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HEMATOLOGICAL BLOOD INDICES IN DOGS ARTIFICIALLY INFECTED WITH OPISTHORCHIASIS

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Abstract
Northern Kazakhstan is known for its high incidence of opisthorchiasis, a parasitic infection affecting both humans and animals. In this study, hematological and biochemical blood indices were assessed in dogs infected with opisthorchiasis to evaluate the disease's impact on the body. The research involved dogs artificially infected with *Opisthorchis felineus* metacercariae, with dogs serving as definitive hosts. Blood samples were taken weekly and analyzed to monitor hematological and biochemical changes throughout the infection. Significant deviations from normal blood indices were observed. Eosinophil counts increased early in the infection, peaking on days 10 and 26, while lymphocyte levels rose, indicating an active immune response. Hemoglobin, erythrocyte, and platelet counts showed complex dynamics, with erythrocyte levels tending to decrease over time. Biochemical analysis on day 53 revealed elevated levels of ALT, AST, GGT, bilirubin, and total amylase, indicating significant liver involvement. The study concludes that the observed alterations in hematological and biochemical parameters hold significant diagnostic and monitoring potential for infections caused by parasites from the Opisthorchiidae family in canines.

Keywords: *Opisthorchis felineus,* metacercariae, dog, hematological and biochemical parameters, animal health.

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INTRODUCTION

One of the notable foodborne trematode (FBT) infections is opisthorchiasis which is caused by parasites from the family Opisthorchiidae. According to the World Health Organisation, it is the eighth most important food-borne parasite (Worl Health Organization 2014). The infection is typically associated with two parasite species, *Opisthorchis felineus* (Rivolta, 1884) and *Opisthorchis viverrini* (Poirier, 1886), with *O. felineus* being more common in Eastern Europe, Russia and Kazakhstan (World Health Organization 2021a). Around 200,000 persons worldwide are affected by FBTs including opisthorchiasis leading to approximately 7,000 deaths annually (World Health Organization 2021b).

The life cycle of *O. felineus* includes two intermediate hosts as well as one definitive host. Definitive hosts include over 33 mammal species, mainly carnivores like domestic cats, dogs, wolves, foxes and bears while humans act as accidental hosts (Aunpromma et al. 2012, Perakanya et al. 2022). Infection in the definitive host occurs when fish infected with metacercariae are consumed. The parasite then migrates in the digestive system into liver bile ducts where it matures and starts providing eggs.

The first intermediate host, mollusks of the family Bithyniidae, consumes the eggs after eggs are excreted into the environment through the host's feces, which must get into the water. The parasite goes through several developmental stages within the mollusks, including sporocysts, rediae, and cercariae. After free-swimming cercariae infect cyprinid fish, the second intermediate host, they encyst and develop into metacercariae, the stage at which they are capable of infecting fish-eating mammals.

Opisthorchiasis is transmitted when raw or undercooked freshwater fish infected with the parasite's metacercariae stage is consumed. Acute symptoms of *O. felineus* infection in humans typically appear 10 to 26 days after exposure and include fever, anorexia, nausea, vomiting, abdominal discomfort, malaise, myalgia, arthralgia, and urticaria (Jang et al. 2023). Pets are frequently infected with parasitic illnesses that affect the liver, circulatory system, digestive system, and other organs. In addition to releasing metabolic by-products into the host, these parasites physically damage tissues, triggering immune responses and metabolic abnormalities. Monitoring hematological and biochemical markers is essential to understanding the impact of parasitic infections on the health of infected animals. Factors such as red blood cell (RBC) concentration, hemoglobin, and hematocrit are highly informative, as anemia often results from chronic liver disease and inflammation. Leukocyte count is also important, as an eosinophilic response to worms can indicate parasitic infection, suggesting the need for diagnostic testing. Furthermore, measuring liver enzymes, such as alanine aminotransferase (ALT), and bilirubin levels helps assess the extent of liver damage and bile duct obstruction caused by parasites (Wysmołek et al. 2020). Thus, the use of veterinary hematological methods is not only valuable for diagnosing but also for evaluating the disease's effects on the animals, aiding in treatment and prognostic decisions.

This study examines the effects of *O. felineus* infection on hematological and biochemical parameters in dogs, providing important preliminary insights into the pathophysiology and impacts of the disease on animal health.

MATERIAL AND METHODS

Ethics approval

Ethical approval for the study involving animals' participants was obtained from the local ethics committee of the Faculty of Veterinary Medicine and Animal Husbandry Technology at Seifullin University (Protocol No. 1, dated April 7, 2015), Astana, Kazakhstan. This was done in compliance with internationally accepted standards for the ethical management, maintenance and feeding of laboratory animals which are stipulated in both the International Guidelines for Biomedical Research Involving Animals (2012) and the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (2005) during design and execution of experiments.

