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CORRELATION BETWEEN CLINICAL SIGNS AND DIFFERENT LABORATORY INVESTIGATIONS IN DOGS DIAGNOSED WITH LEPTOSPIROSIS

Radu CONSTANTINESCU, Victor CRIVINEANU, Gheorghe GORAN, Doina DANEȘ, Dorin ȚOGOE, Mario Darius CODREANU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67, Email: constantinescur.radu@gmail.com; vcrivineanu@gmail.com; gvgoran@gmail.com; danes.doina@gmail.com; dtogoe@yahoo.com; codveterinary@yahoo.com

Corresponding author email: constantinescur.radu@gmail.com

Abstract

Leptospirosis represents a scattered zoonosis determined by antigenically distinct serovars of Leptospira interrogans, a sporadic bacterial disease which causes severe clinical illness in dogs and humans. Leptospira thrive directly within hosts, dogs and humans, and reservoirs hosts, rodents, and indirectly within the environment.
Leptospirosis is an odd disease, with a large variety of symptomatology, or, in some cases, shows no signs or symptoms at all. That can be explained by the dog's organism defense mechanisms against infection. Although, in other cases, the disease may be life threatening.
Even when symptoms and signs are quite specific, in order to confirm the diagnosis it is compulsory to perform laboratory tests, such as dark-field microscopy examination (DFM) and microscopic agglutination test (MAT).
In this study we highlighted the request to link the clinical history with the clinical signs and paraclinical specific tests. The purpose of this paper is to show how to relate the results of different test with the clinical stage of the illness.

Key words*:dark-field microscopy examination, dog, leptospirosis, microscopic agglutination test.*

INTRODUCTION

Leptospirosis is a significant clinical ilness present in canine pathology, known to cause hepatonephric syndrome characterized by acute hemorrhagic diathesis, subacute jaundice or subacute uremia (Greene et al., 1990; Adin et al., 2000).
In the first phase of the disease, leptospira organisms enter the bloodstream causing bacteremia, then the spirochetes may multiply in the kidney, liver, spleen, central nervous system, ocular tissue and genital tract. There are three main forms of the disease, represented by the hemorrhagic, renal and hepatic form. In the hemorrhagic form, the infection is localized in the bloodstream and, usually, causes bleeding. In the renal form, the spirochetes are localized mainly in the kidney, they multiply in the renal tubular epithelial cells causing acute nephritis. When the bacteria is mainly localized in the liver, it causes centrilobular necrosis and bile duct occlusion, inducing the jaundice obstructive syndrome, representing the hepatic form (Barr et al., 2002; Hartmann et al., 2005).
In Bucharest, in the last several years, the diagnosed cases of Leptospirosis in dogs has increased dramatically. This is due to the poor control of rodents and especially of the stray cats population that has reached a high number. It is a well known fact that cats develop an asymptomatic infection. Also, it is important not to neglect the zoonotic aspect of the disease, which intimately is correlated with the improper control isolation and strict measures of infected dogs.

MATERIALS AND METHODS

The study was conducted in the Department of Internal Medicine, Faculty of Veterinary Medicine Bucharest, over a period of 2 years, from October 2012 to October 2014. Six dogs of different ages, genders and breeds

were diagnosed with Leptospirosis, presenting various clinical signs.

The dogs taken for study presented a history of illness during the past days, before they being examined in the Department of Internal Medicine, Faculty of Veterinary Medicine Bucharest. Firstly they were misdiagnosed and an ineffective treatment was started, that led to a worsening of the animals state and disease outcome.

The dogs were clinically examined, blood tests - coagulation profile test, haematology and biochemistry profiles were performed, also urinalysis and imagistic exams, in order to evaluate the internal organ damage. Signalment and results of this tests fully corroborated with the history, led to the clinical suspicion of an infection with *Leptospira* species.

The urine and blood serum were examined under dark field microscopy in all cases in order to provide a strong suspicion diagnosis. The motile organisms were detected in these samples, using dark-field microscopy. In the presented cases, Penicillin was administered at a dose of 40,000 IU/kg I.M.. At the same time, all dogs were hospitalized in an isolation room and supportive and symptomatic treatment was provided. The urine samples were obtained by cystocentesis, in order to avoid the bacterial contamination.

