PATHOLOGIC MORPHOLOGICAL DIAGNOSIS OF HELMINTHIASIS

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Abstract

The article presents the results of research on the differential diagnosis of pathological dogs at various zoonotic helminths. Total for 2015 was dissected 89 corpses of dogs of various breeds and ages, 12 of which various types of helminths were found. The material for the study of differential pathological changes in dogs helminthoides served 12 corpses of dogs brought from veterinary clinics in the city of Almaty, to determine the cause of death. Of those surveyed, eight dogs were brought to agony condition with severe clinical signs. As a result of pathological studies of corpses in the heart and lungs of 2 dogs found helminths nematodes in the genus Dirofilaria, in 3 dogs in the small intestine - nematodes Toxocara canis, in 2 dogs in the small intestine and in the stomach are found nematodes Toxascaris leonina, y one bile ducts in the liver trematode species found Opisthorchis felineus, from 3 - in the front part of the small intestine mature cestode species found Echinococcus granulosus and one - nematodes Trichinella spiralis. Extensiveness of invasion (EI) amounted to 13.4%, the intensity of infection (II) at various helminthoses ranged from 1 to 29 specimen.

Key words: *toxocarosis, toxascaridosis, opisthorchosis, trichinosis, echinococcosis, dirofilariasis, multiceptosis, hydatigenosis, dipilidiosis, trematodes, cestodes, nematodes, extent of infestation, the intensity of infection, etiology, obligate host, the definitive host, epizootology, epidemiology, contamination, pathomorphology, infestation, helminth, dog, scatological practices, enteritis, degeneration, inflammation*

INTRODUCTION

In Kazakhstan has its helminth infections epizootologic features due to the specific climatic and socio-economic conditions. Almost the entire territory - is favorable for mass propagation of helminth, including dedicated to natural homes. The problem is caused by helminth infections wide circulation of pathogens in the environment and the lack of appropriate measures to identify and de-worming of infected animals - obligate definitive host (pet dogs and cats) [1].

The true incidence of dogs in our country helminths unknown because it is not conducted official registration. In respect of Kazakhstan data helminthiasis of animals and people in the available scientific literature, we have not found. Due to the lack of awareness of veterinarians helminth infections are often under different diagnoses of non-parasitic etiology [2].

In recent years, in major cities of Kazakhstan increased the number of domestic carnivores. The accuracy of determining the number of dogs and cats is difficult, as most of them are wandering [3]. Most of the stray dogs and cats is a purebred animals and the majority of purebred animals are pets and kept in private homes and well-appointed apartments. With such a large number of dogs and cats, besides many of them neglect the problem of environmental contamination with faeces of these animals in an urban environment is becoming more acute. Surveys conducted in various countries, established a significant contamination of the soil in the settlements with helminth eggs varying from 2,9 to 60% of the positive samples [4,5]. In recent years, increasing the number of domestic carnivores, contributes to the spread of infectious diseases among animals and humans, including helminth infections [6].

Knowledge of the species composition of helminths in dogs, study of the propagation of helminthiasis, extensiveness and intensity of infestation, as well as the age and seasonal dynamics of the necessary knowledge epizootology of helminth infection in domestic carnivores, pathological features, as well as epizootology and epidemiology of parasitic diseases in Kazakhstan [5].

This will help to more accurately and effectively carry out preventive and curative measures against these invasions. To date, I accumulated a fairly extensive literature on helminth and helminthiasis of domestic dogs [7, 8].

According to the authors, including helminth infections of dogs in the big cities of Kazakhstan dominated such as toxocariasis, toxascaridosis, opisthorchosis, trichinosis, echinococcosis, dirofilariasis, multiceptosis, hydatigenosis, dipilidiosis and others. Particularly three species of helminths carnivores of the above - toxocariasis, hydatidosis and trichinosis pose a serious threat not only to a specific host, but also to humans.

Hence the study of pathological situations on basic helminthological diseases of dogs is not only an urgent problem, but almost necessary. In all regions of the Republic of Kazakhstan the most widespread high incidence and intensity of infection are opisthorchosis, trichinosis, echinococcosis toxocariasis and dirofilariasis [5].