Animal Quarantine

The experimental animals were housed at the veterinary clinic of the Faculty of Veterinary Medicine and Animal Husbandry Technology at Seifullin University. A separate building with an isolated entrance was used for this purpose. The room where the animals were kept measured 7×7 m². Before the start of the experiment, the room was disinfected with a 4% emulsion of the "GLAK" product (BioChemPharm, Raduzhny, Russia).

The experimental group consisted of mixed-breed dogs, approximately 3 months old, all born from the same litter. A total of seven animals were selected, including five experimental and two control subjects. At the start of the experiment, the animals' weights ranged from 10.8 to 11.3 kg.

Each experimental dog was housed separately, with one dog per cage. The cages were equipped with special trays to collect food waste and feces. Feeders and trays were cleaned and disinfected daily using a 2% calcium hypochlorite solution, after which they were thoroughly rinsed with water for reuse. The tray cages were also treated with heat from a blowtorch.

Animal care adhered to the standards outlined in Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Guidelines for animal housing and care were strictly followed.

The dogs were fed 2-3 times daily, with continuous access to water. The room was equipped with natural ventilation to ensure sufficient fresh air circulation, reduce odors from food waste and animal excretions, and control excess heat and humidity, while avoiding drafts.

As the animals had not previously been vaccinated, they were immunized against major infectious diseases, including distemper, parvovirus enteritis, hepatitis, adenovirus infection, and leptospirosis. The "Geksakanivak" vaccine (NPViZC Vetzverotsentr, Moscow, Russia) was administered according to the manufacturer's instructions.

Ten days after vaccination, a routine deworming was carried out using the drug "Vermis-EX" (Aulendorf, Germany).

Infection of Dogs

Heavy helminth eggs Two days after deworming, blood and fecal samples were collected from all the dogs. The animals were infected with the *Opisthorchis felineus* pathogen by feeding them infected fish from the Cyprinidae family (ide, crucian carp, bream, tench, roach, Siberian roach).

Throughout the experiment, two blood samples were taken weekly from the animals for hematological (with heparin) and serological (without heparin) analyses. Blood serum was preserved for future use in serological and immunological studies.

Hematological parameters, such as red blood cell count, hemoglobin concentration, color index, platelet count, leukocyte count, and erythrocyte sedimentation rate, were measured using standard methods. Fecal samples were collected starting on the 15th day after infection.

Fulleborn method (flotation method)

A portion of the feces is mixed with 20 parts of a saturated salt solution (density 1.18), which is gradually added in small portions. Large particles that float to the surface are removed, and the mixture is left undisturbed for 45-60 minutes. During this time, eggs with a lower specific weight than the salt solution rise to the surface. A few drops of the surface film are collected with a wire loop (1 cm in diameter) and transferred to a glass slide for examination. Heavy helminth eggs, including *Opisthorchis* eggs, do not float in the saturated salt solution. Therefore, after removing the surface film, the liquid is drained, and drops are taken from the sediment with the loop, transferred to a slide, and examined under a microscope (Połozowski et al. 2006, Abdybekova et al. 2023).

Sequential washing method (sedimentation method)

A small portion of feces (5-10 g) is mixed with 10 times its volume of water. The mixture is then filtered through a metal sieve or gauze and allowed to settle for 5 minutes. Afterward, the liquid layer is drained, and fresh water is added to the sediment, allowing it to settle again for another 5 minutes. This process is repeated until the top layer of liquid becomes clear. Once the liquid is drained, the sediment is examined under a microscope for the presence of trematode eggs (Moitinho Mda et al. 1992).

Darling method

This method combines sedimentation and flotation procedures. The feces are mixed with water to achieve a semi-liquid consistency and then centrifuged for 3-5 minutes, allowing helminth eggs to settle at the bottom. The liquid is drained from the test tube, and Darling's solution (glycerin mixed in equal parts with a saturated solution of table salt) is added to the sediment. The mixture is thoroughly mixed and centrifuged again for 3-5 minutes. After this, the eggs of parasitic worms float to the surface. The surface film is then removed with a metal loop, placed on a glass slide, covered with a cover slip, and examined under a microscope (Abdybekova et al. 2023).

Blood Analysis

Blood samples were collected from all the dogs in the experimental group before infection and analyzed for the main hematological indices at Olimp (Astana). According to the results, all blood indices of the experimental animals were within the physiological norm. Blood samples were then collected again on days 10, 26, and 37 after the onset of infection. Analysis of these blood results, including hematological and biochemical studies, showed a strong correlation with the progression of the invasive process.