Dark-field microscopy may be useful for observing leptospires in fluids such as culture medium, blood or urine, particularly when they are present in large numbers. The results of dark-field microscopy of clinical material should always be confirmed by specific tests. To confirm a certain diagnostic, microscopic agglutination test (MAT) was performed in all cases. The microscopic agglutination test is a specific serodiagnostic method and represents the an important test for the diagnosis of leptospirosis (Harkin, 2003).

The MAT technique was performed with the following 4 serovars of *Leptospira* as antigen: *pomona*, *icterohaemorrhagiae*, *canicola* and *sejroe*.

RESULTS AND DISCUSSIONS

The highest incidence of the dogs with Leptospirosis is found in adult dogs, ranging from one to five years of age, mean age of 5.05 years. The result of MAT showed 4 cases infected with serovars *L. canicola,* and 2 cases with *L. icterohemorrhagiae*; the samples are considerate positive when the titres are higher than 1:800.

Four of 6 cases were diagnosed between September and December and it seems to be a correlation between the frequency of the cases and seasonality, during the rainfall season.

In cases diagnosed with serovars *L. canicola,* all 4 dogs presented clinical signs of renal dysfunction associated with subacute and acute renal failure. From these 4 cases, 2 of them presented only signs of renal failure, another one presented also clinical signs of hepatic dysfunction and the other case presented also signs of clinical muscle dysfunction (Table 1).

Dogs infected with *L. canicola* presented initially nonspecific signs of lethargic depression (n=4), appetite loss (n=3), dehydration (n=3) and vomiting (n=3). Other signs included polyuria/polydipsia (n=2), lymphadenopathy (n=2), macroscopic hematuria (n=2), microscopic hematuria (n=2), weight loss (n=2), lumbar pain (n=1), intermittent fever (n=2) and muscle pain (n=1) (Table 2). Nephromegaly was detected following abdominal ultrasound examination (n=1).

Serovars *L. icterohemorrhagiae* were diagnosed in 2 dogs, which showed clinical signs of hepatic dysfunction, one of them also developing renal dysfunction. These two dogs initially presented signs of lethargy (n=2), appetite loss (n=2), dehydration (n=2), jaundice (n=1), fever (n=1), diarrhoea (n=1), microscopic hematuria (n=1) and vomiting (n=1). Ascites was observed following abdominal ultrasound examination (n=1).

Table1. Organ injury as indicated by serovars and serum biochemical analysis in studied dogs

	Breed	Gender	Age (yrs.)	Serovars	Clinical syndrome
1	Labrador	M	1.6	*L. canicola*	Renal
2	"mixed breed"	M	3.8	*L. canicola*	Renal/Hepatic
3	"mixed breed"	F	5.8	*L.. canicola*	Renal
4	Doberman	M	6.9	*L. canicola*	Renal/muscle
5	Golden Retriever	M	9.7	*L. icterohemorrhagiae*	Renal/Hepatic
6	German shepherd	F	2.5	*L. icterohemorrhagiae*	Hepatic

Table 2. Predominant clinical signs observed in studied cases

Case nr. / Clinical signs	1	2	3	4	5	6
Lethargic depression	•	•	•	•	•	•
Fever					•	
Intermittent fever		•		•		
Appetite loss	•		•	•	•	•
Dehydration	•		•	•	•	•
Vomiting	•	•		•	•	
Polyuria/ polydipsia	•		•			
Lymphadenopathy		•		•		
Diarrhoea					•	
Macroscopic hematuria	•		•			
Microscopic hematuria		•		•	•	
Lumbar pain	•					
Muscle pain				•		
Jaundice						•

Fever was observed in one case (case 5) when the spirochetes probably were present in the bloodstream. Further complications may arise when spirochetes are localized in the kidney, where the bacteria reproduces, causing inflammation, kidney and liver failure. Other symptoms of the disease are vomiting, hematuria, jaundice.

