

LEUKEMOID REACTION ASSOCIATED WITH CANINE LEPTOSPIROSIS

María José Tintel Astigarraga

Center for Veterinary Specialties. Asuncion,
Paraguay

ORCID 0000-0002-8333-2769

Romina Isabel Marini Benítez

Veterinary Clinic Dr. Brown. Asuncion,
Paraguay

ORCID 0009-0002-9854-7874

Jorge Ramón Rivas Franco

Veterinary Clinic Dr. Brown. Asuncion,
Paraguay

ORCID 0009-0009-5985-0310

Guillermo José Brown Beckelmann

Veterinary Clinic Dr. Brown. Asuncion,
Paraguay

ORCID 0009-0003-5244-8712

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Abstract: Leptospirosis is a zoonosis with worldwide distribution, mainly in tropical regions. It is usually transmitted by direct contact of the skin and mucous membranes with urine, blood, and other organic fluids, contaminated from infected animals. Clinically it is a polymorphic disease that can be confused with multiple infectious diseases and can cause hematological alterations that can show a leukemoid reaction. The objective of the present work is to describe the association of the Leukemoid Reaction in the course of canine leptospirosis. Methodology: 29 canine patients with leptospirosis were analyzed who, in the course of disease progression, developed a leukemoid reaction with a leukocyte count between 25,000/50,000 cells/ μ l and reactive hyperplastic bone marrow cytology. After the initiation of antibiotic treatment, the leukocyte count returned to normal, with a favorable clinical evolution. Conclusion: Extreme leukocytosis or leukemoid reaction should be considered during canine leptospirosis, for timely management and favorable clinical evolution of the patient.

Keywords: Leptospirosis; canine, leukemoid reaction; zoonosis.

INTRODUCTION

Leptospirosis is one of the most important zoonotic bacterial infections worldwide (Haake & Levett, 2015). It most commonly affects low-income populations, resulting in significant morbidity and many deaths. It is estimated that the infection causes one million cases and around 58,900 deaths per year, with a case fatality rate of 6.85% (Costa et al., 2015).

Leptospira, is a Gram-negative bacterium of the order Spirochetales, with a helical, flexible, and fine shape, from 6 to 15 μ m in length and 0-1 μ m in width, its extremities are usually hook-shaped, they are strict aerobic bacteria, slow growing and with a generation

time of 3 to 15 hours (Abgueuen, 2014; Acha and Szyfres 2001; Brenner *et al.*, 1999). Currently, 21 species of the genus *Leptospira* are recognized: 9 pathogenic for humans and animals, 7 free-living saprophytes (they are found in surface waters and are generally not associated with infections in mammals) and 5 intermediate species. In turn, they have been classified into more than 250 serovars, other authors even refer to more than 300, of which the genome of 5 species has been sequenced: *L. interrogans*, *L. santarosai*, *L. bordpetersenii* and *L. licerasiaelas*.

Leptospira interrogans It is the cause of human and animal leptospirosis (Fernandes *et al.*, 2016). This disease is associated with the occurrence of fever, jaundice, vomiting, diarrhea, hemorrhage, uremia caused by kidney failure and, eventually, death, although in most cases they present asymptotically, both in humans and in animals (Van de Maele et al., 2008; Aygün *et al.*, 2016). Rodents and canines are the most important carriers of this disease (Rojas *et al.*, 2010; Guernier *et al.*, 2016), having dogs as the carriers closest to man.

The transmission of the disease is influenced by the presence of asymptomatic carriers (rodents, canids, cattle) in regions with humid tropical and subtropical climates. Infection occurs by direct contact of mucous membranes or skin lesions with urine, blood, water, or soil contaminated with *Leptospira* spp (Vieira *et al.*, 2016). In the case of canids, the infection develops leptospiremia with subsequent invasion of the kidney tissue, excreting leptospire in the urine for several months, contaminating the environment where they live (Monahan *et al.*, 2008). Likewise, environments with high temperatures and humidity allow the survival of leptospire on land and in water for months (Saito *et al.*, 2013; Kurilung *et al.*, 2017), facilitating a greater spread of the disease

(Bronson *et al.*, 2009).

