MONITORING OF THE ASSOCIATED COURSE OF LEPTOSPIROSIS AND BABESIOSIS IN DOGS OF THE SUMY REGION OF UKRAINE

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Abstract

Leptospirosis and babesiosis in dogs may be associated. These diseases often have similar symptoms and mask each other, or are asymptomatic, which complicates the diagnosis, significantly worsens the prognosis and reduces the survival of sick animals. Monitoring of the associated course of leptospirosis and babesiosis in dogs makes it possible to study the frequency of this parasitocenosis in dogs and the peculiarities of its course, which is the basis for improving the diagnosis and treatment of sick animals.

The aim of the research. Monitor the associated course of leptospirosis and babesiosis in dogs.

Methods. The research was conducted in the private veterinary clinic «Vetservice» in Sumy and in the serology department of the Sumy Regional State Laboratory of the Civil Service of Ukraine for Food Safety and Consumer Protection. Blood samples were taken from dogs came for treatment to the «Vetservice» clinic. The diagnosis of babesiosis was established directly in the clinic by the results of erythrocyte microscopy in thin smears of peripheral blood of sick dogs stained by Giemsa. The diagnosis of leptospirosis was established in the serology department of the Sumy Regional State Laboratory based on the results of a study of the serum of sick dogs in the microagglutination reaction. The subjects of the study were clinically ill and suspected dogs with the associated course of leptospirosis and babesiosis, as well as blood samples from these animals. Babesium trophozoites were detected in erythrocytes under an immersion microscope, a magnification of ocular 10×90, and the presence of antibodies to leptospira was detected by the presence of agglutination of leptospira in microagglutination reaction.

Results. In the study of the incidence of dogs associated with leptospirosis and babesiosis during 2014–2019, according to the clinic «Vetservice» found that the number of reported cases of associated leptospirosis and babesiosis annually varies from 9 to 28 cases. This parasitocenosis is registered all year round with peaks in spring and autumn, moderately in summer and rarely in winter. Purebred animals get sick several times more often than non pedigree ones. During the period from 2016 to 2019, the number of diseases in females and males was approximately the same. During the period from 2014 to 2015, males were more often ill.

Conclusions. The prevalence of the associated course of leptospirosis and babesiosis in dogs of the Sumy region of Ukraine was determined with the determination of seasonal, breed and sexual dynamics of this parasitocenosis.

Keywords: associated course of leptospirosis and babesiosis in dogs, laboratory diagnosis of leptospirosis and babesiosis, blood, microagglutination reaction, microscopy.

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1. Introduction

The subjects of these studies were clinically ill and suspected dogs associated with leptospirosis and babesiosis, as well as blood samples from these animals. Babesia trophozoites were detected in erythrocytes under an immersion microscope, magnification of ocular 10×90, and the presence of antibodies to leptospira was detected by the presence of agglutination of leptospira in microagglutination reaction.

Dogs with an associated course of leptospirosis and babesiosis are periodically registered in veterinary clinics around the world, particularly in Europe [1, 2]. These diseases are characterized by a similar course and can mask each other, which delays timely diagnosis, complicates treatment and worsens the prognosis. Under such conditions, it is necessary to study the associated course of leptospirosis and babesiosis in dogs with the study of its course, which will understand the prevalence of this parasitocenosis, help speed up diagnosis and, based on existing clinical cases, develop more effective treatment regimens [3, 4].

Leptospirosis is a common bacterial infection in dogs worldwide, particularly on the European continent [1, 2]. The relevance of research on this disease is also due to the fact that leptospirosis also affects humans and can be transmitted from animals. Dogs become infected with leptospirosis

in the absence of annual vaccination, in contact with sick dogs or their urinary marks in the environment, through murine rodents, as well as from productive animals through meat, offal, dairy products contaminated with leptospira [2, 3]. Leptospirosis can occur in sick dogs acutely or chronically, manifesting itself only in the presence of concomitant pathology. The causative agents of leptospirosis in dogs are most often *L. icterohaemorrhagiae*, *L. cunicola*, *L. grippityphosa*, *L. australis* [5, 6]. Leptospira affect the kidneys, liver, central nervous system, causing endogenous intoxication due to exo- and endotoxins leptospira and their breakdown products, hemorrhagic syndrome, ulcers on mucous membranes and skin, jaundice, renal and hepatic failure, infectious-toxic. Dogs with leptospirosis often have such non-specific manifestations as anorexia, pyrexia, tremor, weakness, vomiting, diarrhea with or without blood impurities, dark urine color [7, 8].