Almost all dogs showed increased urea and creatinine, as well as leukocytosis with neutrophilia and decreased hemoglobin level. The mild decreased hemoglobin, increased packed cell volume and total leukocyte counts can be attributed to toxins released by leptospira organisms, which cause damage to red blood cells. Normal or high leukocyte counts and lower haemoglobin values could potentially indicate a diagnosis of leptospirosis. Two cases presented increased levels of alkaline phosphatase, may indicate hepatic cytotoxicity, which may be caused by the leptospiral endotoxins. Two other cases had evidence of hepatocellular and cholestatic disease, and none had evidence of

Table 3. Biochemical and hematological panel

Parameter	Reference ranges	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Blood urea nitrogen (mmol/L)	3.5-9.0	**27**	**15.8**	**56.9**	**149**	**12.9**	8.2
Creatinine (µmol/L)	20-150	**440**	**398**	**347.7**	**452.6**	**275.9**	62
Total bilirubin (µmol/L)	0-4	2	3	3	2	**6**	**608**
Alkaline phosphatase (U/L)	22-143	33	**148**	56	**456**	**289**	**1190**
Gamma glutamyl transferase (U/L)	0-7	4	**68**	**45**	**8**	**99**	**145**
Alanine aminotransferase (U/L)	19-107 U/L	45	89	57	106	**120**	**445**
Leukocytes ($\times10^9$/L)	4.9-15.4	**18.9**	**16.2**	**15.6**	**38.3**	**18.2**	**17.7**
Neutrophilis ($\times10^9$/L)	2.9-10.6	**36.7**	**19.8**	**12.3**	**17.5**	9.7	**13.1**
Monocytosis ($\times10^9$/L)	0.0-1.1	0.13	0.56	**1.59**	**1.89**	**4.08**	**2.45**
Trombocytes ($\times10^9$/L)	117-418	**56**	**78**	159	175	123	229

hepatocellular damage only, showed by the bilirubin and transaminases levels. The coagulation profile test revealed mild increasing values in all cases probably correlated with thrombocytopenia. Patients which presented early neutrophilia and thrombocytopenia developed severe infection with leptospirosis.

The urine specific gravity value was isosthenuric, with values between 1.004 to 1.010, in all cases. Analysis of urine by dipstick method revealed the presence of occult blood in cases 1, 2, 3, 4 and 5, bilirubinuria in cases 5 and 6, and trace of protein in cases 1, 3 and 5. Urinary sediments included granular casts (cases 1, 4 and 6) and erythrocytes (cases 1 and 5). These values are secondary and reveal the renal injury.

Table 4. Table showing the correlation of dark field microscopy and Microscopic agglutination test results. (* after 7 days from the first tests)

Test / Case nr.	Dark-field microscopy Blood	Dark-field microscopy Urine	Microscopic agglutination test
1	Negative	Positive	Positive
2	Positive	Positive	Positive
3	Negative	Positive	Positive
4	Positive	Positive	Positive
5	Positive	Negative	Negative
5*	Negative	Positive	Positive
6	Negative	Positive	Positive

Leptospiras may be visualized by dark-field microscopy in clinical material, blood or urine, in correlation with the stage of the disease. Thus, in the first week of infection, the spirochetes are observed in blood, but not in urine and MAT test was negative. This suggest that the dog was brought into the clinic in the first stage of infection, when the detection of group-specific antibodies was not possible. In this case the MAT test was repeated after 7 days, the result was positive for *L. icterohemorrhagiae.* Also we repeated dark-field microscopy and it was positive in urine sample and negative for the blood sample. Clinically, the dog presented now jaundice. However, there are two cases, case 2 and 4, in which the dark-field microscopy results are positive both in blood and urine, which can explains the intermittent fever.

This study reveals the importance of correlation between clinical stages of infection and different diagnostic tests, whenever there is a suspicion of leprospirosis, therefore the diagnostic is more accurate when dark-field microscopy (urine and blood samples) and MAT are combined.

The dark-field microscopy test is important to justify the quickly use of antibiotics in order to clear the leptospiremic phase and/or sterilize the urine in first stages of infection, when the MAT has a low sensibility. In this way the use of antibiotics in earlier stages increase the rate of favorable outcome.

The clinical signs and evolution seems to be diverse between different serovars of *Leptospira* species, the virulence and the

CONCLUSIONS

organ targeted by the bacteria, most of them presented signs of renal disease.

Because canine leptospirosis has become increasingly common in recent years and due to the poor control of rodents and asymptomatic cats, the vaccination with a canine vaccine is recommended, especially for dogs which present a high risk of infection.

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