The clinical signs that the disease presents vary in the different species, being in canines and in man where the greatest variation in the forms of presentation is shown (Hagiwara, 2004). The clinical picture in canines may differ, among other causes, according to the infecting serovar (Rubel *et al.*, 1997). In acute cases, a predominance of signs of hepatic and renal dysfunction is generally observed (Faine *et al.*, 1999). Approximately from day 10 post-infection, after the appearance of antibodies, leptospires are located almost exclusively in the renal tubules, causing extensive lesions and elimination in urine. The pathological changes at the renal level persist even when there is clinical improvement. Both animals with subclinical infections and those that have recovered from acute forms excrete leptospires in urine for a prolonged period, constituting a source of environmental contamination of epidemiological importance (Greene *et al.*, 1998). Due to the variations in the form of presentation, the clinical diagnosis of this disease is difficult in most cases and today it is still under-diagnosed (Silva *et al.*, 2007). As it is a polysystemic disease, biochemical and hematological studies are a routine tool to guide clinical diagnosis. Within the hematological alterations, a leukemoid reaction (RL) can be evidenced, which is uncommon in this entity (Leung *et al.*, 2009).

RESULTS

The age range of the dogs analyzed ranged from 1 to 12 years. 17.2% (5/29) located in rural areas and 82.8% (24/29) domiciled in urban areas. 44.8% (13/29) were female and 55.2% (16/29) male. The leukocyte count varied between 25,500 to 50,800 cells/ μ l with a predominance of segmented neutrophils in all cases. **Table 1.** Of the total number of positive sera analyzed by MAT, only 7/29 (24.1%) reacted to one serovar, 19/29 (65.5%)

to more than one serovar (coagglutination), 3/29 (10.3%) with a Negative result to MAT.

Fig.1.

The serological titles found ranged between 1/200 and 1/800. The *Grippotyphosa* and *Icterhaemorrhagiae* serovars agglutinated in 16/29 canines (55.2%), the *Pomona* in 13/29 (44.8%) and the *Canicola* serovar in 7/29 patients (24.1%). A total of 5/29 patients (17.2%), with leukocytosis and low or negative MAT titers, accompanied by hypercreatinemia and positive results by the urine PCR method; they were considered confirmatory cases for Leptospirosis.

Peripheral blood smears showed immature cells and absence of blasts. On the other hand, bone marrow cytology shows an exaggerated increase in both mature and band neutrophils related to Myeloid-type Leukemoid Reaction.

Fig. 2.

Determining the association of RL with leptospirosis, antibiotic therapy and intravenous fluid support were established according to the physiological conditions of each patient, analyzed based on the criteria of the treating clinicians, where normalization of leukocytes and evident clinical improvement were subsequently observed. We must mention that, depending on the severity, the antibiotic treatment established was different: mild cases were managed with oral agents such as doxycycline or ampicillin, and moderate or severe cases used crystalline penicillin G or third generation cephalosporins such as Ceftriaxone. On the other hand, combined protocols with corticosteroids were indicated in patients with AKI to counteract the effects of tubulointerstitial nephritis caused by leptospirosis.

DISCUSSION

Although most of the canines showed agglutination for more than one serovar, the most frequent serovars being

Icterohaemorrhagiae and *Grippityphosa* (55.2%), of which; only patient 9 manifested jaundice. This supports the concept that jaundice is not a pathognomonic sign in leptospirosis, although its presence increases suspicion and suggests a high possibility that the condition is caused by *Icterohaemorrhagiae*.

Using the MAT technique, specific antibodies can be detected from the first week after infection, so a negative result before that date is not a reason to rule out the disease and a new evaluation is required a week later. High titers or the presence of agglutination in more than one serovar are indicative of recent disease (Levett, 2001). For the protocol diagnosis of leptospirosis, 3 criteria are used: 1. Seroconversion of a patient with a previous negative sample, 2. Presence of microagglutination in a 1:400 dilution in a single sample plus suggestive symptoms, and 3. Increased dilution by more than 4 times in samples separated by more than 15 days, this being the most specific, but impractical.

Leptospirosis generally presents with normal or slightly elevated leukocytes. Leukocytosis could indicate acute infection, since according to different authors in the subclinical form they mention that a transient leukopenia can be observed, but in the acute forms, from the 5th day of infection, leukocytosis with a marked deviation to the left is observed (Luna *et al.*, 2008).

