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SEROEPIDEMIOLOGICAL STUDIES ON CANINE LEPTOSPIROSIS IN SHARKIA GOVERNORATE

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SUMMARY

A serological survey for detection of antibodies to leptospira spp. was conducted on stray and owned dogs serum. The samples were collected form different localities (rural, urban and suburban environmental) in Sharkia governorate, Egypt during the period from February, 2001 to March, 2003. A total of 290 dogs were tested by using ELISA and microscopic agglutination test (MAT) against six leptospiral serovars. At the time of blood collection, all the dogs appeared healthy with no clinical signs suggestive of leptospirosis. In addition, urine samples for bacteriological examination and paired serum samples for serological investigation were collected from 18 diseased dogs suspected to suffer from leptospirosis.

Seroprevalence by ELISA was 12.4% (36/290), and by MAT was 10.7% (31/290). The most frequently serotypes identified serologically were Leptospira *Canicola* (38.7%) and *L. Icterohaemorrhagiae* (32.25%), followed by *L. pomona* (12.9%), *L. Hardjo* (9.7%) and *L. Grippotyphosa* (6.45%), while *L. Sejroe* antibodies were not detected. The prevalence in stray dogs was higher (13.9%, 12.1%) than in owned dogs (8%, 6.7%) by ELTSA and MAT respectively. The seroprevalence in females was lesser than in males ($P<0.05$) and in puppies less than 1 year old it was lesser than in older animals ($P<0.01$).

Four-fold rise in titers between serum samples obtained during the acute and convalescent phases were detected in three cases of diseased dogs and indicative to an acute leptospirosis. While, cultural isolation of leptospires from dogs urine was unsuccessful.

It is concluded that, leptospirosis was of significant occurrence in stray and owned dogs in Sharkia governorate and consequently, dogs could be a reservoir source of infection to domestic animals and human.

INTRODUCTION

A large diversity of bacteria can infect dogs, but leptospirae is the most common one because it can persist in wildlife and domestic animals as subclinical infection (Rentko et al. 1992). Leptospirosis is an important disease affecting domestic animals, wildlife and humans world-wide. The infection can spread by recovered animals that shed organisms in their urine for months to year following infection (Stepben and Robert, 1994). The disease is most common in semitropical areas and transmission occurs by direct contact, wounds, veneral and ingestion. Urine from the infected host is the most common source of environmental contamination (Rubel et al. 1997). Numerous serovars of leptospira interrogans have been recognized, at least eight of which are important for dogs (Michael, 2003) Leptospira organisms can change host specificity and virulence in response to selective pressures in the environment (Torten and Marshall , 1994). Leptospira is often difficult to isolate from ill animals and the microscopic agglutination test (MAT) is one of the most commonly used serological technique for detection of serum antibodies (Ciceroni et al., 1997) as well , ELISA is easy, rapid and suitable screening test (William et. al 1997).

Relatively little research work on leptospirosis have been undertaken in the dogs in Egypt. The main purpose of this study was to determine the seroprevalence of leptospiral antibodies in stray and owned dogs at different environments in Sharkia governorate, Egypt by using ELISA and MAT.

MATERIAL AND METHODS

Animals:- Two hundred and ninety stray (215) and owned (75) dogs of different ages, sex and breeds were conducted in this study. They were located at different environments (rural, suburban and urban) in Sharkia governorate, Egypt (Table 1).

Table (1) :- Number of collected sera sample from apparently healthy dogs and their localities:

Animals	Total number	Localities		
		Rural	Suburban	Urban
Stray dogs	215	135	80	-
Owned dogs	75	-	-	75
Total	290	135	80	75

Samples :-

Serum samples :- 290 sera sample were collected from the previously mentioned dogs during the period from February, 2001 to March, 2003 and stored at - 20°C until tested by ELISA and microscopic agglutination test (MAT). At the time of blood collection all the examined dogs appeared healthy and present no clinical signs suggestive of leptospirosis

Leptosipiral antigens:- six leptopsiral serovar antigens which were used in MAT were supplied by the Center for Molecular Medicine and Infectious Diseases, College of Vet. Med., Virginia, USA.

