34.4 ± 1.19
15th day	37.6 ± 1.42	33.0 ± 1.54*
20th day	37.4 ± 1.35	31.7 ± 1,60**
25th day	37.5 ± 1.40	30.4 ± 1.55**
30th day	37.6 ± 1.33	30.7 ± 1.20**

Note: * – $P < 0.05$; ** – $P < 0.01$***; – $P < 0.001$

The table shows that in infected dogs the causative agent of toxocariasis is moderate changes in the cellular immune system. It was found that the T-lymphocytes count in the blood of dogs of the (E) group on n the 10th day of the experiment decreased by 3.3 %, and on the 15th day – by 4.6 % relative to the (C) group of dogs. Subsequently, a more probable reduction in T-lymphocytes was observed, respectively, on the 25th day of the research, their number declined to 30.4 ± 1.55 %. On the 30th day of the test, the T-lymphocytes count in the blood of infected animals of the (E) group increased slightly compared to the previous day, but relative to the (E) group was lower by 6.9 %.

In the study of the B-lymphocytes count in the blood of dogs of the (E) group found a reduce from 5 days of the experiment. On the 10th day of the study in the blood of infected the puppies found a decrease in the B-lymphocytes count by 1.9 % relative to the (C) group of animals.

Table 2
The B-lymphocytes count in the blood of dogs in experimental toxocariasis (M ± m, n = 6)

Blood test time (days)	B-lymphocytes, %	
	Groups of animals	
	Control (C)	Experimental (E)
Before infection	17.2 ± 1.23	17.4 ± 1.20
5th day	17.5 ± 1.16	16.6 ± 1.10
10th day	17.3 ± 1.18	15.4 ± 1.28
15th day	17.7 ± 1.20	14.7 ± 1.32*
20th day	17.9 ± 1.21	13.8 ± 0.98*
25th day	17.6 ± 1.11	12.6 ± 1.28**
30th day	17.7 ± 1.20	13.0 ± 1.00**

The lowest B-lymphocytes count was found in the blood of dogs of the (E) group on the 25th day of the experiment, compared with the (C) group of animals, it decreased by 5.0 %, respectively. On the 30th day of the study, the B-lymphocytes range in the blood of the (E) group was 13.0 ± 1.00 %, while in the (C) group – 17.7 ± 1.20 %.

The fall of T- and B-lymphocytes count in dogs with toxocariasis, we explain the action of helminths and their

metabolites on the immune system of the puppies in the (E) group.

Thus, the development of toxocariasis in dogs is accompanied by the suppression of cellular immunity.

Along with the decrease in cellular immunity in infected dogs, suppression of the nonspecific immune system was established, which is manifested by a decline in the PhA of neutrophils and the reduction in the PhI (Table 3–4).

Table 3
The phagocytic activity of neutrophils in the blood of dogs in experimental toxocariasis (M ± m, n = 6)

Blood test time (days)	PhA, %	
	Groups of animals	
	Control (C)	Experimental (E)
Before infection	32.1 ± 2.5	32.3 ± 2.0
5th day	32.4 ± 2.2	31.9 ± 2.6
10th day	32.0 ± 2.0	30.4 ± 2.5
15th day	32.2 ± 1.5	29.3 ± 1.8
20th day	32.4 ± 2.3	28.5 ± 2.0
25th day	32.1 ± 2.0	27.2 ± 2.5*
30th day	32.5 ± 1.7	27.7 ± 1.9*

In the study of PhA of neutrophils and PhI in dogs, which were experimentally invaded by the causative agent of toxocariasis, a decrease in these indicators was found throughout the test. Thus, on days 15 and 20 of the research, the PhA of neutrophils reduced by 1.6 and 3.9%, while the PhI – by 5.4 and 6.9 %, respectively.

On the 25th day of the study in the blood of dogs of the (E) group found a decrease in PhA of neutrophils to 27.2 ± 2.5 %, while in the (C) group of dogs this figure was 32.1 ± 2.0 %.

Table 4
The phagocytic blood index of dogs in experimental toxocariasis (M ± m, n = 6)

Blood test time (days)	PhI, %	
	Groups of animals	
	Control (C)	Experimental (E)
Before infection	44.1 ± 3.3	44.0 ± 2.7
5th day	44.0 ± 3.0	41.4 ± 3.5
10th day	44.3 ± 2.7	40.2 ± 4.0
15th day	44.1 ± 3.1	38.7 ± 3.8
20th day	44.2 ± 2.8	37.3 ± 3.5*
25th day	44.4 ± 3.0	36.5 ± 1.9*
30th day	44.2 ± 3.2	37.1 ± 4.0*

The lowest PhI in the animals of the (E) group was on the 25 and 30 days of the test, compared with the values of the (C) group, it decreased by 7.9 and 7.1 %.

Therefore, with the experimental development of toxocariasis in dogs during 30 days, parasites and their metabolites reduce the protective properties of animals, including indicators of nonspecific immunity.

In the study of antimicrobial activity of serum of infected dogs *T. canis* found inhibition of bactericidal and lysozyme activity, which reflects the suppression of the physiological state of the humoral immune system of the puppies with the development of toxocariasis.

