Serological survey of canine leptospirosis in the tropics of Yucatan Mexico using two different tests

Matilde Jimenez-Coello a,*, Ignacio Vado-Solis b, Maria F. Cárdenas-Marrufo b, Jorge C. Rodríguez-Buenfil c, Antonio Ortega-Pacheco c

Abstract

Blood samples were taken from 400 stray dogs. The microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay (ELISA) test were implemented using Leptospira interrogans serogroups canicola, hardjo, pyrogenes, panama, pomona, tarassovi, icterohaemorrhagiae, gryppotyphosa, wolfii and bratislava. For the ELISA test, sonicated antigen from above mentioned cultures was used. A conjugate AP-labeled anti-dog IgG antibody was used, the optimal cut-off point of ELISA was set at 1.34. Concordance between ELISA and MAT titers was measured by kappa (κ).

Overall prevalence was 35%. The most prevalent serogroups were canicola and icterohaemorrhagiae. Positive samples showed titers between 1:100 and 1:25,600, with higher titers found in serogroups canicola and icterohaemorrhagiae. Positive serum samples fell within a range of 1.36–1.65. A correlation index of 96% was found between MAT and ELISA. The sensitivity of ELISA was 98.6% and specificity 95.8%.

Seroprevalence of canine leptospirosis and titers were high as a direct consequence of environmental conditions in the studied area. The ELISA test showed a good sensitivity, resulting in a good alternative test for the detection of leptospiral antibodies in dog serum.

Keywords: Canine leptospirosis; Prevalence; Tropics; Diagnostic tests

1. Introduction

Canine leptospirosis is an infectious disease with world-wide distribution. Due to the coexistence and close contact between dogs and humans, the transmission of the etiological agent may occur among veterinarians, dog breeders and dog owners (Greene et al., 1998).

Cases of canine leptospirosis are produced by spirochete bacteria of the species Leptospira interrogans sensu lato, of which around eight serovars (from about 240) are important in dogs. Although dogs are only definitive hosts of serovar canicola, clinical signs are also commonly associated with serovar icterohaemorrhagiae (Sessions and Greene, 2004).

The distribution patterns of canine leptospirosis in the canine populations can greatly vary according to what has been observed in other countries. In the United States for instance, serovar gryppotyphosa has been related to clinical cases of canine leptospirosis (Brown et al., 1996), while in New Zealand serovar copenhageni is most commonly associated with clinical signs in dogs (Hilbink et al., 1992).

Leptospirosis can be diagnosed by bacterial isolation or by the presence of specific antibodies (Sessions and Greene, 2004). More recently, amplification of leptospiral DNA by PCR for diagnosis in dogs has been described (Harkin et al., 2003).

Microscopic agglutination test (MAT) is regarded as the “gold standard” test of reference for the detection of leptospiral anti-
bodies. The MAT has been used for the study of outbreaks and global epidemiology of the disease and provides a crucial pool of clinical strains for studies of pathogenesis (Wuthiekanun et al., 2007). However, the MAT has some limitations since, during acute-phases, elevated antibody titers may be non-specific. Furthermore, MAT requires highly trained personnel and living organisms as antigens. In addition, the cost of strain maintenance is high, making it difficult to use as a routine method in diagnosis laboratories (Faine, 1982).

On the contrary, the enzyme-linked immunosorbent assay (ELISA) has been used for the diagnosis of several diseases including leptospirosis in different species including cattle (Thiermann and Garrett, 1983) and dogs (Ribotta et al., 2000), since it is easier to implement and highly sensitive, therefore yielding reliable results. The knowledge of the presence, distribution and predominant serovars of leptospirosis in natural hosts is important to understand the epidemiology of the disease in any region.

The objectives of this study were to determine the seroprevalence of canine leptospirosis and frequency of the main serovars of 

Leptospira spp. in the population of stray dogs in the tropics of Yucatan Mexico, to demonstrate the usefulness of an ELISA test to be used as an initial screening for the detection of IgG antibodies of the Leptospira serogroups in dogs and to establish the correlation between the diagnosis test ELISA and MAT, used as an aid for the serological determination of canine leptospirosis.