Babesiosis is a widespread blood-borne parasitic disease of dogs, endemic on all continents, where parasitic mites are registered, which is acute and chronic. The causative agents of the disease are *Babesia canis* and its subspecies: *B. canis canis*, *B. rossi*, *B. vogeli*, as well as *B. gibsoni* and *B. conradae*. Carriers and definitive hosts of babesia are parasitic Ixodes mites of the genera *Ixodes*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Rhipicephalus*, which inoculate babesia to warm-blooded intermediate hosts during blood suction [9, 10]. *Babesia* affects erythrocytes, liver, kidneys, spleen, causing hypoxia and intoxication of all organs and tissues [11, 12]. Babesiosis is characterized by hyperthermia, intoxication, dehydration, anemia, jaundice, splenomegaly, neutrophilia, hemoglobin and thrombocytopenia, hyperbilirubinemia, hemoglobinuria, tachycardia, tachypnea; recently, the presence of bloody diarrhea in patients with babesiosis dogs has been increasingly registered [13, 14].

Babesiosis, like leptospirosis, can be acute or chronic, with clinical manifestations similar to those of leptospirosis, and in fact affecting the same organs and tissues of sick dogs, especially the liver and kidneys, which significantly complicates diagnosis and significantly impairs prognosis. Complications of both leptospirosis and babesiosis have been shown to be the development of systemic inflammatory response syndrome (SIRS) and multiorgan dysfunction syndrome (MODS), which is often fatal. In the case of the associated course of leptospirosis and babesiosis, the dog's body actually receives a double lesion of the same organs and tissues, which often ends in death. In the associated course of leptospirosis and babesiosis, in addition to treatment, diagnosis is also difficult, because these diseases have similar symptoms and can mask each other. For example, relatively often leptospirosis in dogs is chronic, manifesting itself only in acute babesiosis [15, 16].

Leptospirosis is diagnosed most often by the reaction of microagglutination of blood serum in the laboratory [17, 18]. Urine microscopy is also performed in a darkened field of the microscope, but this method is imperfect, because in the absence of leptospiuria in a sick dog or with the previous administration of antibiotics to the animal, the diagnosis will be false negative. The method of PCR diagnostics also has its drawbacks, as it can give a false-negative result in the accidental absence of the leptospira genome in the test sample of biological material [19, 20].

Therefore, the main **aim of the study** was to monitor the associated course of leptospirosis and babesiosis in dogs in the Sumy region during 2014–2019. The objectives of the study were to determine the number of sick animals, to establish the seasonal, breed, sexual dynamics of the incidence of dogs on this parasitocenosis.

2. Materials and methods

The studies were conducted during 2014–2019 in a private veterinary clinic «Vetservice» Sumy, Ukraine (reception and clinical examination of animals, blood samples, epidemiological evaluation and statistical processing of data) and in the serology department of the Sumy Regional State Laboratory State Service of Ukraine for Food Safety and Consumer Protection (study of dog sera in microagglutination reaction).

All experimental procedures and surgical interventions were performed in accordance with the «Regulations on the use of animals in biomedical research» and in accordance with the recommendations of the «European Convention for the Protection of Animals Used for Experimental and Scientific Purposes».

Blood samples were taken from dogs who got to the «Vetservice» clinic for treatment. The diagnosis of babesiosis was established directly in the clinic by the results of erythrocyte micro-

scopy in thin smears of peripheral blood of sick dogs stained by Giemsa. The diagnosis of leptospirosis was established by the study of serum of sick dogs in the microagglutination reaction using 8 serogroups of leptospira (*L. icterohaemorrhagiae*, *L. canicola*, *L. grippityphosa*, *L. romona*, *L. tarassovi*, *L. hebdomadis*, *L. sejroe*, *L. bratislava*) in the serological department of the Sumy regional state laboratory. The subjects of the study were clinically ill and suspected dogs with the associated course of leptospirosis and babesiosis, as well as blood samples from these animals. *Babesia* trophozoites were detected in erythrocytes under a microscope using immersion oil, a magnification of ocular 10 x 90, and the presence of antibodies to leptospira was detected by the presence of agglutination of leptospira in microagglutination reaction.

Statistical processing of the results was performed by methods of mathematical statistics, using Microsoft Excel. For each test item, if necessary, the arithmetic mean (M) and the standard error of the arithmetic mean (m) were determined. Differences with a significance level of more than 95 % were considered significant. The degree of probability was assessed by Student's criteria.

3. Results

Studies were conducted to identify the dynamics of the associated course of leptospirosis and babesiosis in dogs (**Table 1**).

It has been experimentally established that the number of reported cases of the associated course of leptospirosis and babesiosis varies from 9 to 28 cases annually.

Table 1

The incidence of dogs in the associated course of leptospirosis and babesiosis for the period from 2014 to 2019

Year	Number of sick dogs
2014	15
2015	11
2016	9
2017	28
2018	20
2019	16

Analyzing the monthly incidence of dogs in the associated course of leptospirosis and babesiosis, we see that the largest number of cases is registered annually in April-June (**Table 2**).

