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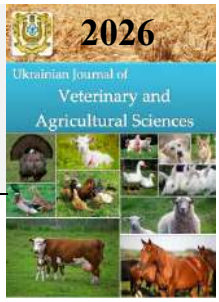

Antioxidant and immune status of puppies spontaneously infected with toxocariasis

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Contents

Abstract

Toxocariasis is one of the most widespread helminth infections in dogs and has significant veterinary, sanitary-epidemiological, and social importance, particularly due to the high susceptibility of puppies to infection. The disease is accompanied by damage to various organs and systems, the development of metabolic and immunological disorders, and may remain latent for a long time, complicating timely diagnosis and prevention. Processes of lipid peroxidation and dysfunction of the antioxidant defense system play an important role in the pathogenesis of toxocariasis and are closely associated with changes in the immune reactivity of the organism. The aim of this study was to comprehensively assess the antioxidant and immune status of puppies spontaneously infected with toxocariasis. The study was conducted on puppies aged 4–6 months, divided into a control group (clinically healthy animals) and an experimental group (puppies spontaneously infected with *Toxocara* spp.). Blood samples were analyzed to determine the intensity of lipid peroxidation processes, the activity of key enzymes of the antioxidant system and the glutathione system, as well as indicators of cellular, humoral, and nonspecific immune resistance. The results demonstrated that toxocariasis infection in puppies is accompanied by the development of pronounced oxidative stress, manifested by a significant increase in the levels of diene conjugates by 2.7 times and thiobarbituric acid-reactive substances by 1.7 times compared with clinically healthy animals. At the same time, a substantial decrease in the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and the content of reduced glutathione was observed, indicating depletion of both enzymatic and non-enzymatic components of the antioxidant defense system. Immunological studies revealed suppression of the cellular, humoral, and phagocytic components of the immune system, including a decrease in the number of T and B lymphocytes, bactericidal and lysozyme activity of blood serum, phagocytic activity of neutrophils, and phagocytic index, against the background of a significant increase in circulating immune complexes. The obtained data indicate that toxocariasis in puppies leads to the formation of oxidative-immune imbalance and the development of secondary immunodeficiency, which substantiates the feasibility of a comprehensive assessment of the body's protective systems and may be used as a scientific basis for developing approaches to the prevention and pathogenetic correction of toxocariasis in dogs.

Keywords: toxocariasis; puppies; antioxidant system; immune status; oxidative stress; immune reactivity.

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1. Introduction

Canine toxocariasis is one of the most widespread helminth infections and has significant veterinary, sanitary-epidemiological, and social importance (Despommier, 2003; Daryani et al., 2009; López-Osorio et al., 2020; Zheng et al., 2020; Mengarda et al., 2023). The causative agent of the disease, Toxocara canis, is characterized by high invasiveness, long-term environmental persistence of eggs, and a complex life cycle, which ensures prolonged maintenance of infection in dog populations, especially among puppies (Fenoy et al., 2001; Dantas-Torres, 2020; Overgaauw & Nijsse, 2020; Ardekani et al., 2022). Young animals are particularly susceptible to toxocariasis due to the functional immaturity of the immune system, intensive larval migration, and the pronounced toxic effects of larval metabolic products on the host organism (Degouy et al., 2001; Auer & Walochnik, 2020; Rostami et al., 2020; Li et al., 2022).

The pathogenesis of toxocariasis is shaped by the combined action of mechanical, toxic, immunological, and metabolic factors (Woodruff, 1987; Stybel et al., 2021b). Following ingestion of infective eggs, larvae hatch in the gastrointestinal tract, penetrate the intestinal wall into the bloodstream, and undergo hepatopulmonary migration. During migration, larvae cause mechanical tissue damage, microcirculatory disturbances, and the development of local inflammatory reactions. These changes are accompanied by the release of inflammatory mediators and the formation of cellular infiltration, which serve as one of the triggering mechanisms of systemic pathological processes (Schnieder et al., 2011; Strube et al., 2013; Ketzis & Lucio-Forster, 2020).

The disease is associated with damage to the digestive, hepatobiliary, immune, and hematopoietic systems, negatively affects animal growth and development, and may exhibit a latent or low-symptom course while being accompanied by profound metabolic and immunological disorders (Overgaauw, 1994, 1997; Gharekhani, 2014; Chidumayo, 2020; Jenkins, 2020; Mazur-Melewska et al., 2020; Miller, 2020).