Diseased dogs :- 18 cases of acutely ill dogs suspected to suffer from leptospirosis were examined and the clinical signs were reported. Paired serum samples were collected from diseased dogs during acute and convalescent phases (2 weeks apart) and were tested by MAT.

Serological examination :-

a- ELISA :-

ELISA was performed according to Terpstra et al (1985). Microwell plates coated with patoc leptospira antigens which were chemically inactivated suspensions of internationally recognized strains of leptospira and ELISA kits were provided by Difco Laboratories, Detroit, Michigan, USA.

b- Microscopic Agglutination test (MAT

Sera were tested using MAT according to Cole et al. (1983). All sera were tested against the antigens of the following six serovars: *Leptospira Canicola, L. Icterohaemorrhagiae, L. pomona, L. Hardjo, L. Grippotyphosa* and *L. Sejroe.* These sera were diluted with Sorensen's phosphate buffered saline solution at pH 7.2 to give final sero dilutions of 1:50 and 1:100. Sera showing 50 percent agglutination or more in 1:100 dilution were further titrated to determine the end point titers using two-fold serial dilutions. A presumptive positive reaction was based on a 1: 100 dilution or greater to any of the test antigens.

Urine samples :- Also 18 urine samples were collected from the diseased dogs during the acute phase for bacteriological examination according to Herr et al. (1982). Urine was diluted with liquid Ellinghausen, McCullough, Johnson, Harris (EMJH) medium (Difco Laboratories Inc. Detroit, Mich.) and 0.5ml aliquots of these dilutions were used to inoculate tubes containing 10ml EMJH semisolid medium. The cultures were maintained at 22°C for 20 weeks and examined periodically by darkfield microscopy for leptospires.

RESULTS

Table (2):- Prevalence of leptospiral antibodies in tested sera by ELISA:-

Animals	No. of tested sera	No. of positive	Positive %
Stray dogs	215	30	13.9
Owned dogs	75	6	8
Total	290	36	12.4

Table (3):- Prevalence of leptospiral antibodies in tested sera by MAT at titer of > 100.

Animals	No. of tested sera	No. of positive	Positive %
Stray dogs	215	26	12.1
Owned dogs	75	5	6.7
Total	290	31	10.7

Table (4) :- Distribution of positive reactors among apparently normal dogs to main leptospiral antigens used :-

Animal	Total No. of positive	Number of positive to main antigen used					
		L. Canicola	*L. Icterohaemorr-higiae*	*L. Pomona*	*L. Hardjo*	*L. Grippo-typhosa*	*L. Sejroe*
Stray dogs	26	10	8	3	3	2	-
Owned dogs	5	2	2	1	-	-	-
Total	31	12	10	4	3	2	-
Percentage		38.7%	32.25%	12.9%	9.7%	6.45%	-

Table (5) : Seroprevalence of leptospiral antibodies in relation to age and sex.

Young less than 1 year			Adult			Female			Male		
No. of Exam.	No. of +ve	+ve %	No. of Exam.	No. of +ve	+ve %	No. of Exam.	No. of +ve	+ve %	No. of Exam.	No. of +ve	+ve %
118	4	3.4%	172	27	15.7%	143	10	7%	147	21	14.3%

* X^2 between young and adult dogs was highly significant ($P<0.01$)

* X^2 between females and males was significant ($P<0.05$)

Result of diseased dogs :-

The most clinical signs appeared on 18 affected cases of diseased dogs were anorexia, fever, diarrhea, vomiting, lethargy,depression, icterus and abdominal pain.

The results of MAT on paried serum samples revealed four-fold increase or more in three dogs (Table 6).

The bacteriological examination of urine samples which were collected from diseased dogs revealed negative results and I failed to isolate any of the etiologic serovars.