In recent years, there are more cases of deaths of dogs diseases caused by helminthiasis. History confirms that the dogs were observed fallen in contact with the parasite [8.9]. However, the analysis of the literature data revealed that helminths and, accordingly, they cause disease almost domestic carnivores have not been studied in the cities of Kazakhstan. In this regard, we consider it necessary to present the scientific and the general public the results of our observations.

RESEARCH METHODS

The work was done on the basis of the laboratory "Antiparasitic Biotechnology" the department "Biological Safety" of the Kazakh national agrarian university.

The material for the study is based on fresh blood, feces and organs from patients with various helminth dogs. Blood tests for the presence of microfilariae was performed by the modified Knott. For this purpose, a standard tube to which to prevent clotting was added a few drops of 20% sodium citrate solution and 1 ml of fresh blood, and mixed well. The blood of a dog taken from the saphenous veins of the forearm. Then, 1 ml of blood was poured into the centrifuge tube and 10 ml of 2% formalin. The resulting mixture was centrifuged at 1500 rev / min for 5 minutes. The supernatant was decanted, the precipitate was mixed with 0.1% solution of methylene blue, hematoxylin in equal amounts, then wet smear made and examined under a microscope. Fixation was carried out in a mixture of formalin and rectified alcohol in a ratio of 1:10.

For faecal examination studies have used the system for the collection and processing of parasites in the feces «Parasys» and hubs for collecting and filtering of fecal «Parasep SF» samples, excluding any researcher contact with infested material. Pathologic method included mortem autopsy cadaver dogs to identify and describe the macroscopic changes in the organs and tissues for A.V.Zharov`s, I.V.Ivanov`s and A.P.Strelnikov`s methods (2003). Autopsy of animals was carried out with all precautions, instructing the past, pathologists, specialists, animal carcasses after opening disposed of. Histopathological examination of organs and tissues was performed to detect microscopic changes, as well as for the study of the relation of the internal organs. Wiring and fill material was carried out by conventional methods in Pathomorphology by management G.A.Merkulova material for histological examination were fixed in 10% neutral formalin and Carnoy's fluid. Thereafter, serial paraffin sections were prepared thicknesses of 5 and 6 mm. Ultrathin sections were prepared on a semi-automatic microtome HEOTION ERM 3100 and microtome MC-2. Celloidin prepared, paraffin and frozen sections. Sections were stained with conventional and some histological methods: hematoxylin-eosin, Van Gieson processor apparatus for coating slices Leica №S4040 / №000000358.

Histological slides are studied binocular microscope MBI-6 at different magnifications. If you suspect trichinosis conducted a study of digestion methods in simulated gastric fluid for the detection trichinella larvae.

Results of the study. We investigated 89 of these 12 dogs dogs afflicted with worms: from 2 found nematodes in the genus Dirofilaria, have 3 dogs - nematodes Toxocara canis, at first - fluke Opisthorchis

felineus, at 3 Echinococcus granulosus and even in 3-s dogs - nematode Trichinella spiralis. Extent of infestation (EI) was 13,4%, the intensity of infection (II) at different helminthoses ranged from 1 to 29 copies. The results are shown in Table 1.

Table 1. Prevalence of dogs different species of helminths (according to post-mortem autopsies)

Number of autopsies	Infected by helminths		Types of detected of helminths:									
			Toxocara		Echinococcosis		Dirofilariosis		Trichinella		Opistorchiasis	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
89	12	13,4	3	25,0	3	25,0	2	16,6	3	25,0	1	8,3

From Table №1 shows that out of 89 affected dogs exposed worms 12 or 13,4%. Because detected at autopsy helminth species toxocara reached 25,0%; echinococcus- 25,0%; dirofilaria – 16,6%; Trichinella – 25,0%; opisthorchis – 8,3%. Here are the most characteristic pathological changes in helminthiasis registered dogs at autopsy. Pathological changes in the showdown with heartworm dogs were similar and typical for this invasion. When dirofilaria infection in dogs clinically manifested: ascites and edema of the extremities, shortness of breath, mild dry cough, shortness of breath, when listening to the lungs - rattling. And also marked non-specific clinical signs as: loss of appetite, lethargy, weight loss, general weakness, fatigue and drowsiness.