Oxidative stress plays an important role in the pathogenesis of toxocariasis and develops as a result of excessive production of reactive oxygen species and disruption of the balance between prooxidant and antioxidant defense mechanisms (Said et al., 2020a, 2020b). Activation of lipid peroxidation leads to the accumulation of toxic lipid peroxidation products that damage cellular membranes, alter cellular functional status, and promote the progression of pathological processes in infected animals. In turn, the antioxidant system is a key component in maintaining cellular homeostasis and protecting the organism from oxidative damage (Stybel et al., 2021a).

Disturbances in the prooxidant–antioxidant balance are closely associated with changes in immune reactivity (Tokar et al., 2024a, 2024b). During parasitic infections, the immune system undergoes continuous antigenic stimulation, leading to strain on the cellular, humoral, and nonspecific components of immunity. Prolonged exposure to Toxocara antigens and their metabolic products may result in depletion of immune reserves, development of secondary immunodeficiency states, and a decrease in the overall resistance of the puppy's organism.

Despite the availability of isolated reports on immunological changes in canine toxocariasis, the issue of comprehensive evaluation of antioxidant defense status in relation to the immune status of puppies under spontaneous toxocariasis infection remains insufficiently studied. This determines the relevance of research aimed at deepening the understanding of pathogenetic mechanisms of toxocariasis and providing a scientific basis for approaches to correcting the identified disorders.

Aim of the Study – to comprehensively assess the state of protective systems in puppies spontaneously infected with toxocariasis by determining the intensity of lipid peroxidation processes, the functional activity of enzymatic and nonenzymatic components of the antioxidant defense system, as well as indicators of cellular, humoral, and nonspecific immune resistance.

2. Materials and methods

All experimental studies were conducted in accordance with the "General Ethical Principles of Animal Experiments" (Ukraine, 2001), in compliance with the Law of Ukraine "On the Protection of Animals from Cruel Treatment," as well as the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985).

To study the pathogenesis of toxocariasis in dogs, two groups of puppies aged 4–6 months were formed, with 10 animals in each group. Puppies in the control group were clinically healthy and free of helminth infections. Puppies in the experimental group were spontaneously infected with Toxocara spp. The main indicator of toxocariasis infection in dogs was the extent of invasion (EI, %).

For morphological and biochemical studies, blood samples were collected from the subcutaneous vein of the forearm into sterile tubes in a volume of 2–3 mL. To prevent blood coagulation, the inner walls of the tubes were moistened with a heparin solution.

The state of the antioxidant defense system was assessed based on the activity of antioxidant enzymes and parameters of the glutathione system. Superoxide dismutase (SOD) activity was determined using the method of Dubinina et al. (1983), catalase activity according to Koroliuk (1988), glutathione peroxidase and glutathione reductase activity according to Lemeshko et al. (1985), and reduced glutathione content according to Butler (1963). The intensity of lipid peroxidation was evaluated by measuring the levels of diene conjugates and thiobarbituric acid-reactive substances (Vlizlo et al., 2012).

Immunological parameters included the determination of neutrophil phagocytic activity and phagocytic index using the method of Hostev (1950), circulating immune complex levels by polyethylene glycol precipitation, and the total number of T and B lymphocytes by the spontaneous rosette formation method. Bactericidal and lysozyme activity of blood serum were determined using methods adapted in the Immunology Laboratory of the State Scientific Research Control Institute of Veterinary Medicinal Products and Feed Additives (Vlizlo et al., 2012).

Statistical analysis was performed using standard computer software (Statistica Version 6, StatSoft, Inc., SPSS Statistics 17.0) with determination of the arithmetic mean (M) and the standard error of the mean (m). Differences between groups were considered statistically significant at a probability level of $P < 0.05$ (ANOVA).

3. Results and discussion

Lipid peroxidation is a physiologically determined process that continuously occurs in the animal body and involves the direct transfer of oxygen to lipid substrates with the formation of peroxides, ketones, aldehydes, and other intermediate and final oxidation products. It should be noted that lipid peroxides are characterized by low stability and readily decompose with the formation of more stable secondary or intermediate products of lipid peroxidation, including alcohols, aldehydes, dialdehydes, and diene conjugates. Thiobarbituric acid-reactive substances (TBARS) are considered the final markers of peroxidation processes. Therefore, it is important to investigate the levels of diene

conjugates and TBARS in the blood of puppies spontaneously infected with toxocariasis.

