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R.R. Maronpot, I.S. Barsoum and E. Ezzat



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#### Canine Leptospirosis in Cairo

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The worldwide distribution of canine leptospirosis has been documented by isolation of *Leptospira*<sup>1</sup> and serologic surveys [1–4]. Frequencies of leptospiral antibodies in canine populations range from 3 to 65% [1–4]. Infections of dogs with *Leptospira* serotype canicola and *Leptospira* serotype icterohemorrhagiae produce serum agglutinins which may be detectable for years [5].

Naturally occurring canine leptospirosis has been associated with interstitial nephritis [6, 7], but this relationship is not always easily demonstrated experimentally [8–11]. Recently, Anderson [12] and Taylor et al. [13] have produced subacute interstitial nephritis following experimental infection of dogs with *canicola*. However, canine leptospiral infection has not been proved to cause chronic interstitial nephritis [14, 15].

Canine leptospirosis in Egypt, U.A.R., has been poorly studied. McGuire and Myers [16] reported that five of 30 sera from Cairo dogs, when tested by complement fixation (CF), had antibody titers of 1:8 to *Leptospira* but were negative by the microscopic agglutination test.

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The experiments reported herein were conducted according to the principles set forth in "Guide for Laboratory Animal Facilities and Care" prepared by the National Academy of Science, National Research Council.

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<sup>1</sup> Galton, M. M. 1966. Leptospiral Serotype Distribution Lists. Communicable Disease Center Zoonoses Surveillance. U. S. Department of Health, Education, and Welfare, Public Health Service. Atlanta, Georgia, 130 p More recently, Hamed [17] documented that prevalence of leptospiral scropositivity in 98 dogs in Cairo was 15%. Leptospiral agglutinating antibodies have been reported in man and in noncanine domesticated and wild mammals in Egypt [16–19].

We undertook this study to assess the serologic status of dogs in Chiro with respect to *Leptospira* and to determine whether there is any consistent relationship between their serologic status and interstitial nephritic lesions.

#### **Methods**

Jugular blood was obtained from 170 unselected stray dogs from different areas of Cairo; sera were harvested and stored at -20 C until used for leptospiral serology. Blood from 68 of these dogs was cultured by aseptic addition of two drops of whole blood to two tubes each of Fletcher's and Ellinghausen's media. All dogs were killed and necropsied. Urine was aseptically obtained from the urinary bladders of the same 68 dogs immediately after death and was inoculated with and without dilution into three tubes each of Fletcher's and Ellinghausen's media. Kidney and liver specimens were fixed in 10% formalin buffered at neutral pH and processed routinely. Sections were stained with hematoxylin and cosin; Warthin-Starry stains were made on kidney sections.

All sera were tested by the microscopic agglutination (MA) test [20, 21] with use of the following serotypes of *Leptospira: canicola, autumnalis, sentot, djasimana, bataviae, australis, javanica, grippotyphosa, icterohemorrhagiae, wolffi, hebdomadis, pomona, tarassovi, pyrogenes, ballum, butembo,* and *cynopteri.* The highest final twofold dilution of serum with at least 50% agglutination was the endpoint.

Significant interstitial nephritis is defined as active diffuse inflammation producing histologic

disruption of at least 20% of the renal parenchyma. Occasional foci of interstitial cellular aggregates and damage to the renal parenchyma resulting from vascular thrombosis were not considered to represent significant interstitial nephritis. Evaluation of renal lesions was based on examination of histologic sections from six blocks of renal tissue from each dog.

#### Results

Leptospiral agglutinins were detected in 41 of 170 sera. Thirty-six of the 41 seropositive samples reacted against *canicola*, two against *icterohemorrhagiae*, two against *javanica*, and one against *autumnalis*. The frequency distribution of end titers is presented in table 1.

All cultures of blood were negative for leptospires, but two of 68 samples of urine were culturally positive for *canicola*. One isolate was from a 2-year-old male dog with a serum titer of 1:8192 against *canicola*, Hond Utrecht IV. The second isolate was from a 3-year-old male dog with a serum titer of 1:16,384 against *canicola*, Hond Utrecht IV.