For the detection of parasite larvae, we took blood from peripheral parts of the body affected dogs, making thick smears and microscopic examination at a magnification of 140 times. Erythrocytes were observed between single helminth larvae stained blue. With the opening of all dogs mentioned average fatness, anemia mucous membranes with mild jaundice. The surface of the peritoneum was dull, slightly swollen with grayish overlays. All the cavities of the body contained seroplastic exudate. First, in the abdominal and thoracic cavities. All dogs had increased heart, the right heart chamber expanded, characterized by changing the ratio of the thickness of the myocardium of the right half to the left, as 1: 8 at a rate of 1: 3. At the heart cavities contained poorly clotted blood with the presence of friable blood clots dark red with a bluish tint, in the right half of the heart and pulmonary arteries were observed mass of adult nematodes. Their number reached 20 to 26. They were of different sizes, some specimens of helminthiasis in length reaches 23 cm, is in the blood freely. In the cavity of the heart, they formed coils, wrapping her heart valves. The morphological characteristics, taking into account localization nematodes found were related to the genus Dirofilaria (Figure 1).

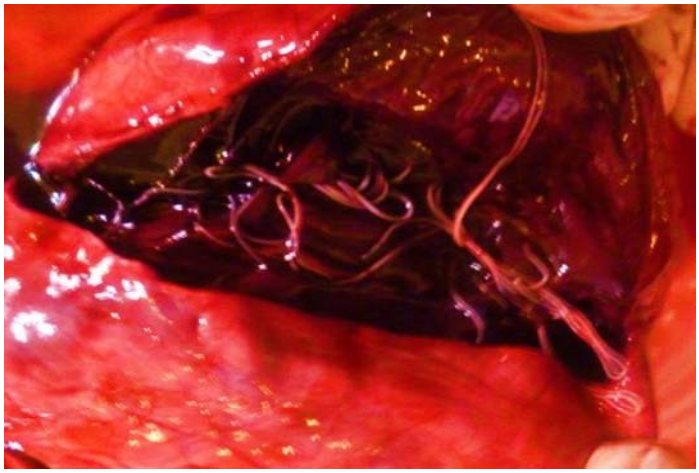


Figure 1. Dirofilaria in of the dog ventricle of the heart cavities

The myocardium dull, grayish, loose consistency on the cut fibrous structure flattened. Noted necrotizing ulcerous endocarditis. These were accompanied by a change of heart blood circulation in all the other organs in the form of venous plethora, cyanosis.

In the lungs marked venous congestion and edema. The apical lobe of both lungs was determined by focal serous-catarrhal bronchopneumonia.

The liver was observed chronic venous congestion and parenchymal degeneration. The liver is increased in size, with the cut surfaces and unevenly dyed. In general, red-brown background visible grayish-brown areas with indistinct borders. Figure lobes is not expressed.

The gall bladder is stretched a lot of thick, dark green bile. The stomach and intestines were observed catarrh. The stomach was not the content, its mucosa, swollen, reddened and folded, covered with small amount of muddy, viscous mucus. Similar, but less pronounced changes were found in the mucosa of the small intestine.

The spleen is reduced in size. In the kidney marked protein and fatty degeneration and venous congestion. Mediastinal, mesenteric, gastric, portal and perirenal lymph nodes were enlarged in size, gray-yellow, thick consistency. On the surface of the cut running down their muddy gray-yellow liquid, the figure was flattened. Microscopic examination of parenchymal organs and tissues of dogs, as well as studies of samples of animal blood have shown that under the influence of parasites of the genus Dirofilaria in dogs serious body changes.

In histopathological examination revealed myocardial dystrophic and atrophic changes of certain groups of cardiomyocytes. Many cardiomyocytes were able granular dystrophy. Noted fiber muscle symplast, fragmented plots disintegration of muscle fibers. The individual muscle fibers were observed karyolysis.

The endocardium was observed necrotizing process and deposition of fibrin. In the kidneys, along with venous congestion, hemorrhage, edema the inter-channel connective tissue, serous and sero-hemorrhagic glomerulitom, marked protein dystrophy, plasmolysis proximal tubule epithelium and foci of necrotic collapse of unresponsiveness.