RESULTS

Feces were sampled separately from each dog, and the presence of helminth infections in the experimental animals was determined using the Fulleborn method (10 g of feces). The studies revealed the presence of *Toxocara* eggs (round in shape with a cellular outer shell) in the experimental dogs: Dog No. 1 had 19 eggs, Dog No. 2 had 37 eggs, Dog No. 3 had 11 eggs, Dog No. 4 had 8 eggs, Dog No. 5 showed a negative result, Dog No. 6 had 14 eggs, and Dog No. 7 showed a negative result.

Thus, coprological studies demonstrated that 5 out of the 7 experimental dogs were infected with toxocariasis (*Toxocara canis*), with eggs of this parasite found in the fecal samples (Fig. 1).

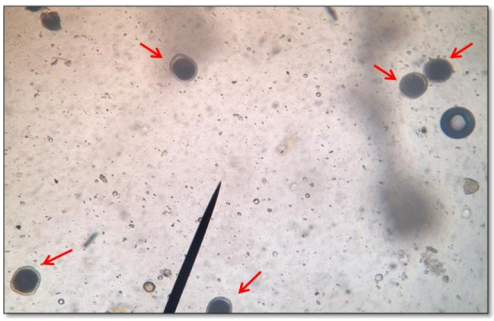

Figure 1. Results of coprological Studies of dogs: arrows indicate *Toxocara canis* eggs (×25). Image courtesy V. Kiyan.

Twenty-four hours after deworming, fecal samples were collected from each dog, and helminthoscopy was performed. This examination revealed mature forms of roundworms from the Anisakidae family (Fig. 2): Dog No. 1 had 15 specimens, Dog No. 2 had 12 specimens, Dog No. 3 had 17 specimens, Dog No. 4 had 8 specimens, Dog No. 5 showed a negative result, Dog No. 6 had 19 specimens, and Dog No. 7 showed a negative result.

The coprological studies confirmed that all dogs were infected with toxocariasis, caused by round helminths of the Anisakidae family. After deworming, further coprological studies were conducted to check for the presence of helminths. The results are presented in Figure 2.

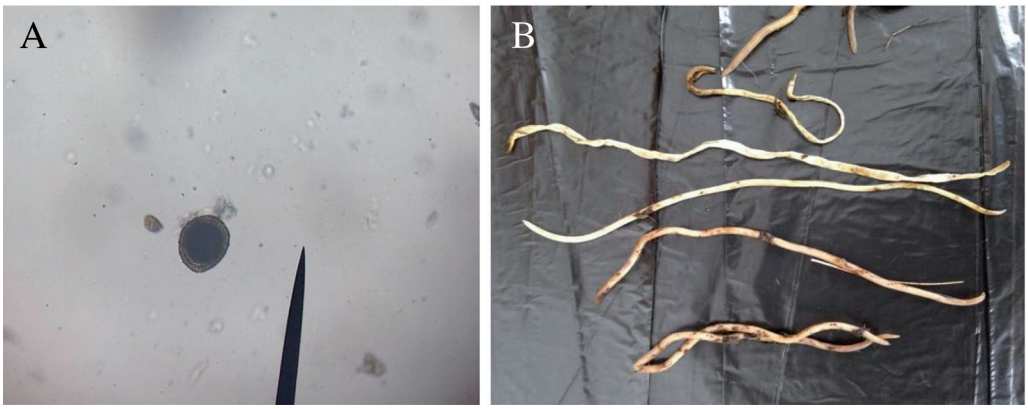

Figure 2. Helminths of the *Anisakidae* family: A – helminth eggs (×25), B – mature form. Images courtesy V. Kiyan.

Mature worms, gray-yellow in color, were observed, with females measuring 10-18 cm in length (males are smaller). At the anterior end of the body, there are cuticular wings that protrude noticeably in a half-arc. The male's tail end is curved, and a distinctive feature of the species is the presence of a "stomach" located between the esophagus and intestines. The eggs are dark gray and round (0.068-0.075 mm) with a well-defined cellular structure.

A second round of deworming and coprological studies was conducted, confirming that the animals were completely cleared of the infection. However, further coprological studies revealed the presence of mature *Toxocara* worms exiting the digestive tracts of the dogs for 4 days and *Toxocara* eggs for 5 days following the initial deworming.

On the 6th day after the beginning of deworming, control coprological studies were performed using the Fulleborn method. The results revealed *Toxocara* eggs in the following dogs: Dog No. 1 had eggs, Dog No. 2 had 8 eggs, and Dogs No. 3, No. 4, No. 5, No. 6, and No. 7 showed negative results.