Examining the BABS of animals in toxocariasis invasion, it was found that on the 10th day of the experiment this indicator decreased to 28.4 ± 2.3 %. On days 15th and 20th of the study, the (BABS) of infected puppies relative to control fell down by 3.1 and 4.5 %. On the 25th day of the research, we note a decrease in BABS to 24.0 ± 2.7 %, while in the (C) group of dogs this figure was 30.6 ± 1.9 %. On the 30th day of the experiment, was a slight increase in BABS of dogs of the (E) group compared with the indicators taken on the 25th day of the study.

Table 5
Bactericidal activity of blood serum of dogs in experimental toxocariasis (M ± m, n = 6)

Blood test time (days)	BABS, %	
	Groups of animals	
	Control (C)	Experimental (E)
Before infection	30.7 ± 2.3	30.4 ± 1.7
5th day	30.9 ± 1.9	29.3 ± 2.5
10th day	30.5 ± 1.8	28.4 ± 2.3
15th day	30.7 ± 1.6	27.6 ± 2.0
20th day	30.8 ± 2.2	26.3 ± 2.0*
25th day	30.6 ± 1.9	24.0 ± 2.7**
30th day	30.7 ± 2.3	24.6 ± 2.0*

In the study of the LABS of dogs infested with *T. canis*, it was found that on the 10th and 15th day of the experiment, this figure decreased by 1.9 and 2.4 % relative to control values. Subsequently, LABS in animals of the (E) group continued to decline and, accordingly, on the 20th day of the examination was 21.6 ± 2.6 %, while in the (C) group this figure was 26.2 ± 1.9 %. The lowest LABS was on the 25th day of the test in dogs that were experimentally infected with the pathogen toxocariasis, compared with the (C) group, this figure reduced by 6.0 %, respectively. On the 30th day of the research, the LABS of dogs of the (E) group increased slightly and was 20.4 ± 3.0 % (table 6).

Table 6
Lysozyme activity of dog serum in experimental toxocariasis (M ± m, n = 6)

Blood test time (days)	LABS, %	
	Groups of animals	
	Control (C)	Experimental (E)
Before infection	26.1 ± 2.4	26.3 ± 2.0
5th day	26.4 ± 2.1	25.5 ± 1.8
10th day	26.3 ± 2.1	24.4 ± 2.4
15th day	26.3 ± 2.5	23.9 ± 3.0
20th day	26.2 ± 1.9	21.6 ± 2.6*
25th day	26.1 ± 2.0	20.1 ± 1.5**
30th day	26.3 ± 2.7	20.4 ± 3.0**

In the study of humoral immunity in dogs, it was found that in the blood of both the (E) and (C) groups, the level of CIC before infection was 0.15 ± 0.01 – 0.14 ± 0.02 mg/ml. At toxocariasis invasion in animals on the 10th day of the experiment, the level of CIC increased to 0.18 ± 0.04 mg/ml, while in the control group of dogs this figure was 0.14 ± 0.03 mg/ml. On the 15th, 20th and 25th days of the experiment, the level of CIC in the blood of

puppies of the (E) group was high and compared with the (C) group, it grew by 50.0, 53.3 and 78.6 %, respectively.

Table 7
The level of circulating immune complexes in the blood of dogs in experimental toxocariasis (M ± m, n = 6)

Blood test time (days)	CIC, мг/мл	
	Groups of animals	
	Control (C)	Experimental (E)
Before infection	0.15 ± 0.01	0.14 ± 0.02
5th day	0.13 ± 0.01	0.16 ± 0.02
10th day	0.14 ± 0.03	0.18 ± 0.04*
15th day	0.14 ± 0.02	0.21 ± 0.04***
20th day	0.15 ± 0.01	0.23 ± 0.03***
25th day	0.14 ± 0.01	0.25 ± 0.05***
30th day	0.15 ± 0.02	0.24 ± 0.06***

The high level of CIC in the serum of dogs infected with *T. canis* indicates the suppression of the immune system of their body due to the attachment of specific antibodies to the products of metabolism of toxocara, which act as antigens.

4. Conclusions

At the clinical display of toxocariasis invasion at dogs the cellular, humoral and nonspecific links of the immune system are suppressed and the secondary immunodeficiency comes. Suppression of cellular immunity was accompanied by a decrease in T- and B-lymphocytes count in the blood of animals of the (E) group, which indicates the suppression of the lymphoid immune system and a reduction in the resistance of animals. Serum antimicrobial activity decreased throughout the study, as indicated by a fall in bactericidal and lysozyme serum activity in infected dogs. The high level of circulating immune complexes in the serum of puppies of the (E) group indicates the suppression of the body's immune system. Suppression of the nonspecific part of the immune system is manifested by a decline in the phagocytic activity of neutrophils and a decrease in the phagocytic index.

According to the analysis of the obtained results, it can be predicted that the signs of immunosuppression in dogs may be a consequence of the toxic effects of toxocara on their body, which further requires not only effective antiparasitic treatment, but also immunobiological correction.

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