In the liver - necrosis, protein and fatty degeneration and venous congestion. At the same time, they looked swollen hepatocytes, gaps intralobular capillaries reduced the cytoplasm of liver cells became cloudy, the cytoplasm contained a large number of granules of protein nature. The boundaries of the modified cells and the outlines of the nuclei were not always distinct. We cannot be seen or been in a state of pyknosis and lysis in the most affected by the core cells. Separate groups of hepatocytes in a state of fatty degeneration. Vascular liver Network strongly hyperemic in the lumen of the vessel revealed a lot of broken red blood cells. In acute lung marked venous congestion and edema. Capillaries

and veins the inter-channel connective partitions interlobular connective tissue expanded and filled with blood. The lumina of the alveoli and bronchioles are filled with a homogeneous mass, eosin stained in a light pink color and air bubbles (Figure 2).

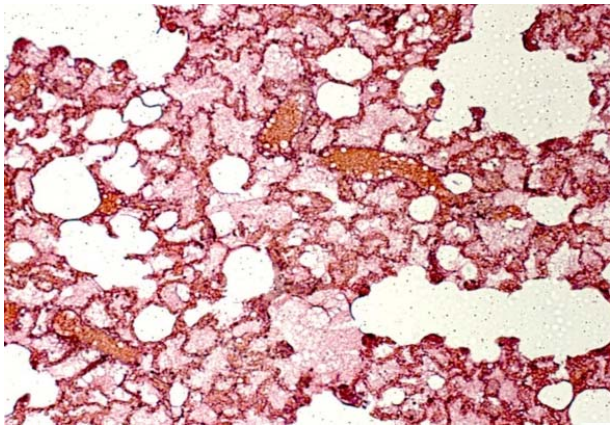


Figure 2. Edema of and venostasis in the lungs of dogs with dirofilariasis

In the stomach and small intestine marked serous-catarrhal. The mucous membrane of the stomach and small intestine is partially desquamated. intestinal cells were in granular, and hydropic degeneration of the mucosa. The blood vessels of the villi and submucosa dilated, filled with erythrocytes. On the mucosal surface and between the fibers contained catarrhal exudate. The mesenteric, gastric, portal and Kidney lymph nodes - serous inflammation.

In the spleen, characterized by changes in venous congestion, depletion of lymphocytes pulp, reduction of Malpighian cells, increased hemosiderosis. Pathological changes at autopsy echinococcosis when dogs were typical for this invasion. Before opening the 3 dogs that died from echinococcosis, it was revealed from the anamnesis from the owners that dogs fed raw meat and internal organs of sheep. All the dogs were hunting. Clinically, the disease manifested decreased appetite, diarrhea, vomiting, weakness, fever, at the end of the disease from lack of appetite and signs of nervous system excitation.

With the deterioration of general condition of clinically sick animals in a veterinary clinic were conducted laboratory tests and found that in the blood decrease in the number of red blood cells, hemoglobin, a sharp slowdown in leukocytosis and eosinophilia, and in studies of feces in the system for parasite collection and processing in the stool «Parasys» samples and hubs for collecting and filtering of fecal «Parasep SF» found echinococcus eggs. With the opening of the main dogs pathomorphologic changes found in the small intestine. We all studied dogs vstrechali imaginal forms of echinococcus (Figures 2, 3).

There was marked congestion of the mucous membrane of the small intestine, especially the villi, as well as catarrhal or hemorrhagic inflammation. Connective basis of their loosened and desquamated epithelium.

