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State Veterinary and Food Administration of the Slovak Republic
Veterinary Chamber of Slovak Republic
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O KONFERENCII

Šiesty ročník medzinárodnej vedeckej konferencie je jedným z najvýznamnejších podujatí v oblasti infekčných a parazitárnych chorôb zvierat. Cieľom konferencie je ponúknuť odbornej a vedeckej verejnosti najaktuálnejšie informácie z oblasti infekčných a parazitárnych chorôb zvierat a ľudí ako aj informovať o najmodernejších trendoch v ich diagnostike, terapii a zdolávaní. Na predchádzajúcich ročníkoch sa nám dosiaľ vždy podarilo vytvoriť príjemnú atmosféru pre diskusiu a výmenu skúseností a názorov. Veríme, že si nájdete čas a budete si môcť spríjemniť septembrové dni odbornými a priateľskými stretnutiami, a rozhovormi na zaujímavé témy a podelíte sa s ostatnými účastníkmi konferencie o výsledky vášho výskumu. Organizačný výbor a všetci účastníci sa tešia na Vaše zaujímavé odborné príspevky, ktoré infektológia a parazitológia v praxi a vo výskume ponúkajú.

ABOUT THE CONFERENCE

Sixth International Scientific Conference is one of the most important events in the field of infectious and parasitic diseases. The aim of the conference is to offer expert and scientific public the most up-to-date information from infectious and parasitic diseases of animals and humans as well as to inform about the latest trends in their diagnostics, therapy and treatment. At previous years, we have always managed to create a pleasant atmosphere for discussion and exchange of experience and opinions. We believe that you will find time to make your September days more professional and friendly, and talk about interesting topics, and share the results of your research with other conference participants even in today's hasty time. The Organizing Committee and all participants will look forward to your interesting expert contributions that infectology and parasitology offer in practice and in research.

VISCERAL TOXOCAROSIS IN THE MODEL OF WHITE MICE: EFFECT ON THE BODY

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ABSTRACT
The article presents the results of a hematological and histological study of laboratory white mice infected with invasive eggs of *Toxocara canis* (Werner, 1782). It was found, that during migration of Toxocara larvae through the body of non-specific hosts they are causing a complex harmful effect. So, indicators of infected animals' blood are characterized by violations such as erythrocytopenia, hypogemaglobinemia, leukocytosis, eosinophilia, the decrease in the total protein concentration, especially the albumin fraction, an increase in the concentration of total bilirubin, as well as the activity of the enzymes ALT and AST. Infected animals had violations of the beam structure and points of necrosis in the liver, signs of proliferative bronchitis and myositis.

INTRODUCTION
Toxocarosis is a parasitic disease, the pathogen of which is *Toxocara canis* (Nematoda, Ascaridata) in dogs, and *Toxocara cati (Toxocara mystax)* in cats. Researches of parasitologists are indicate a significant spread of toxocarosis invasion in Ukraine, both among animals and among humans [4]. *Toxocara* is geohelminth, infection of susceptible animals occurs as a result of ingestion of invasive eggs, which mature in the soil [6].
During migration in the host's organism, toxocara's larvae become the cause of visceral toxocarosis [2]. We decided to investigate the effects of *Toxocara* invasion on changes in hematological parameters and morphology of target organs of the larvae – liver, lungs and skeletal muscle. This is due to the fact that these organs in the process of hepatopulmonary migration of larvae are exposed to toxic, trophic, mechanical and inoculatory effects [1].
It is best to do an experimental reproduction of visceral toxocarosis, using laboratory rodents. These animals are non-specific hosts for toxocara, their larvae are capable to migrate through the body, but mature individuals of the parasite are not formed [2, 3]. Laboratory white mice were selected for infestation with the culture of invasive toxocara eggs, due to their small size and carelessness.