Table (6): Result of MAT on paired serum samples collected from diseased dogs:

Total No. of examined dogs	No. of serological reactors	Four-fold rise or more		Predominant serovars
		Acute phase titer	Convalescent phase titer	
18	3	100	800	L. Canicola.
		200	1600	L. Canicola.
		100	400	L. Icterohaemorrhagiae

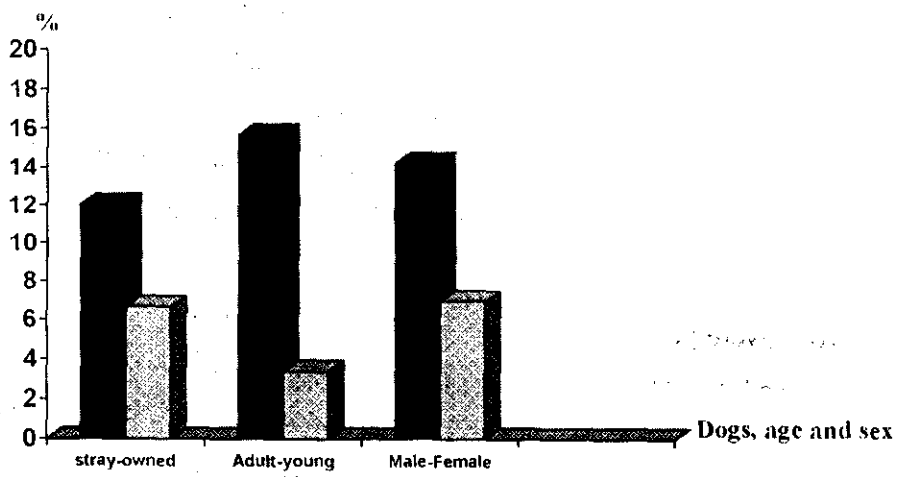

Fig. (1): Results of MAT in relation to stray & owned dog, age and sex.

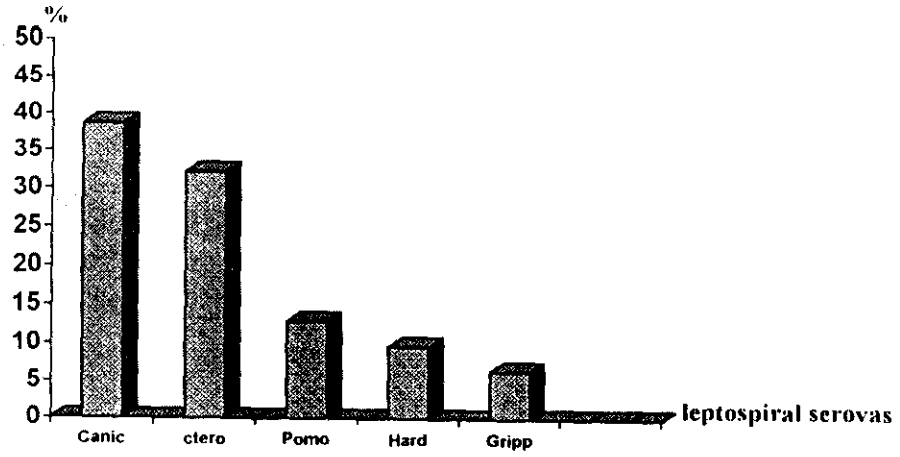

Fig. (2): Results of leptospiral serovas detected by MAT.

DISCUSSION

Leptospirosis is an important clinical and zoonotic disease of worldwide significance known to occur in dogs, humans and many other animal species. Rodents and wild animals are the most common reservoirs for domestic animals and hummans (Micheal and William, 1997).