2. Materials and methods

2.1. Study area

This study was performed in the dog pound of the city of Merida Yucatan, Mexico (19°30' and 21°35'N latitude, and 87°30' and 90°24'W longitude). The climate is characterized as tropical sub-humid with a mean annual temperature of 25–28 °C (range of 15–40 °C during the winter and summer, respectively) and relative humidity of approximately 80%. Annual rainfall is 400–1500 mm.

2.2. Animals

Four hundred stray dogs from the Canine and Feline Control Centre from the municipality of Merida Yucatan were studied. The sample size was determined by considering the average annual population of dogs captured and euthanized, which is 2500 (Ortega, 2001), with a prevalence of 50% and error of 5% and a 95% confidence interval (Thrusfield, 1990). The sample was calculated using the program WinEpiscope 2.0 (De Blas et al., 2000). According to the policy of the Mexican Health Ministry (NOM-033-200-1995) dogs not claimed or adopted within 3 days after arrival at the centre, are painlessly killed using an IV overdose of barbiturates.

2.3. Sample collection

Blood samples (5 ml) were taken by either the cephalic or the saphenous vein into sterile tubes and centrifuged at 400 × g for 15 min. Serum was separated and stored at −80 °C until use.

2.4. Microscopic agglutination test (MAT)

The MAT was considered the reference test for leptospirosis in the sampled population and was performed using live antigens as previously described (Turner, 1968). The antigens used were from serogroups: canicola, hardjo, pyrogenes, panama, pomona, tarassovi, icterohaemorrhagiae, grypottypothesa, woffii and bratislava, all commonly associated in previous studies with illnesses in humans and animals in Yucatan (Zavala-Velazquez et al., 1984). The MAT was performed as described (Brandao et al., 1998). The criterion for diagnosing positive samples at MAT is described by Brandao et al. (1998). The sera showing at least 50% of agglutination with one or more serogroups were further titrated in serial twofold dilutions, and titers from 1:100 upward were recorded. Cases were not considered as positive if maximum MAT titers of <100 were detected, or if titers of ≤100 were detected, with equal titers directed against one or more serogroups (Levett, 2003).

2.5. Enzyme-linked immunosorbent assay (ELISA)

The ELISA test was carried out according to the methodology described (Da Silva et al., 1990). The antigen was prepared according to the methodology described (Adler et al., 1980). The antigen employed was a mixture of Leptospira serovars used in the MAT. All the cultures were maintained in the liquid medium of Kortoff, centrifuged 17 days after the pass to 10,000 × g during 30 min, and then were washed twice in phosphate buffer saline (PBS) pH 7.2. Later on, the cultures underwent ultrasonication and the concentration of proteins of the antigen was determined.

Polystyrene plates (NUNC) were coated with 100 µl of a mix of dilute antigen in buffer of carbonates with pH 9.6, and left overnight at 4 °C to incubate in a moisture chamber. Later on they were washed three times during 3 min with PBS-Tween 20 (PBST). Each serum sample was diluted 1:100 in PBS 2% skimmed milk and tested in duplicate. Hundred microliters of each serum dilution were added to each well and incubated for 1 h at 37 °C in a moisture chamber. The plates were washed three times with PBST, incubated with 100 µl/well peroxidase-conjugated rabbit anti-dog IgG (SIGMA, USA), and diluted appropriately 1:5000 in PBST or PBS 2% skimmed milk for 1 h at 37 °C.

Five dilutions of peroxidase-conjugated anti-dog IgG, ranging from 1:300 to 1:5000, were made to determine the best titer under our laboratory conditions. From these dilutions, the 1:5000 was selected and used thereafter. After washing three times with PBST, 50 µl of a substrate solution consisting of 0.04% o-phenylenediamine-dihydrochloride (OPD) (SIGMA, USA) was added and incubated for 30 min at 37 °C. The enzymatic reaction was stopped by adding 50 µl of 2.5 M sulfuric acid and the optical density (OD) was determined in a plate-spectrophotometer at a wavelength of 490 nm. The cutting-off