MATERIAL AND METHODS
In order to receive *T. canis* invasive eggs for further infecting laboratory mice with them, we used the «Method of cultivating of invasive eggs Toxocara genus and infecting of laboratory animals with them» [3].
To study the peculiarities of the pathogenesis of visceral toxocarosis, we formed 2 groups of laboratory white mice weighing 18–22 g (n=15). Animals of the 1st group served as controls. Mice of the 2nd group were infected at the rate of 1000 ± 12.0 *T. canis* invasive eggs per animal, mixing the suspension with food. Euthanasia of mice was carried out by intraabdominal administration of the drug Sedazin (Biowet Pulawy, Poland) at a dose of 1 mg of xylalin per 10 g of body weight.
The morphological parameters of blood were determined using the automatic hematologic analyzer "Medonic-Ca 620". Biochemical parameters of blood serum were determined using a semi-automatic biochemical analyzer "Rayto-1904C" closed type with a running cuvette.
To investigate histological changes, we took bits of the liver, lungs and skeletal muscle of white mice. For the manufacture of histological sections, fixed pieces of organs were poured in paraffin according to the generally accepted scheme. From each organ were made from 5 to 8 paraffin

blocks, of which 3–4 gistological sections (up to 10 microns thick) were performed at the microtome MS. Histopreparations were stained with hematoxylin and eosin. Morphometric studies were carried out in accordance with generally accepted methods [5].

RESULTS

Non-invasive Toxocara eggs were obtained by isolating them directly from the uterus of females obtained by deemillization of invasive puppys with piperazine adipinat (0.5/1 kg body weight). Later the eggs were incubated for 28 days at a temperature of 24 °C. In control of development, at the 7th day of the incubation process, it was found that evolving eggs had a dimming of the kidney-shaped form. When the incubation period was 14 days, the larvae had a ring-shaped form, and for 21 days, they were elongated and wound in a shell in the form of a spiral. On the 28th day, under the microscope, invasive, fully-formed *Toxocara* eggs were visible. The larvae became even more elongated and tightly placed in the shell. The movements of larvae became active and diverse – both in separate parts, and throughout the body.

Infestation of laboratory animals (white mice) with a suspension of invasive eggs allowed us to study the effect of *Toxocara* larvae in experimental conditions under visceral toxocarosis.

We conducted a study of blood of clinically healthy white mice and infected with *Toxocara* eggs.

As can be seen from the data given (Table 1), in the group of infected mice there was a sharp decrease in the number of erythrocytes (29.0%, p<0.001), an increase in the number of leukocytes (17.2%, p<0.001), eosinophils (9.5 times, p<0.001), a steady increase in the number of neutrophils of different (banded – by 26.7%, p<0.01, segmented – 57.8%, p<0.001), relative lymphocytopenia, increased monocyte levels by 94.0% (p<0.001) compared with healthy mice.

Table 1

Morphological parameters of laboratory white mice's blood, M ± m (n = 15)

Parameters, units of measure		Healthy animals	Animals, infected with *T. canis* eggs (30 days after experiment start)
Erythrocytes, T/L		9,39±0,21	6,71±0,18**
Leukocytes, G/L		9,07±0,28	10,55±0,21**
Basophils, %		-	0,60±0,30
Eosinophils, %		1,80±0,21	17,20±0,40**
Neutrophils, %	Young	-	-
	Banded	3,20±0,47	3,80±0,40*
	Segmented	19,80±1,15	32,20±3,16**
Lymphocytes, %		71,80±0,91	39,60±1,65**
Monocytes, %		3,40±0,33	6,60±0,58**

Notation: *p<0,01, **p<0,001 – compared with the animals of the control group.

The results of the biochemical study of experimental mice's blood are presented in Table 2.

Table 2

Biochemical parameters of laboratory white mice's blood, M ± m (n = 15)

Parameters, units of measure	Healthy animals	Animals, infected with *T. canis* eggs (30 days after experiment start)
Hemoglobin, g/L	144,50±5,96	98,00±4,01**
Total protein, g/L	49,90±1,02	38,60±0,89**
Albumin, g/L	27,70±0,42	20,90±0,31**
Globulin, g/L	22,20±0,78	17,70±0,83*
Albumin : Globulin	1,25:1	1,18:1
Total bilirubin, µmol/L	4,60±0,23	6,90±0,30**
ALT, U/L	28,60±3,93	69,80±4,19**
AST, U/L	49,40±2,14	95,90±3,84**

Notation: *p<0,01, **p<0,001 – compared with the animals of the control group.