It is worth mentioning that vaccination reduces the occurrence and severity of Leptospirosis, emphasizing that it does not prevent subclinical infection or elimination through urine (Luna *et al.*; 2008). Subclinical leptospirosis or in a renal carrier state can present with acute interstitial nephritis, producing only asymptomatic urinary disorders or producing a typically non-oliguric acute renal failure (ARF). Although patients usually recover kidney function, some may develop chronic renal failure

(CRF). Sometimes, the patient may present CRF of unknown or unknown etiology and based on the clinical history it could be useful to determine if they have anti-*Leptospira* antibodies or to detect *Leptospira* in urine by PCR (Yang, 2018; Riefkohl *et al.*, 2017).

On the other hand, RL consists of an increase in leukocytes above 30,000 mm³, which can be confused with chronic myeloid leukemia. To differentiate a leukemoid reaction from acute and chronic leukemia, it is important to remember that acute myeloid leukemia presents with a high percentage of blasts in the peripheral blood smear, bone marrow examination shows an excess of blasts that displace the rest of the cells. the hematopoietic series: elements that give the hematological spectrum of leukosis, that is, anemia, leukocytosis and/or leukopenia and thrombocytopenia (Quintero *et al.*, 2023).

The main causes of RL are serious infections (*Clostridium difficile*, disseminated tuberculosis, severe shigellosis), malignant neoplasms (Hodgkin lymphoma, melanoma, sarcoma, and lung, oropharyngeal, gastrointestinal, and genitourinary carcinoma), drugs (corticosteroids, minocycline, and factors hematopoietic growth), poisoning (ethylene glycol), severe bleeding or acute hemolysis (Sakka *et al.*, 2006).

The RL, is presented by; a) stimulation of the bone marrow by physical, chemical or allergic agents; b) bone marrow response to an overwhelming demand for leukocytes; and c) ectopic hematopoiesis, by destruction or invasion of the bone marrow space (Hill, 1941). Velasco *et al.*, 2005 mention that the presence of *Leptospira* in the bone marrow suggests that it is a microorganism capable of crossing membranes up to 0.22 mm, behaving like a filterable virus, which, by different mechanisms, can destroy hematopoietic cells, produce oncogenes, as well as repeated

mutations, which could lead to neoplasia and/or disorganize the genome of the host cell, capable of producing leukemoid syndromes, which can mimic leukemia and aggravate the patient's condition.

CONCLUSIONS

The adaptability of the *leptospira* is evidently high, making an immunocompromised dog, or without competent antibody levels, a safe target. For this reason, it is important to include leptospirosis as a differential diagnosis in patients with non-specific clinical symptoms and relevant hematological abnormalities such as RL.

METHODS

CANINES

Indeterminate sex and age with non-specific clinical symptoms, compatible with infectious diseases such as fever, weakness, loss of appetite, myositis, ocular congestion with a leukocyte count between 25,000/50,000 cells/ μ l. **Table 1.**

PLACE OF STUDY

This study had a retrospective design. The data was collected from various private veterinary clinics in Asunción, Paraguay during the period from January 2022 to January 2023.

COLLECTION AND PROCESSING OF SAMPLES

Blood samples, with and without anticoagulant, were obtained by aseptic puncture of the saphenous or antibrachial cephalic vein. The blood samples obtained with anticoagulant (EDTA) were used to perform a complete blood count using a hematological counter by electromagnetic impedance. The serological diagnosis of leptospirosis was made using the Microscopic Agglutination

Test (MAT), internationally recognized as the technique of choice for determining the kinetics of antibodies in this disease (Myers, 1985). Each sample was tested for the following *L. interrogans* serovars: *Icterohaemorrhagiae*, *Canicola*, *Pyrogenes*, *Grippotyphosa*, *Castellonis*, *Pomona*, and *Wolffi*.