Given the high level of infection in the

experimental animals with the causative agent of toxocariasis, a second deworming was conducted 7 days after the initial treatment. The drug "Cestem" (Libourne, France) was administered at a dose of 1 tablet per 10 kg of body weight, in the form of a water suspension. The composition of the drug includes 50 mg of praziquantel, 150 mg of febantel, and 50 mg of pyrantel.

Coprological studies conducted on the 11th day after the first deworming and the 3rd day after the second deworming showed no presence of *Toxocara* eggs or other helminths in the feces of the experimental dogs.

Following the start of the infection in the animals, we initiated coprological studies on the 32nd day post-infection. These studies employed the Fulleborn method, as in previous experiments, along with additional methods – the Darling method and the sequential washing method. The results of the coprological studies obtained after infecting the experimental animals are presented in Table 1.

From Table 1, it is evident that coprological examinations of feces from the infected dogs using the Fulleborn method did not reveal eggs of the causative agent of opisthorchiasis during the experiment. Similar results were obtained using the Darling method. However, the sequential washing method confirmed the infection of the dogs with the causative agent of opisthorchiasis on the 47th and 48th days post-infection by detecting the pathogen's eggs in their feces. This result further highlights the inadequacy and low sensitivity of the other diagnostic methods.

Table 1. Results of coprological studies of dogs after infection with *Opisthorchis felineus* metacercariae.

Method	Days After Infection	Experimental dog number					Helminth species
		No.1	No.2	No.3	No.4	No.5	
Fulleborn	32	-	-	-	-	-	*O. felineus*
	33	-	-	-	-	-	*O. felineus*
	34	-	-	-	-	-	*O. felineus*
	35	-	-	-	-	-	*O. felineus*
	36	-	-	-	-	-	*O. felineus*
	37	-	-	-	-	-	*O. felineus*
Darling	38	-	-	-	-	-	*O. felineus*
Fulleborn	40	-	-	-	-	-	*O. felineus*
Darling	41	-	-	-	-	-	*O. felineus*
Fulleborn	42	-	-	-	-	-	*O. felineus*
Darling	43	-	-	-	-	-	*O. felineus*
Fulleborn	44	-	-	-	-	-	*O. felineus*
	46	-	-	-	-	-	*O. felineus*
Sedimentation Method	47	+	+	+	+	+	*O. felineus*
	48	+	+	+	+	+	*O. felineus*

On the 10th day after infection, blood samples were collected from the experimental dogs for hematological analysis. The results of these blood tests are presented in Appendix 1.

When considering the general leukocyte counts in the blood, it is important to note that the data are inconsistent, with values varying among individual animals. This variation in leukocyte counts may be related to changes in the proportions of different types of leukocytes. A comprehensive blood test identifies the percentages of various leukocyte types, including neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Certain diseases may cause an increase in some leukocyte types, while others decrease proportionally.

In our case, we observed that the levels of various types of leukocytes fluctuated with the course of infection. The key indicator in this experiment is the eosinophil count, as this marker typically remains elevated and plays a significant role in combating parasitic infections. Eosinophils are characterized by their expression of Fc receptors specific for IgE, which is crucial for their cytotoxic properties and active involvement in antiparasitic immunity. The data related to eosinophil counts in the blood of the experimental animals are presented in Figure 3.

Regarding the data presented in Figure 3, the eosinophil count in the dogs' blood on the 10th day after infection showed a sharp increase, ranging from 7.9% to 31.3%. Another spike was recorded on the 26th day post-infection, after which the eosinophil levels began to decrease by the 37th day.

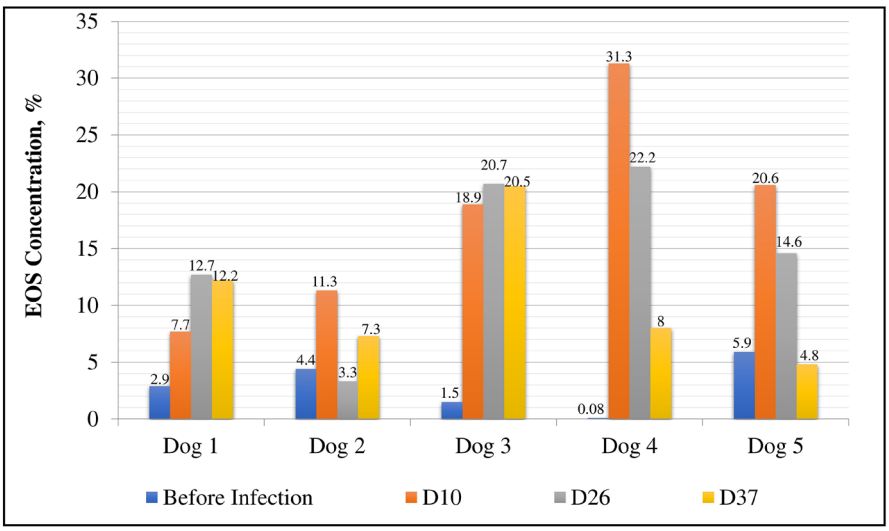

Figure 3. Eosinophil concentration in the blood of dogs. D = days.