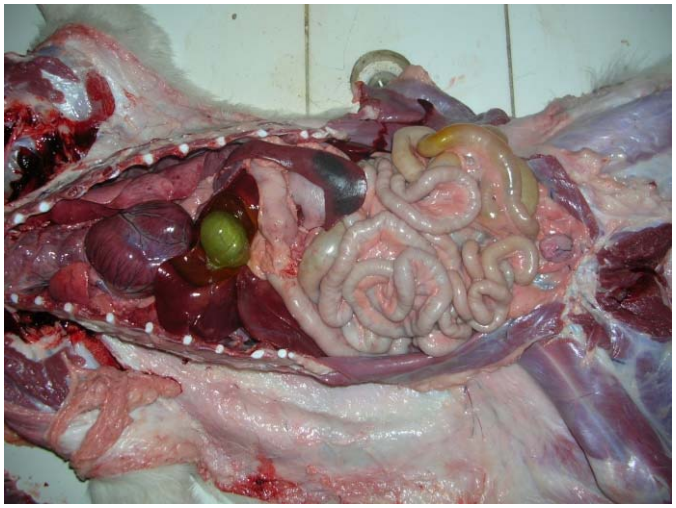


Figure 2. Changes in the small intestine of dogs with echinococcosis

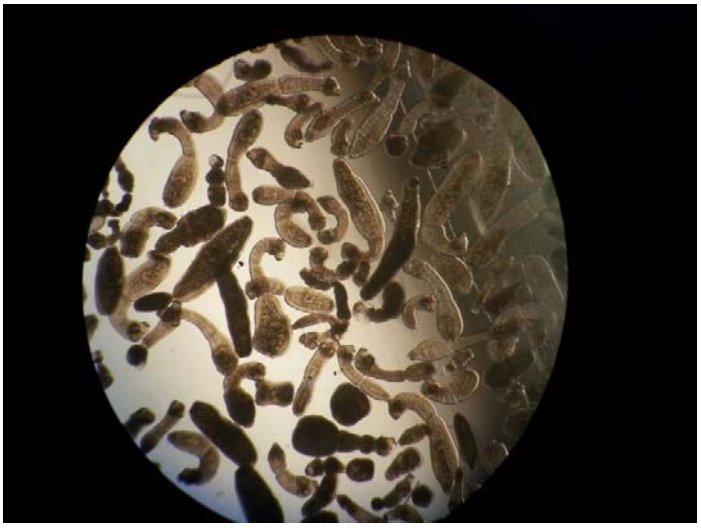


Figure 3. The imaginal stage of echinococcus of the dogs bowel

Clinical and pathological changes at autopsy at toxocarosis were typical for this invasion. Toxocarosis dogs clinically emaciation, anemia, a disorder of the digestive tract activity. Appetite weakened, marked diarrhea alternating with constipation, colic and vomiting, nervous phenomena in the form of seizures. The vomit found toxocara.

With the opening of dogs infected with Toxocara canis, observed subcutaneous tissue edema. All sections of the intestine observed petechial hemorrhage around them accumulated neutrophils, eosinophils and histiocytes, fibroblasts. Intestine red, swollen, reveal focal catarrhal enteritis, in some areas - small ulceration (Figures 4,5).



Figure 4. Catarrhal enteritis at of the dog toxocarosis



Figure 5. Toxocara Toxocara canis in the dog's intestine

Research histological preparations fine department intestine showed that the vessels of mucous and submucosal layers expanded with blood. Surface epithelium and crypt epithelium in some areas necrotic or desquamated. Connective tissue mucosa abundantly infiltrated neutrophilic leukocytes, eosinophils and fibroblasts. Submucosal loose as a result of accumulation of edema fluid; marked by cellular proliferation of connective tissue around the blood vessels. In some parts of the lung tissue expressed cell proliferation and thickening of the connective tissue septa interalveolar, capillaries filled with blood in them, diapedetic bleeding, eosinophils and infiltrated by lymphoid cells. Around the vessels - loosening connective tissue, collagen fibers between - eosinophils and fibroblasts. In some areas of lung tissue destroyed interalveolar partitions. In places of localization of larvae toxocara - foci of necrosis with eosinophils. The bronchi dilated, their swollen mucous membrane, fold in the lumen - the accumulation of exfoliated epithelial cells and red blood cells. Lymphocytes, plasma cells distributed uniformly throughout the lung tissue, but their maximum amount is in the peribronchial and perivascular tissue. On the pleura in places where larvae toxocara entered the capillary walls and into the lumen of the alveoli are seen small hemorrhages and inflammatory lesions of gray. And in some places visible light parasitic nodules of gray color, the size of a millet grain. On microscopic examination, but the larvae of the parasite, noted neutrophils, eosinophils, white blood cells, histiocytes and fibroblasts. The connective tissue necrotizing calcifications center. All parenchymal organs in a state of degeneration.