Serology plays an important role in the diagnosis of leptospirosis. Of the total 290 dog sera tested by ELISA, 36 (12.4%) were seropositive reactors. Of them ,30 (13.9%) and 6 (8%) of stray and owned dogs respectively,were positive ,Table (2). While, the seroprevalence by microscopic agglutination test (MAT) demonstrated 10.7% (31/290) leptospiral antibodies among surveyed dogs. The seropositive results were 12.1% and 6.7 for stray and owned dogs respectively, Table (3). These results agree with Hartman (1984), Greene and Shotts (1990), Harkin and Gartrell (1996), Mohamed et al. (2000) and Ozdemir and Erol (2002), ELISA was sufficiently sensitive test to be used as an initial screening test for the detection of leptospiral antibodies in canine sera, with subsequent confirmation of positive test results with the MAT (Ribotta et al. 2000,a). The prevalence by ELISA was higher than MAT, this may be due to that the ELISA is considered more sensitive than MAT which detect both IgM and IgG, while MAT is a more sensitive detector of IgM antibody than IgG (Scanziani et al. 2002).

The most frequent infecting serovars found by MAT were *Leptospira Canicola* (38.7%), *L. Icterohaemorrhagiae* (32.25%), *L. Pomona* (12.9%), *L. Hardjo* (9.7%) and *L. Grippotyphosa* (6.45%), while *L. Sejroe* was not demonstrated.The MAT is one of the most commonly used serological techniques for the detection of scum

antibodies to leptospira. A comparison of the titers obtained testing a serum sample with different serotypes of leptospira is the conventional way for the identification of the causative agent (Rathinam and Namperumalsamy, 1999) It should be noted that these serovars predominated in both human and animal populations. These results agree with Grant et al (1988), John et. al (1993), Cathy et al. (1996), Lomar et al. (2000) and Harkin et al. (2003). The serovars responsible for human infections usually reflect the serovars, which are present in the prevaling animal species (Faine, 1982). The highest rates and titers were to L. Caincola and L. Icterohaemorrhagiae, which suggests that these serovars are the most common etiologic agents of leptospirosis in dogs ,Table (4). Similar findings were reported by Weekes et al.(1997) and Ribotta et al (2000, b). The leptospira interrogans serovars reported in this study, has previously been recovered from domestic animals including dogs, cattle, sheep, goats and horses and from wildlife (Miller et al. 1990, Donahue et al. 1992, Dickeson and Love, 1993 Ciceroni et al. 2000 and Sasaki, 2000).

High significant difference in seroprevalence of leptospiral antibodies ($P< 0.01$) was recorded between adults (15.7%) and puppies (3.4%), while it was significant ($P<0.05$) between males (14.3 %) and females (7 %);Table (5).It seems that male outdoor adults are more frequently exposed to the infection. The difference in prevalence rates may be attributed to ecological and environmental reasons. The effect of environmental conditions such as pH, temperature and moisture on distribution and survival of leptospires had been previously studied by many authors (Yasuda et. al, 1980 , Tangkanakul et al. 2001, and Ward, 2002).

Dogs of any age,breed or sex can be affected. Disease occurs in dogs from both rural, urban and suburban environments. Street behavior in the dog, the presence of stagnant water, contact with trash deposits, hunting behavior and the presence of rodents are the most important of the risk factors (Douglin et al 1997).

Moreover, the most common clinical features in 18 diseased dogs were anorexia vomiting, fever, lethargy,depression, diarrhea,icterus and abdominal pain. Four-fold increase in titers between serum samples obtained during the acute and convalescent phases of the disease was indicative in 3 cases to an active infection. This result indicates and confirms acute leptospirosis. The four-fold change in titers were to *L. Canicola* (2 cases) and *L. Icterohaemorrhagiae* (one case).

The results of bacteriological examination were negative and I failed to isolate any lepospiral serotype from urine samples collected from acutely-ill cases. Culture of leptospiral organisms is technically difficult and may require weeks before a conclusive result is obtained. Recent advances in PCR techniques (De Caballero et al., 1994) have

allowed specific leptospira serovars to be identified more rapidly and easily . However, these techniques are not yet readily available. The diagnosis of leptospirosis must be inferred from the serologic titer and assumes that the highest titer reflects the etiologic serovar (Saravanan et. al. 1999, Christopher and Larry 2000, Levett, 2001 and Kontos 2002),

Finally, it is concluded that, the high prevalence of leptospiral antibodies in sera of dogs as shown in this study in Sharkia governorate indicate that dogs may well constitute a reservoir source of infection to human and other domestic animals.