According to the obtained results, the level of intermediate lipid peroxidation products in the blood of puppies from the experimental group significantly increased by 2.7 times ($P < 0.001$), while the level of final products increased by 1.7 times ($P < 0.001$). The elevated levels of diene conjugates and TBARS in the blood of puppies spontaneously infected with toxocariasis indicate activation of oxidative stress (Table 1).

At the same time, these puppies demonstrated a significant decrease in the activity of the main antioxidant defense enzymes. In particular, catalase and superoxide dismutase activities in the blood of toxocariasis-infected puppies decreased by 42.1 % ($P < 0.001$) and 34.3 % ($P < 0.001$), respectively, compared with the control group.

Regarding the glutathione component of antioxidant defense, puppies spontaneously infected with toxocariasis showed a decrease in glutathione peroxidase activity by 28.2 % ($P < 0.01$) and glutathione reductase activity by 22.2 % ($P < 0.01$) compared with control values. In addition, the content of reduced glutathione in the blood of the experimental group was significantly lower by 31.3 % ($P < 0.01$), indicating depletion of the non-enzymatic component of the antioxidant system in toxocariasis-infected puppies.

Table 1

State of the antioxidant defense system in puppies spontaneously infected with toxocariasis (M ± m, n = 10)

Parameters	Control	Experimental
Diene conjugates, OD units/mL	0.275 ± 0.013	0.748 ± 0.034***
TBARS, μmol/L	24.73 ± 0.23	41.46 ± 0.35***
Superoxide dismutase, arb. units/mg protein	15.84 ± 0.63	10.41 ± 0.71***
Catalase, mg H_2O_2	0.183 ± 0.011	0.106 ± 0.009***
Glutathione peroxidase, μmol $NADPH_2 \cdot h^{-1}$/mg protein	17.7 ± 0.87	12.7 ± 0.91**
Glutathione reductase, μmol $NADPH_2 \cdot h^{-1}$/mg protein	6.40 ± 0.32	4.98 ± 0.24**
Reduced glutathione, mmol/L	0.457 ± 0.023	0.314 ± 0.027**

Note: significance compared with control group: $P < 0.05$ – *; $P < 0.01$ – **; $P < 0.001$ – ***.

Thus, summarizing the obtained results, it can be stated that toxocariasis in puppies contributes to intensification of lipid peroxidation processes against the background of suppression of enzymatic and non-enzymatic antioxidant defense mechanisms, confirming the development of pronounced oxidative stress in infected animals.

Analysis of immune system parameters in puppies spontaneously infected with toxocariasis (Table 2) indicates the development of marked immunological disorders compared with clinically healthy control animals. In particular, puppies in the experimental group showed a significant decrease in the number of T lymphocytes by 7.2 % ($P < 0.01$) and B lymphocytes by 5.0 % ($P < 0.01$), indicating suppression of cellular immunity. In addition, infected puppies demonstrated a significant reduction in bactericidal and lysozyme activity of blood serum by 6.6 % ($P < 0.01$) and 6.1 % ($P < 0.05$), respectively, indicating weakening of nonspecific resistance factors.

A particularly notable finding was a significant increase in circulating immune complex levels in the blood of experimental group puppies by 74 % ($P < 0.001$) compared with the control group, reflecting intense antigenic stimulation and immune response tension during toxocariasis infection. Moreover, spontaneously infected puppies exhibited decreased neutrophil phagocytic activity to 27.4 ± 1.11 % and a reduced phagocytic index to 36.3 ± 1.71 % ($P < 0.05$), indicating suppression of cellular mechanisms of nonspecific defense.