Additionally, it is important to note that lymphocyte levels increased in all experimental animals as early as the 10th day post-infection. Lymphocytes, which are key components of the immune system, release antibodies into the bloodstream that neutralize and remove antigen molecules from the body. This indicator, along with the eosinophil count, confirms the presence of an invasive process in the experimental animals.

When examining segmented neutrophil counts, the results varied among the animals. Three of the five dogs exhibited an increase in segmented neutrophils during the infection, while the

levels in the remaining two dogs showed a significant decrease.

The total basophil counts also presented ambiguous results. In dogs No. 1, 4, and 5, basophil levels exhibited an inverse relationship with segmented neutrophil counts. When neutrophil levels increased, basophil counts in these animals decreased as the infection progressed. In contrast, dog No. 3 had normal basophil levels on the 10th day, a sharp decrease by the 26th day, followed by an increase by the 37th day.

Monocyte levels in the blood of the experimental animals, except dog No. 2, showed a decrease as the invasive process developed. In terms of plasma cells, a reduction in their total count was observed in all dogs throughout the infection.

Thus, a detailed analysis of the different types of leukocytes in the experimental animals suggests that each cell type reacts uniquely to the presence of the Opisthorchiasis pathogen and the progression of the invasive process.

When examining platelet counts, two of the experimental dogs exhibited an increase during the invasion, whereas the other animals showed a decrease. A similar pattern was observed in thrombocyte levels, which reflect the percentage of blood volume occupied by platelets. The average platelet volume, however, decreased across all animals throughout the experiment.

Significant changes were also seen in erythrocytes, hemoglobin, and its distribution by volume, with all these indicators showing an increase during the invasive process. Erythrocytes, which are formed in the red bone marrow, contain hemoglobin responsible for transporting oxygen from the lungs to tissues and organs, and carbon dioxide back to the lungs. An increase or decrease in hemoglobin volume leads to polycythemia or anemia, respectively. These three indicators are closely interconnected, meaning a shift in one trigger correlative change in the others. The variations in erythrocyte and hemoglobin levels in the dogs during the infection are presented in Figure 4.

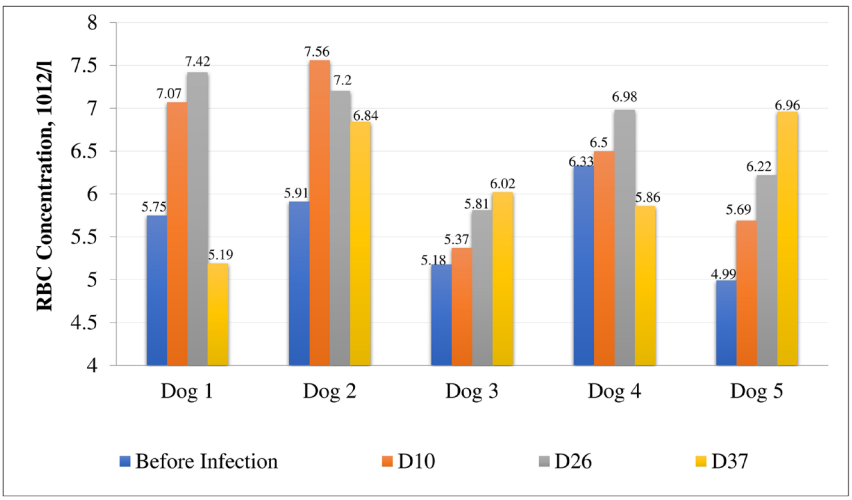

Figure 4. Erythrocyte indices in the blood of animals. D = days.

Interestingly, indices such as the average volume of an erythrocyte, the distribution of erythrocytes by volume, and the average hemoglobin content per erythrocyte remained largely within normal ranges, with only minor deviations. However, the average concentration

of hemoglobin in erythrocytes stayed within normal limits at the early stages of the experiment but increased by the 37th day of infection. The blood color index, which reflects the relative hemoglobin content in erythrocytes, also showed minor fluctuations. A close correlation was observed between erythrocyte indices and hematocrit levels. Hematocrit progressively increased alongside the total erythrocyte count throughout the experiment.

The dynamics of total hemoglobin content in the animals' blood increased in line with erythrocyte levels, closely matching the changes seen in hemoglobin distribution by volume, where a similar rise was noted.