With the opening of two dogs registered trihinelllez. When dogs study on trichinosis, history data were collected at the beginning. According to the veterinarian clinical picture trichinosis manifested itself in the second week after a possible infection and manifested by fever, swelling of the muzzle, conjunctival

hemorrhage, retinal and nail beds, mottled rash, cough, dyspnea, dysphagia, muscle weakness. After 3 weeks after the onset of the clinical picture of the dog fell. With the opening of the dogs who died of trichinosis larvae Trichinella were found in the diaphragm muscle, especially in the chewing muscles, peritoneum and the muscles of the hindquarters. Histologically, in places of penetration of larvae observed accumulation of edema fluid, hemorrhage, accumulation of lymphoid cells. Muscle fibers are damaged. Mostly sarcolemma in the muscle fibers torn and encapsulated by fibrous connective tissue. Trichinella larvae in capsules whitish color, the size of a millet grain. The nodules foci of calcification seen. The mucosa catarrhal inflamed, swollen, reddened, with hemorrhages. To clarify the diagnosis for trichinosis carried out sampling of the diaphragm muscles of legs, skeletal muscle and studied by provarivaniya muscles in simulated gastric fluid (Figure 6).

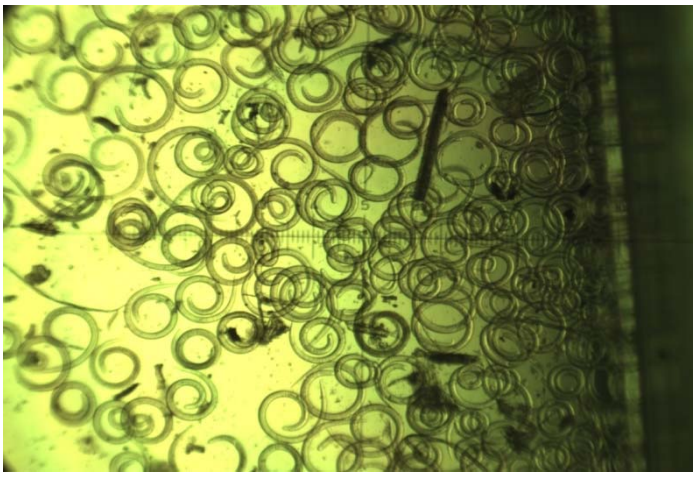


Figure 6. Trichinella larvae obtained by digestion of the muscles in the artificial gastric juice

With the opening of the dogs at one in the bile ducts of the liver and gall bladder were found fluke Opisthorchis felineus, which are hazardous to human infection. With the opening of the main changes were characterized by an increase, jaundice, liver uneven coloring, seals liver consistency, extension of the bile ducts, gallbladder wall thickening, filled with a yellowish greenish bile. The bile ducts and gall bladder encountered parasites - opisthorchis (Figures 7,8).



Figure 7. The gall bladder is filled with a yellowish-greenish bile opisthorchosis of dogs



Figure 8. Mature opisthorchosis of the bile ducts of the liver and gall bladder of the dog

CONCLUSION

In recent years, among the dogs in Kazakhstan are often recorded worms. Among them, mainly recorded nematodes in the genus Dirofilaria, Toxocara canis, Toxascaris leonina, Tricinella spiralis; trematodes - Opisthorchis felineus; cestodes - Echinococcus granulosus, which can often be fatal in dogs. The clinical picture of the disease depends on the number of worms in the body, toxicity toxins isolated from them, the age and physiological status of the animals. With the opening of the dogs died from helminth infections, pathological changes were observed in all the internal organs, but especially in the gastro-intestinal tract. In dogs, the fallen from heartworm, basic localized pathological changes in the heart and were presented atrophic, dystrophic and necrobiotic processes. In addition, products of metabolism of nematodes causing circulatory disorders, degenerative and inflammatory processes in parenchymal organs. When trichinosis dogs marked atrophy and necrosis of the muscles. When toxocara infection characterized by redness, lobular proliferation of lymphoid histiocytic liver cells lymphoextravasatis. The main pathologic manifestations are opisthorchosis cholangitis, cholecystocholangitis and cirrhosis.

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