Twenty-nine of 170 dogs had significant histologic interstitial nephritis affecting 20% or more of the renal parenchyma. The inflammatory process was subacute, primarily cortical, and characterized by focal and linear infiltrates of lymphocytes and histiocytes between tubules and around glomeruli. Plasma cells and fibroblasts were included in cellular infiltrate of some lesions. The inflammatory infiltrate was associated with localized degeneration of renal parenchyma.

Twenty-seven (66%) of 41 seropositive dogs had significant interstitial nephritis; 14 (34%)had no significant nephritic lesions. Of the 27 seropositive dogs with nephritis, 26 had titers against *canicola* and one against *autumnalis*. Only two (1.6%) of 129 seronegative dogs had significant interstitial nephritis. Leptospires were present in Warthin-Starry stained renal tissue from six dogs, each of which was scropositive to *canicola* and had severe nephritic lesions. Two of these six dogs had cultures of urine positive for *canicola*.

While nephritic lesions were not dependent on age or sex, and seropositivity was not dependent on sex, there was a 2:1 ratio of seropositivity in dogs over one year of age compared to younger dogs. Seropositive dogs were from all the areas of Cairo that were sampled.

There were no significant hepatic lesions in any of the 170 dogs.

#### Discussion

The 25% frequency of seropositivity obtained in this study is within the range of other canine leptospiral serologic surveys published [1-4]. Comparison of our results with those of McGuire and Myers [16] for dogs in Cairo is difficult since they did not state which serotypes were used, and tested only five of 30 canine sera by the MA test. While their 30 canine sera were positive by a CF test, the titers were all 1:8. The significance of low leptospiral CF titers has not as yet been determined [22]. We agree with Turner [22] that in surveys, low MA titers may be indicative of past infection. Hamed [17] reported only leptospiral titers of 1:200 or greater in a serologic survey of dogs in Cairo. Isolation of serotype canicola from urine samples of two dogs is the first isolation of this leptospiral serotype in Egypt. Agglutinins against canicola have been reported in Egyptian domesticated and wild animals other than canines [18, 19].

The renal interstitium of many dogs in Cairo over one year of age had small cellular accumulations which technically represent interstitial nephritis. The common occurrence of focal interstitial nephritis must be considered in relation to the significance of nephritic lesions. The criteria for

Table 1. Frequency distribution of microscopic-agglutination titers of antibodies to Leptospira scrotypes of 41 scropositive dogs in Cairo

	Reciprocal of titer											
Serotype	32	64	128	256	512	1024	2048	4096	8192	16,384	32,768	
canicola	1	4		7	6	3	4	6	3	1	1	
javanica	1	• • •	ĩ									
icterohemorrhagiae		1		1		• • •						
autumnalis		1					• • •	• • •				

significant interstitial nephritis for this study were established to avoid the probability of associating cases of spontaneous or noninfectious nephritis with leptospiral seropositivity.

This study was intended to document the prevalence of leptospiral seropositivity and associated interstitial nephritis in dogs in Cairo, not to prove or disprove a causal relationship between naturally occurring canine leptospirosis and interstitial nephritis. The results do document a higher prevalence of severe interstitial nephritis associated with leptospiral seropositivity thrai with seronegativity. Although specimens that are positive by the Warthin-Starry stain and isolation of leptospires from seropositive dogs with severe nephritis are not absolute proof of a causal relationship between leptospiral infection and canine interstitial nephritis, such a relationship is at least suggested. Other factors may causally lead to interstitial nephritis.

#### Summary

Sera of 170 dogs in Cairo were tested for leptospiral antibodies by the microscopic agglutination test. Forty-one sera had detectable leptospiral agglutinis, most reacted against *Leptospira* serotype *canicola*. Subacute interstitial nephritic lesions were present in renal tissue from 27 seropositive and two seronegative dogs. *Leptospira* serotype *canicola* was isolated in cultures from urine of two dogs.

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