These findings indicate that opisthorchiasis infection in animals leads to deviations from the physiological norm in hematological parameters, with the most pronounced changes observed in eosinophils, lymphocytes, erythrocytes, hemoglobin concentration, and its overall distribution by volume.

In addition to hematological parameters, biochemical blood markers were studied on the 53rd day post-infection. The results of these analyses are presented in Table 2.

This table shows that, compared to the control group, the serum indices of the experimental animals exhibited an increase in almost all of the studied parameters. Notably, there was an elevated content of ALT and AST transaminases, GGT, total amylase, total protein, and bilirubin in the blood of the experimental dogs. These indices are key markers in the diagnosis of liver diseases, including opisthorchiasis.

In conclusion, the study of the invasive process in animals infected with opisthorchiasis indicates that the main hematological and biochemical indices are highly likely to reveal the presence of an invasive process in the body.

Table 2. The main biochemical parameters of experimental animals on the 53rd day after infection.

Index	Norm	Experimental dog number				
		Control	1	2	4	5
Total protein	56-75 g/l	47.5	58.5	53.8	49.7	55.3
ALT	0.00-39.0 IU/l	20	212	95	41	62
AST	0.00-56.0 IU/l	41	84	58	45	54
Bilirubin (total)	0.00-17.0 µmol/l	<2.5	<2.5	<2.5	<2.5	<2.5
Bilirubin (direct)	0.00-5.0 µmol/l	<1.5	0.6	<1.5	0.8	0.6
Bilirubin (indirect)	1.50-17.0 µmol/l	1	1.9	1	1.7	1.9
GGTP	0.00-87.0 IU/l	0	3	2	1	4
Total amylase	28.00-100.0 IU/l	515	886	737	782	486
Cholesterol	3.11-5.18 µmol/l	6.82	6.68	7.17	5.54	6.21

Opisthorchiasis infection leads to an increase in the levels of eosinophils, lymphocytes, erythrocytes, hemoglobin concentration, ALT and AST transaminases, GGT, total amylase, total protein, and bilirubin in the blood.

DISCUSSION

Opisthorchiasis is a foodborne trematode (FBT) infection (Bouvard et al., 2009; Pakharukova et al., 2023). The pathology of opisthorchiasis is significant in both humans and animals, with symptoms ranging from gastrointestinal discomfort and fever to more severe liver damage. Understanding the hematological and biochemical changes in infected animals, particularly domestic dogs, can provide critical insights into the disease's progression and its impact on health (Qian et al. 2024).

Studies on the hematological parameters of dogs infected with helminths such as *T. canis*, *T. leonine*, and *E. granulosus* have shown notable changes. Trunova et al. (2009) reported an increase in leukocytes by 1.8-1.3 times, band neutrophils by 2.3-2.6 times, eosinophils by 2.1-3.1 times, and lymphocytes by 16.3-18.1%. Conversely, there was a 20% decrease in segmented neutrophils, hemoglobin concentration dropped by 22.4-29.9%, erythrocytes reduced by 12.2%, and young neutrophils appeared consistently at 0.8-1.4%.

Similar results were observed by Polshkova E.V. (2005), who noted leukocytosis with eosinophilia up to 9.6%, a decrease in total serum protein by 1.1 times, increased AST activity by 1.4-1.5 times, a reduction in ALT activity by 1.1 times, a decline in the phagocytic index by 6-7%, and a reduction in T- and B-lymphocytes by 4-6.4%.

In cases of opisthorchiasis, the blood parameters of infected dogs also undergo significant changes. Hematological changes were detected as early as the 15th day of infection, gradually intensifying and peaking by the 90th day of invasion. On the 30th day, erythrocyte counts were 9.45% lower, and hemoglobin was 10.7% lower compared to control animals. By the 60th day, these reductions reached 14.4% and 31%, respectively.

Leukocyte counts increased by 1.5 times on the 30th day, 1.6 times on the 60th day, and 1.4 times on the 90th day compared to controls. In the leukocyte formula, segmented neutrophils decreased by 23.3-31.4%, basophils by 18.8-38.9%, and monocytes by 7.9-5.4%, while band neutrophils increased by 2.2-2.3 times, eosinophils by 1.6-1.95 times, and lymphocytes by 11.9-22.3% (Shinkarenko et al. 2005).

The study reveals significant hematological changes in dogs infected with opisthorchiasis. Chronic liver damage, which often accompanies the disease, manifests in various blood abnormalities (Karbysheva et al. 2021). Among these, elevated white blood cell counts – especially eosinophils – are characteristic of parasitic infections, with levels increasing sharply within 10 days of infection (up to 31.3% in some cases). These trends are characteristic of eosinophils during an invasive process, as most eosinophils do not remain in the blood for long. After migrating into tissues, they tend to stay there for extended periods (Novitskiĭ et al. 2008, Litvinova et al. 2008).