Peripheral blood cytology: material collected by microcapillary with heparin obtained by cytopuncture of the inner face of the auricle, after local disinfection with alcohol at 70°C. Fine Needle Aspirate (FNA) bone marrow was carried out by means of aseptic aspiration of the manubrium area of the sternum with a 5 ml syringe with a 21 Gx1" needle. Materials obtained from both peripheral blood and bone marrow were cytologically extended on previously identified coverslips, subsequently stained with 10% Giemsa stain and analyzed under an optical microscope.

Obtaining urine samples: material collected by ultrasound-guided cystocentesis using a 10 ml 21 Gx1" syringe, after local disinfection.

VARIABLES

Vaccinated dogs or dogs without knowledge of their vaccination status were considered positive if they had titers greater than or equal to 1:400. This cut-off point was used taking into account that vaccine titers do not exceed 1:400 and titers due to *Leptospira* spp infection can exceed 1:1600 (Andre-Fontaine *et al.*, 2013). Canines with low MAT titers and positive urine PCR were also considered confirmed cases.

LIST OF ABBREVIATIONS

ARF: Acute renal failure

CRF: Chronic renal failure

FNA: Fine needle aspiration

MAT: Microscopic agglutination test

N: Negative

NDR: No determined race

PCR: Polymerase chain reaction

RL: Leukemoid reaction

TABLE

Case	Age	Race	Gender	Location	Leukocytes	MAT	Serovar	PCR
1	12	Rottweiler	Male	Urban	25,500	1/400	<i>Grippytyphosa</i>	-
2	11	Boxer	Male	Urban	26,200	N	-	Urine
3	2	NDR	Male	Rural	31,200	1/100	<i>Grippytyphosa</i>	-
						1/ 800	<i>Pomona</i>	
4	3	Beagle	Male	Urban	28,400	N	-	Urine
5	5	Fox Terrier	Female	Urban	36,000	1/200	<i>Grippytyphosa</i>	-
						1/400	<i>Canicola</i>	
6	2	Pitbull	Male	Urban	32,900	1/400	<i>Grippytyphosa</i>	-
7	2	Border Collie	Male	Urban	42,100	1/200	<i>Grippytyphosa</i>	-
						1/200	<i>Pomona</i>	
8	5	Teckel	Female	Urban	38,100	N	-	Urine
9	6	Poodle	Female	Urban	50,800	1/200	<i>Grippytyphosa</i>	-
						1/200	<i>Icterhaemorrhagiae</i>	
						1/400	<i>Pomona</i>	
10	8	NDR	Male	Urban	48,800	1/100	<i>Icterhaemorrhagiae</i>	-
						1/800	<i>Grippytyphosa</i>	
11	9	Beagle	Male	Urban	47,500	1/400	<i>Grippytyphosa</i>	-
						1/100	<i>Icterhaemorrhagiae</i>	
12	2	Chihuahua	Female	Rural	27,200	1/800	<i>Canicola</i>	-
						1/200	<i>Pomona</i>	
						1/200	<i>Icterhaemorrhagiae</i>	
13	4	Teckel	Female	Urban	43,500	1/200	<i>Grippytyphosa</i>	-
						1/800	<i>Icterhaemorrhagiae</i>	
14	3	French Bulldog	Male	Urban	33,400	1/100	<i>Grippytyphosa</i>	-
						1/200	<i>Canicola</i>	
						1/800	<i>Pomona</i>	
						1/400	<i>Icterhaemorrhagiae</i>	
15	5	NDR	Female	Urban	41,200	1/200	<i>Grippytyphosa</i>	-
						1/400	<i>Canicola</i>	
						1/800	<i>Pomona</i>	
16	7	NDR	Female	Urban	30,200	1/800	<i>Icterhaemorrhagiae</i>	-
17	6	Pinscher	Male	Rural	44,100	1/200	<i>Grippytyphosa</i>	-
						1/800	<i>Canicola</i>	
18	7	Poodle	Male	Urban	29,800	1/800	<i>Icterhaemorrhagiae</i>	-
19	10	Husky	Female	Urban	41,800	1/800	<i>Grippytyphosa</i>	-