Table 2

Seasonal monthly incidence of dogs in the associated course of leptospirosis and babesiosis for the period from 2014 to 2019

Number of the month from the beginning of the calendar year	Year					
	2014	2015	2016	2017	2018	2019
1	0	0	0	0	2	1
2	0	0	0	0	0	1
3	1	0	1	4	0	1
4	1	3	2	4	3	7
5	3	4	1	6	6	0
6	2	1	0	5	2	1
7	1	0	1	3	0	0
8	3	1	1	1	0	1
9	0	1	0	2	2	2
10	4	0	1	1	2	2
11	0	1	2	2	2	0
12	0	0	0	0	1	0

Analyzing the general seasonal dynamics of dogs in the associated course of leptospirosis and babesiosis for 6 years, we can say that this parasitocenosis is most often registered in spring (47 cases), to a lesser extent – in autumn (24 cases) and summer (22 cases), and rarely in winter (5 cases), which is probably due to favourable conditions for the survival of leptospira in the environment in spring and autumn (warm humid weather), and Ixodes mites, for which hot and cold weather are unfavourable (**Table 3**).

Table 3

Seasonal incidence of dogs in the associated course of leptospirosis and babesiosis for the period from 2014 to 2019

Season	Number of animals
Winter	5
Spring	47
Summer	22
Autumn	24

Analyzing the pedigree dynamics of the incidence of dogs in the associated course of leptospirosis and babesiosis, we see that purebred dogs get sick much more often than non-pedigree, which is probably due to a stronger immune system of non- pedigree animals, as well as the fact that owners keep purebred dogs more often (**Table 4**).

Table 4

Breed dynamics of the incidence of dogs in the associated course of leptospirosis and babesiosis for the period from 2014 to 2019

Pedigree	Year					
	2014	2015	2016	2017	2018	2019
Non-pedigree	1	3	3	8	6	2
Purebred	14	8	6	20	14	14

Analyzing the sexual dynamics of the incidence of dogs in the associated course of leptospirosis and babesiosis, we see that for the period from 2016 to 2019, the number of diseases in females and males was approximately the same. During the period from 2014 to 2015, males were more often ill (**Table 5**).

Table 5

Sexual dynamics of the incidence of dogs in the associated course of leptospirosis and babesiosis for the period from 2014 to 2019

Sex	Year					
	2014	2015	2016	2017	2018	2019
Males	10	9	5	15	11	8
Females	5	2	4	13	9	8

4. Discussion

The experiment provides an opportunity to determine the prevalence of the associated course of leptospirosis and babesiosis in dogs of the Sumy region of Ukraine. However, depending on the conditions of keeping dogs and the availability of vaccination against leptospirosis and treatment with insecticides, the degree of infection of animals may differ each year [1, 2].

As a result of monitoring the incidence of dogs with the associated course of leptospirosis and babesiosis, it is proved that the disease is registered year-round with rapid peaks in spring and autumn, to a lesser extent – in summer and rarely – in winter.

It has been experimentally established that purebred dogs suffer from the associated course of leptospirosis and babesiosis much more often than outbred ones, which is probably due to the strong immune response of non-pedigree animals, as well as the fact that owners keep purebred dogs more often. The severity of the disease also depends on many factors, including the immune status of the animal, its age, housing conditions and quality of feeding, the duration of the disease and the presence of comorbidities [4, 15].

During the period 2016–2019, the incidence of the associated course of leptospirosis and babesiosis in males and females was approximately the same. During the period of 2014–2015, males fell ill with the associated course of leptospirosis and babesiosis more than 2 times more often than females.

The practical value of the experiments is to monitor and determine the prevalence of the associated course of leptospirosis and babesiosis among dogs in Sumy region in the period from 2014–2019 with the establishment of seasonal, breed and sexual dynamics.

Study limitations. A limitation of the study is the incomplete coverage of the dog population with this monitoring, in cases of asymptomatic leptospirosis in animals, in cases of latent babesiosis, which forms immunization.

Prospects for further research. Investigation of the mechanism of pathogenesis of the associated course of leptospirosis and babesiosis in dogs and improvement of therapeutic regimens for this parasitocenosis.

5. Conclusions

The prevalence of the associated course of leptospirosis and babesiosis among dogs of the Sumy region of Ukraine in the period from 2014 to 2019 with the determination of seasonal, breed and sexual dynamics of this parasitocenosis.

Through practical research, it has been proven that for the last 6 years the associated course of leptospirosis and babesiosis in dogs is registered annually in the amount of 9 to 28 cases, and throughout the calendar year with the prevalence of clinical cases in spring and autumn. According to the general seasonal dynamics of the incidence of dogs associated with leptospirosis and babesiosis for 6 years, we can say that this parasitocenosis is most often registered in spring (47 cases), to a lesser extent in autumn (24 cases) and summer (22 cases), and rarely in winter (5 cases). Purebred animals get sick more often than non-pedigree ones. The incidence of dogs depending on sex is about the same.

The practical value of the experiments is to determine the prevalence of the associated course of leptospirosis and babesiosis among dogs in Sumy region.

Conflict of interest

The authors declare that they have no conflicts of interest.

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