We found a decrease in the hemoglobin content in the blood of infected mice (33.3%, $p<0.001$), a decrease in the total protein concentration (22.6%, $p<0.001$), including albumin (24.5%, $p<0.001$) and globulin (20.3%, $p<0.01$). According to the ratio of albumin and globulin, it can be seen that a sharp decline in the protein level occurred, primarily due to albumin. It was stated the increase in the concentration of total bilirubin (by 50%, $p<0.001$), as well as the activity of ALT (2.4 times, $p<0.001$) and AST enzymes (94.1%, $p<0.001$) in serum of mice, which were infected with *T. canis* invasive eggs.
We also conducted a study of histological sections from the organs of white mice, infected with *Toxocara* invasive eggs, as compared to those in clinically healthy animals. At the 30th day after infestation, the mice's liver has disturbances in placement of hepatocytes (they are remote from each other, the links between them are lost). The areas of necrosis in the liver were clearly visible. Throughout the lobe of the liver, or only in the central part of it, blood clots were observed. Between the lobules were marked enlargement of the connective tissue and degeneration of the liver tissue. The bile ducts are enlarged, their walls are thickened by edema and inflammation. Intercellular arteries of the liver of infected mice were enlarged in diameter, their walls thickened. At the site of the triad of bile ducts, atrophy of the connective tissue of the liver beams was observed.
In the parenchyma of lungs of infected mice, we observed expanded blood capillaries filled with blood. Small bronchial tubes were enlarged, surrounded by lymphoid infiltrate, indicating the development of the inflammatory process. The middle and large diameter bronchial tubes were enlarged, some of them filled with blood. Alveolar cavities had thinned walls, sometimes filled with an exudate, in which extinct epithelial cells were found. There are also areas of necrosis and enlargement of the connective tissue.
During the visceral toxocarosis, *Toxocara* larvae are able to migrate to the skeletal muscle, where they are subsequently encapsulated [1]. We decided to investigate how the process of larvae migration affects the histological structure of muscle tissue. Muscles of infected mice had a significant difference in the fact that myofibrils lose their course. Infiltration of lymphoid cells was observed between muscle fibers. Some muscle fibers were swollen, the borders between them were smoothed out.
The results obtained by us are indicate that *T. canis* larvae migration causes deep mechanical and toxic lesions of various organs of laboratory white mice, in particular liver, lungs and skeletal muscle.

DISCUSSION

Erythrocytopenia and hemoglobinemia of infected animals indicate the inhibition of the hematopoietic function of the bone marrow under the influence of the metabolites of *T. canis* larvae, as well as the tissue destruction products resulting from the migration of parasites through the body. Leukocytosis of infected mice is observed due to the stimulation of leukopoiesis by products of decomposition of tissue proteins that enter the bloodstream due to the mechanical effects of larvae, as well as *Toxocara* toxins. Acute eosinophilia in infected mice can be explained by the allergic effects of larval metabolites. A uniform increase in the number of neutrophils and bright monocytosis indicate the mild course of the overall inflammatory process.
A significant reduction in the total protein in serum of infected mice, and especially the albumin fraction, indicates, first of all, a violation of liver function. This pathology is also confirmed by the increased concentration of total bilirubin. The increased activity of the enzymes ALT and AST indicates not only the pathological state of the liver, but also the development of generalized myositis.
Results of the histological examination of *Toxocara* target organs during migration indicate severe mechanical and toxic lesions of the liver and lungs (as intermediate migration points), as well as skeletal muscles (as the "destination" of *Toxocara* larvae in an organism of a non-specific host).
Since the mice served as a model of visceral toxocarosis, we can imagine, how the migratory larvae syndrome is extremely dangerous for the body of specific and non-specific hosts of *T. canis*.

CONCLUSION

As a result of experimental reproduction of visceral toxocarosis, in the blood of white mice were registeredn: erythrocytopenia (6.71 ± 0.18 T/L), hypogemaglobinemia (98.0 ± 4.01 g/l), leukocytosis (10.55 ± 0 , 21 g/l) and eosinophilia (17.20 ± 0.40 %) ($p < 0.001$). Among the changes in the biochemical parameters of blood serum of mice, infected with invasive *T. canis* eggs, the most pronounced was the decrease in the total protein concentration (38.60 ± 0.89 g/L), especially the albumin fraction (20.90 ± 0.31 g/L), an increase in the concentration of total bilirubin (6.90 ± 0.30 µmol/L), as well as the activity of the enzymes ALT (69.80 ± 4.19 U/L) and AST (95.90 ± 3.84 U/L). Such animals had violations of the beam structure and points of necrosis in the liver, signs of proliferative bronchitis and myositis.

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