20	5	NDR	Male	Urban	36,400	1/400	<i>Grippotyphosa</i>	-
						1/200	<i>Pomona</i>	
21	2	French Bulldog	Male	Urban	25,400	1/400	<i>Pomona</i>	-
22	1	French Bulldog	Female	Urban	30,600	1/400	<i>Icterhaemorrhagiae</i>	-
23	3	Poodle	Male	Urban	38,500	1/400	<i>Pomona</i>	-
						1/100	<i>Icterhaemorrhagiae</i>	
24	2	NDR	Female	Urban	43,200	1/400	<i>Icterhaemorrhagiae</i>	-
						1/200	<i>Pomona</i>	
25	2	NDR	Female	Rural	50,000	1/400	<i>Icterhaemorrhagiae</i>	-
26	4	NDR	Male	Rural	48,700	1/400	<i>Icterhaemorrhagiae</i>	-
						1/200	<i>Pomona</i>	
27	5	Labrador	Female	Urban	49,200	1/100	<i>Grippotyphosa</i>	-
						1/100	<i>Canicola</i>	
						1/400	<i>Pomona</i>	
						1/200	<i>Icterhaemorrhagiae</i>	
28	15	Poodle	Female	Urban	48,500	1/100	<i>Grippotyphosa</i>	Urine
						1/200	<i>Canicola</i>	
						1/100	<i>Pomona</i>	
						1/200	<i>Icterhaemorrhagiae</i>	
29	2	French Bulldog	Male	Urban	30,500	1/100	<i>Icterhaemorrhagiae</i>	Urine

NDR: No determined race.

N: Negative

Table 1. Data of the canines analyzed.

FIGURES

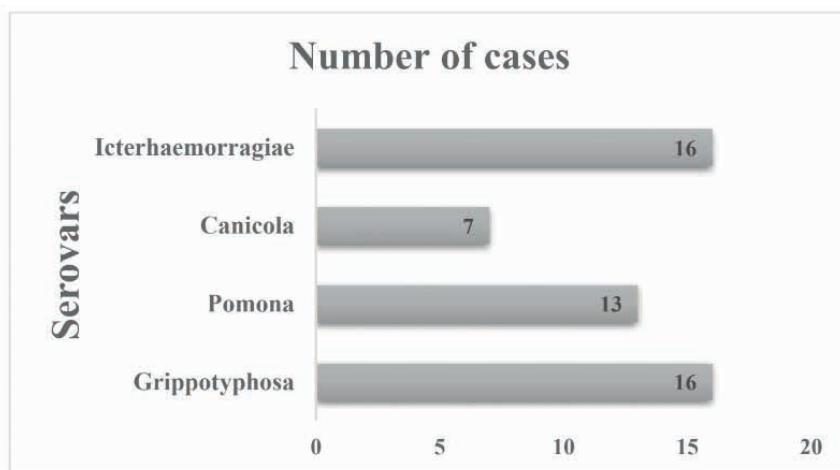


Fig 1. Frequency of canine leptospirosis serovars.

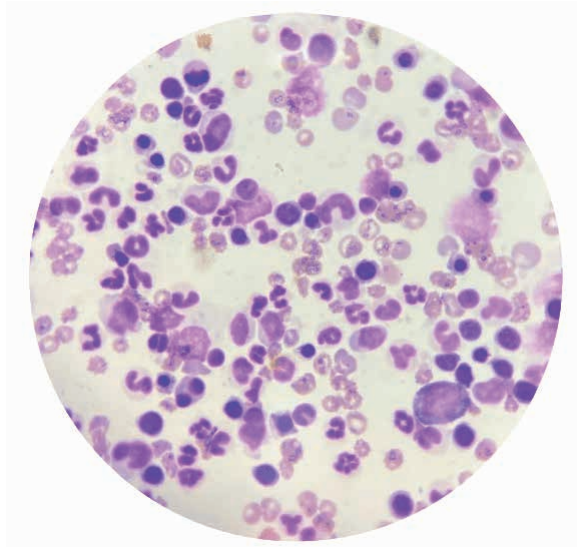


Fig. 2.FNA bone marrow with myeloid medullary hyperplasia with a predominance of neutrophils in the arch. Giemsa. 100X.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The owners of the patients gave their approval by means of a written informed consent

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

The owners of the veterinary clinics refused to disclose the details of the patients' data. All data generated in this study are included in this article.

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CONFLICT OF INTERESTS

The author declare that they have no competing interests

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