A decline in red blood cells (RBCs) and hemoglobin concentration was observed during the infection, likely due to chronic inflammation and liver damage (Karbysheva et al. 2021, Lim et al. 2021). The average hemoglobin concentration fluctuated but eventually increased by the 37th day, suggesting a dynamic response to the infection.

The eosinophilic response is particularly noteworthy due to its role in parasitic immunity (Saijuntha et al. 2019, Doanh et al. 2016). The expression of IgE receptors on eosinophils and their cytotoxic properties against parasites underscores their importance in managing opisthorchiasis (Sripa et al. 2017). Meanwhile, the fluctuating levels of lymphocytes, neutrophils, and basophils indicate that different components of the immune system are variably activated throughout the infection process.

In addition to hematological markers, biochemical parameters related to liver function showed significant findings. Liver enzymes (ALT and AST) were elevated, indicating liver injury and inflammation, typical of opisthorchiasis due to bile duct obstruction and liver tissue damage.

Increased levels of gamma-glutamyl transpeptidase (GGT) further highlighted biliary injury, while elevated bilirubin levels suggested impaired liver function and bile flow, likely due to the parasite's presence in the bile ducts, obstructing bile secretion.

The study also highlights challenges in diagnosing opisthorchiasis. Traditional coprological methods commonly employed in parasitology, such as the Fulleborn and Darling methods, were unable to detect parasite eggs until the 47th day post-infection. To address this limitation, an additional sedimentation method was implemented, which demonstrated superior effectiveness in detecting parasite eggs earlier and more reliably. This highlights the importance of refining diagnostic techniques to improve the accuracy and timeliness of parasite detection.

Hematological and biochemical testing, however, proved highly informative, revealing distinct changes that could serve as reliable indicators of infection. These markers can aid veterinarians and researchers not only in diagnosing the disease but also in monitoring its progression and response to treatment (Smout et al. 2011).

CONCLUSIONS

Opisthorchiasis significantly alters both hematological and biochemical parameters in infected dogs, particularly affecting the liver and immune response (Srithai et al., 2021). The study identifies key diagnostic markers, such as elevated eosinophil counts, liver enzymes (ALT, AST, GGT), and bilirubin levels, which reflect the severity of the disease. These findings are critical for diagnosing and managing opisthorchiasis in domestic animals and could help in developing more effective diagnostic and treatment protocols. Understanding these changes is essential for improving animal health outcomes and controlling the spread of this parasitic disease.

Building on the findings of the study, further research could focus on the following areas:

- Comparative Studies Across Host Species. Comparing the hematological and biochemical profiles of opisthorchiasis in different host species (e.g., dogs, cats, and other susceptible animals) to identify species-specific variations in disease presentation and severity;
- Development of Early Diagnostic Methods. Focusing on the development of more sensitive diagnostic techniques, such as molecular assays (e.g., PCR or qPCR), overcoming the limitations of traditional coprological methods;
- Evaluation of Treatment Protocols. Conducting clinical trials to evaluate the efficacy of existing anthelmintics and explore new therapeutic agents.

These directions could collectively contribute to a holistic understanding of opisthorchiasis and support the development of integrated approaches to its diagnosis, treatment, and prevention.

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Appendix 1. Hematological parameters of dog's blood post infection with *Opisthorchis felineus*. Cell colors show their indicators in comparison with the parameters before infection: red - higher. blue - lower. white - within average.

Index	Norm	Average index before infection	Days After Infection	Experimental dog numbers				
				1	2	3	4	5
Hemoglobin	110-140 g/l	122.8±12.01	10	156	165	120	134	118
			26	161	155	131	147	131
			37	117	151	136	138	158
RBC	3.5-4.8×10^{12}/l	5.632±0.55	10	7.07	7.56	5.37	6.5	5.69
			26	7.42	7.2	5.81	6.98	6.22
			37	5.19	6.84	6.02	5.86	6.96
Color Index	0.86-1.05	0.652±0.02	10	0.66	0.65	0.67	0.64	0.62
			26	0.65	0.65	0.68	0.63	0.63
			37	0.68	0.66	0.68	0.71	0.68
Hematocrit	29.0-41.0%	42.3±4.87	10	53.6	56.6	41.2	47.8	42.1
			26	56	50.8	43.3	49.5	45.4
			37	38.2	49.5	39.4	43.2	52.7
Mean corpuscular volume	77.0-108.0 fl	75.02±2.35	10	75.9	74.8	76.7	73.6	73.9
			26	75.5	70.6	74.5	70.8	72.9
			37	73.6	72.4	65.4	73.8	75.8
Mean corpuscular Hb content	25.0-35.0 pg	21.74±0.65	10	22	21.8	22.3	21.5	20.7
			26	21.8	21.5	22.6	21	21.1
			37	22.6	22	22.6	23.5	22.7
Mean corpuscular Hb concentration	300-360 g/l	290±10.07	10	290	292	291	292	281
			26	288	305	303	297	289
			37	307	304	346	318	299
RBC distribution by volume	11.5-14.5%	15.64±0.84	10	15	15.1	13.6	14.2	14.6
			26	14.4	14.9	14.1	15.2	15.1
			37	14.3	14.7	22.2	14.1	14.3