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REFERENCES

Cathy A., Wayne R.M., Meri, A., Davis d. Scott B., Carole, A. and Craig E. (1996) : Leptospira interrogans serovar grippotyphosa infection in dogs. J. Amer. Vet. Med. Assoc., 209 (7) 1265-1267.

Christopher A. and Larry D. (2000) : Treatment and outcome of dogs with Leptospirosis ; 36 cases (1990 - 1998). J. Amer. Vet. Med. Assoc. 216 (3) 371-375.

Ciceroni, L., Bartoloni, A., Pinto, A., Valdez, V., Roselli, M. and Paradisi. F. (1997)†: Serological survey of leptospira infections in sheep, goats and dogs in Cordillera province, Bolivia. New Microbiol., 20 (1) 77-81.

Ciceroni L., Lombardo D., Pinto A., Ciarrocchi S. and Simconi J. (2000): Prevalence of antibodies to leptospira serovars in sheep and goats. J. Vet. Med. B Infect. Dis. Vet. Publ. Health, 47 (3) 217-223.

Cole J.R., Sulzuer C.R. and Pusell A.R. (1983): Applied Microbiology 25: 976.

De Caballero L. Dias E and Koury M. (1994)†: Low-stringency PCR with diagnostically useful primers for identification of leptospira serovars. J. Clin. Microbiol. 32†: 1369-1372.

Dickeson, D. and Love D.V. (1993) : A serological survey of dogs, cats and horses in south-eastern Australia for leptospiral antibodies. Aust. Vet. J. 70 (10) 389-390.

Donahue J.M., Smith B.J. and Donahue J.K. (1992) : Prevalence and serovars of leptospira involved in equine abortions in central Kentucky during the 1990 foaling season. J. Vet. Diagn. Invest., 4: 279-284.

Douglin C.P., Jordan C., Rock, R., Hurley A. and Levett P.N. (1997)†: Risk factors for severe leptospirosis in the parish of St. Andrew, Barbados Emerging Infections diseases. 3, 78-80.

Faine S. (1982): Guide lines for control of leptospirosis. Offset Publication, No. 67 World Health oxganisation (WHO) Geneva pp 115- 116.

Grant H., Smith G. and Schloss (1988): Seroprevalence of leptospiral antibodies in the jamaican livestock population. Vet. Rec. 122, 419-420.

Greene C. and Shotts E. (1990): Leptospirosis. Clinical Microbiology and Infectious diseases of dog and cat. Philadelphia : WB Saunders Co. 498-507.

Harkin K.R. and Gartrell C.L. (1996): Canine leptospirosis in New Jersey and Michigan J. Amer Anim. Hosp. Assoc. 32 (6) 495-501.

Harkin K.R., Roshto X.M. Sullivan J.T., Purvis T.J. and Chengappa M.M. (2003). Comparison of PCR, bacteriologic culture, and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs J. Amer. Vet. Med. Assoc. 222 (9): 1230-1233.

Hartman E.G. (1984): Epidemiological aspects of canine leptospirosis in the Netherlands. Zentralbl Bakteriol Mikrobiol-Hyg., 258 (2-3) 350-359.

Herr S., Riley A.E., Neser J.a., Roux, D. and Delange J.F. (1982) : Leptospira interrogans serovar pomona associated with abortion in cattle : isolation methods and laboratory ainmal histopathology. Onderst. J. Vet. Res., 49, 57-62.

John F., David, A., John E., Russell C., Louis, A. and Theodore G. (1993) : Isolation of leptospira interrogans serovar grippotyphosa from the skin of a dog. J. Amer. Vet. Med. Assoc. 203 (11) 1550-1551.

Kontos B. (2002): Serologic evidence of mixed infection involving the zoonoses lishmaniasis and leptospirosis in Greek dogs. New Microbiol, 25 (4) 455-462.

Levett P.N. (2001): Leptospirosis. Clin. Microbiol., 14 (2) 296-326.