Table 2

Immune system parameters in puppies spontaneously infected with toxocariasis (M ± m, n = 10)

Parameters	Control	Experimental
T lymphocytes, %	37.7 ± 1.42	30.5 ± 1.27**
B lymphocytes, %	17.4 ± 0.73	12.4 ± 0.80**
Bactericidal activity of serum (BAS), %	30.8 ± 1.25	24.2 ± 1.18**
Lysozyme activity of serum (LAS), %	26.3 ± 1.48	20.2 ± 1.23*
Circulating immune complexes, mg/mL	0.146 ± 0.005	0.254 ± 0.008***
Phagocytic activity, %	32.3 ± 1.20	27.4 ± 1.11*
Phagocytic index, %	44.2 ± 2.45	36.3 ± 1.71*

Note: significance compared with control group: $P < 0.05$ – *; $P < 0.01$ – **; $P < 0.001$ – ***.

Thus, the presented data indicate that toxocariasis in puppies is accompanied by the development of a secondary immunodeficiency state characterized by decreased functional activity of cellular, humoral, and phagocytic components of the immune system against the background of elevated circulating immune complex levels.

The obtained results demonstrate that spontaneous toxocariasis infection in puppies is associated with pronounced oxidative stress, which represents one of the key pathogenetic mechanisms of helminth-induced damage. A significant increase in diene conjugates and TBARS in the blood of infected puppies indicates intensification of lipid peroxidation processes and accumulation of toxic lipoperoxidation products capable of damaging cellular membranes, disrupting biomembrane permeability, and altering the activity of membrane-bound enzymes.

The decreased activity of superoxide dismutase and catalase in the blood of experimental puppies indicates depletion of the enzymatic antioxidant defense component under conditions of excessive reactive oxygen species formation. It is

known that during parasitic infections, activation of phagocytes and inflammatory cells is accompanied by an intense "oxidative burst," which, in the absence of an adequate antioxidant response, leads to destabilization of the prooxidant–antioxidant balance. In this context, reduced catalase and superoxide dismutase activity may be considered a consequence of functional overload and inactivation by lipoperoxidation products.

The observed suppression of the glutathione antioxidant system, including reduced glutathione peroxidase and glutathione reductase activity as well as decreased reduced glutathione levels, indicates impaired mechanisms of peroxide compound neutralization and maintenance of intracellular redox homeostasis. Since the glutathione system plays a leading role in free radical detoxification and cellular protection from oxidative damage, its depletion in toxocariasis creates conditions for further progression of dystrophic and immunopathological processes in puppies.

Disruption of antioxidant homeostasis is closely associated with changes in immune reactivity. The decreased number of T and B lymphocytes in the blood of infected puppies indicates suppression of cellular and humoral immunity, which may result from both the direct toxic effects of Toxocara metabolites and the indirect impact of oxidative stress on immunocompetent cells. Excess reactive oxygen species are known to induce lymphocyte apoptosis and impair proliferative activity, which is consistent with the obtained results.

Reduced bactericidal and lysozyme activity of blood serum, as well as suppression of neutrophil phagocytic activity and phagocytic index, indicate weakening of nonspecific resistance mechanisms in puppies during toxocariasis infection. This creates favorable conditions for secondary microflora involvement and complicates the disease course. Concurrent significant elevation of circulating immune complexes reflects intense antigenic stimulation and immune response tension, which during prolonged infection may contribute to the development of immunopathological reactions.

Thus, the obtained data confirm that toxocariasis in puppies is accompanied by the formation of closely interrelated oxidative and immune disorders underlying the development of secondary immunodeficiency. The identified changes substantiate the feasibility of including antioxidant and immunomodulatory agents in treatment and prevention regimens for toxocariasis in puppies to correct the revealed pathogenetic disturbances.

4. Conclusions

Toxocariasis infection in puppies is accompanied by the development of pronounced oxidative stress, manifested by a significant increase in diene conjugate levels by 2.7 times and TBARS by 1.7 times, against the background of a substantial decrease in the activity of key antioxidant enzymes – superoxide dismutase (by 34.3 %), catalase (by 42.1 %), glutathione peroxidase (by 28.2 %), and glutathione reductase (by 22.2 %) – as well as a 31.3 % reduction in reduced glutathione content. Simultaneously, spontaneously infected puppies exhibit suppression of cellular, humoral, and phagocytic components of the immune system, characterized by decreased numbers of T and B lymphocytes, reduced bactericidal and lysozyme activity of blood serum, diminished neutrophil phagocytic activity and phagocytic index, against the background of a significant 74 % increase in circulating immune complex levels, indicating the development of a secondary immunodeficiency state in puppies with toxocariasis.

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Conflict of interest

The authors declare no conflict of interest.

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