Index	Norm	Average index before infection	Days After Infection	Experimental dog numbers				
				1	2	3	4	5
HGB distribution by volume	22.0-32.0 g/l	18.3±2.94	10	20.7	21.2	20.3	23.2	22.2
			26	22.6	17.8	21.8	24.8	22.7
			37	23.3	23.9	50.7	22.1	22.5
Platelets	180-400×10^9/l	423.8±139.63	10	485	401	484	315	457
			26	533	316	413	369	434
			37	382	346	237	370	378
Thrombocrit	0.15-0.40%	0.452±0.14	10	0.44	0.35	0.44	0.29	0.45
			26	0.46	0.32	0.37	0.33	0.39
			37	0.38	0.31	0.23	0.32	0.33
Mean platelets volume	7.4-10.4 fl	10.74±0.77	10	9.1	8.8	9.1	9.2	9.9
			26	8.6	10.2	9	9	8.9
			37	10	9.1	9.5	8.6	8.7
Leukocytes	5.5-12.5×10^9/l	14.538±1.79	10	12.84	10.69	13.37	18.42	18.7
			26	11.01	10.33	12.63	11.94	12.71
			37	10.37	15.98	10.97	11.93	10.96
NEU%3	%	39.56±26.63	10	56.4	41.2	50.8	44.1	52.1
			26	42.9	63.4	46.5	44.4	49.9
			37	44.4	69.7	43.7	51.6	51.1
NEU#2	2.0-5.5×10^9/l	5.572±3.43	10	7.24	4.4	6.78	8.12	9.74
			26	4.73	6.55	5.88	5.3	6.34
			37	4.6	11.14	4.79	6.16	5.6
EOS%3	0.5-6.0%	2.956±2.3	10	7.7	11.3	18.9	31.3	20.6
			26	12.7	3.3	20.7	22.2	14.6
			37	12.2	7.3	20.5	8	4.8
EOS#2	0.02-0.3×10^9/l	0.4486±0.39	10	0.99	1.21	2.52	5.77	3.86
			26	1.4	0.34	2.62	2.65	1.86
			37	1.26	1.16	2.25	0.95	0.52
BAS%3	0.00 -1.00%	0.44±0.29	10	0.4	0.5	0.2	0.2	0.2
			26	0.4	0.4	0.03	0.2	0.2
			37	0.2	0.1	0.5	0.3	0.2
BAS#2	0.00-0.065×10^9/l	0.066±0.05	10	0.05	0.05	0.02	0.04	0.03
			26	0.04	0.04	0.03	0.02	0.03
			37	0.03	0.02	0.05	0.03	0.03

Index	Norm	Average index before infection	Days After Infection	Experimental dog numbers				
				1	2	3	4	5
MON%3	2.0-12.0%	32.5±23.38	10	10.7	10.3	4.6	6	9.8
			26	9.2	6.5	3.8	5.8	8.9
			37	9	7.2	8	5.9	11.1
MON#2	0.09-0.60×10⁹/l	4.882±3.62	10	1.37	1.1	0.61	1.11	1.82
			26	1.02	0.67	0.48	0.7	1.13
			37	0.94	1.16	0.88	0.7	1.22
LYM%3	42.0-74.0%	15.06±4.29	10	16	29	19.8	14.1	13.1
			26	27.3	21.2	22	23.3	19.7
			37	27.5	12	23.2	27.3	24.7
LYM#2	1.20-3.00×10⁹/l	2.194±0.71	10	2.05	3.1	2.65	2.61	2.45
			26	3.01	2.19	2.78	2.78	2.5
			37	2.85	1.91	2.54	3.26	2.7
PLA%	≥0	9.3±1.5	10	8.9	7.7	5.8	4.2	4.3
			26	7.4	5.3	6.6	4.1	6.6
			37	6.6	3.7	4.6	6.9	8.1
ESR	4-10 mm/h	2±0	10	2	2	2	2	2
			26	2	2	2	2	2
			37	2	3	2	2	2