Lomar A.V., Diament D. and Torres J.R. (2000) : Leptospirosis in Latin America Infect. Dis. Clin. North. Amer., 14 (1) 23-39.

Michael, S. (2003) : Clinical Medicine of the Dog and Cat. Manson publishing Ltd. ISBN, 1st Ed., Spain.

Michael, s. and William E. (1997) : Practical Small Animals Internal Medicine. 2nd. Ed. W.B. Saunders Company.

Miller D.A., Wilson M.A. and Owen W.J. (1990): Porcine Leptospirosis in Iowa. J. Vet. Diagn. Invest. 2: 171-175.

Mohamed, A., Eisa M. and Saad M. (2000) : Leptospirosis in cattle and their human contacts. J. Egy. Vet. Med. Assoc. 60 (3) 93-102.

Ozdemir V. and Erol E. (2000): Leptospirosis in Turkey. Vet. Rec. 150 (8) 248-249.

.Rathinam S.R. and Namperumalsamy P. (1999) : Leptospirosis. Ocul . Immunol.Inflamm. 7 (2): 109-118.

Rentko V., Clark N. and Ross L. (1992): Canine leptospirosis : a retrospective study of 17 cases. J. Vet. Intern. Med 6 : 235-244..

Ribotta M., Higgins, R., Gottschalk M. and Lallier R. (2000,a): Development of an indirect ELISA for detection of leptospiral antibodies in dogs. Can. J. Vet. Res. 64 (1): 32-37.

Ribotta M., Fortin M., Higgins R. and Reaudin S. (2000,b): Canine leptospirosis, serology. Can. Vet. J. 41 (6) 494-495.

Rubel D., Seijio, A., Cernigoi, B., Viale, A. and Wisnivesky C. (1997): Leptospira interrogans in a canine population of greater buenos airies : variables associated with seropositivity. Rev. Panam Salud Publica, 2 (2) 102-105 .

Saravanan R., Rajendran P. and Garajan S. P. (1999): Clinical, bacteriological and histopathologic studies on induced leptospirosis in stray dog pups. Indian J. pathol. Microbiol. 42 (4) 463-469.

Sasaki D.M. (2000): Questions stated prevalence of leptospirosis in dogs. J. Amer. Vet. Med. Assoc., 220 (10) 1452-1453.

Scanziani, E. Iacchia G., Vasino A., Pirovano G. Scarpa P. and Tagliabue S. (2002): Serological survey of leptospiral infection in kenneled dogs in Italy. J. Small Anim Pract. 43 (4) 154-157.

Stepben J. and Robert G. (1994): Small Animals Practics. 1st. Ed. W.B. Sannders Company.

Tangkanakul w., Siriarayaporn P. Pool T, Ungchusak K. and Chunsuttiwat S. (2001) Environmental and travel factors related to leptospirosis in Thailand. J Med. Assoc. Thai. 84 (12): 1674 ñ 1678.

Terpstra, W.J., Ligthart G.S. and Schoone G.J. (1985) : ELISA for the detection of specific IgM and IgG in human leptospirosis. J. Gen. Microbiol., 131, 377-385.

Torten M. and Marshall R. (1994) : Leptospirosis In : Baron GW, ed. Handbook of zoonoses. 2nd ed. Boca Raton, Fla : CRC Press Inc, 245-264.

Ward M.P. (2002): Clustering of reported cases of leptospirosis among dogs in the United States and Canada Prev. Vet. Med. 56 (3) 215-226.

Weekes C.C., Everard C.O., and Levett P.N. (1997) : Seroepidemiology of canine leptospirosis on the island of Barbados. Vet. Microbio., 51, 215-222.

William, E. David J. Moira, L. and peter 1. (1997): Evaluation of a commercial ELISA for detection of IgM antibody in diagnosis of human leptospiral infection. J. Clini. Microbiol., 35 (8) 1938-1942.

Yasuda P.H. Santa R.C., Myers D.M. and Yanaguita R.M. (1980): The isolation of leptospires from stray dogs in the city of Sao Paulo. Brazil. Int. J. Zoon 7, 131-134.