Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [Special Issue V] 2014: 158-162

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Survey on Seroprevalence of *Leptospira* serotypes in household dogs using MAT method in Urmia, Iran

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ABSTRACT

Leptospirosis is a zoonotic disease with worldwide distribution. It is seen in many animals but mainly dogs are affected. As some of these animals have direct contacts with humans so it can influence their health too. Because of unknown prevalence of this disease in Urmia city (Iran), the aim of this study was to determine the seroprevalence of leptospira serotypes in household dogs using microscopic agglutination test (MAT). In this survey, from May 2010 until November 2010, serum samples were collected from ninety three dogs and evaluated against antigens of serovars: canicola, grippotyphosa, icterohaemorrhagiae, Pomona, hardjo, automnalis, ballum. Out of the 93 dogs investigated, 6 dogs (6.5%) had a titer 1:100 or 1:200 against different leptospiral serovars. The predominant serovars were icterohaemorrhagiae (33.3%), grippotyphosa (33.3%), hardjo (33.3%) and canicola (16.7%) respectively. Antibody titer against more than one serovar was detected in one dog only. Fortunately, prevalence of infection was low in dogs in Urmia region that can be due to specialized geographical and climatic condition and also can be due to regularly and annually vaccination against this disease in household dogs.
Key Words: seroprevalence, MAT, Leptospirosis, household dogs.

Received 11.06.2014 Revised 20.07.2014 Accepted 21.08. 2014

INTRODUCTION

Leptospirosis is caused by Leptospira which is thin, filamentous, aerobic spirochete bacteria measuring approximately 6-12 µm long. More than 200 serovars of leptospira were identified worldwide; most of them are pathogenic in dogs including *Leptospira* serovars *bratislava*, *canicola, icterohemorrhagica, pomona*, and g*rippotyphosa*. Infected animals become bacteremic and leptospira organisms multiply in the kidney, liver, spleen, central nervous system, ocular tissue and genital tract. In dogs, serovars canicola and grippotyphosa results in more renal dysfunction, whereas serovars icterohemorrhagiae and pomona produce more hepatic damage [1,2]. Reservoir hosts may be sub clinically infected and shed organisms for months to years after recovery [1].

Leptospirosis has been recognized as an important matter emerging global public health problem because of its epidemic proportions and increasing incidence in both developing and developed countries [3, 4]. It is an acute bacterial infection caused by spirochetes, with different pathogenic species of the genus *Leptospira* [5, 4]. Leptospirosis has wide geographical distribution and occurs in tropical, subtropical and temperate zones. Leptospirosis is a direct zoonosis and is maintained in nature by a large variety of animal hosts. These include both wild and domestic animals. Leptospires shed in the urine of these carrier animals can survive in the environment for prolonged period. The source of human *leptospiral* infection is infected from animal urine. Hence, the commonly considered risk factors and behaviours are those that expose people to animal reservoirs or contaminated environment. Contact with

various species of animals, animal tissue, animal urine and wet environment and occupational and recreational exposure to contaminated water bodies have been implicated as risk factors [4].

It is caused by spirochetes of the genus *Leptospira*, which brings together two species: *L. interrogans* and *L. biflexa*. The first is hombrey pathogenic for animals and the second is free-living, found in surface waters and rarely associated with infections in mammals [6, 7].

Leptospirosis continues to be a significant clinical presence in canine medicine. In addition to an increased number of cases, more diverse clinical presentations are being recognized. Selection of appropriate vaccines and inter-pretation of serological results in the presence of vaccinal titers are emerging issues in clinical practice [1].

Leptospirosis is spread through contact with water, soil, vegetation or any part of a moist environment contaminated by urine or tissue of infected animals or humans. These bacteria can be inactivated by drying, but can survive in a moist environment for weeks or months. Humans and animals can become infected through direct contact with infected urine or other body tissues. The Leptospira organism can enter the body through broken skin (cut or scratch) or mucous membranes (lining of the mouth, eyes, nose or genitalia). Infection can also occur through ingestion of contaminated water or food. If an animal is infected with a serovar or type of Leptospira that is adapted to that species of animal, then the animal will not show clinical signs of illness, but will excrete the bacteria in its urine for months or even years contaminating the environment. This serovar adaption often occurs in rats, mice and wildlife like raccoons, opossums and skunks. Animals will show clinical signs of leptospirosis when infected with a serovar to which it is not adapted [10].

MATERIALS AND MTHODS

Sample collecting

In this study which lasted from May to November in year 2010, 93 pet dogs were randomly sampled. For blood sampling after the disinfection position using cotton and alcohol, 2 ml of blood was collected. Test tubes transferred into the centrifuges and with specific protocols were centrifuged (3 min at 1500rpm, at 3000 rpm for2 minutes, and then 2 minutes at 4500rpm and finally 3 min at 6000rpm). After complete sampling, Samples were sent for leptospirosis MAT test into the Research Laboratory of Veterinary Faculty of Tehran University.

Microscopic Agglutination Test (MAT)

The MAT which was originally described by Galton et al [8] and modified by Cole et al [9] is the most widely used serological test for leptospirosis [11, 12]. The MAT is best used as a screening test when investigating the possibility of *L. hardjo* infection in groups or herds of cattle. At least 30 animals (or 10% of large groups) should be bled and animals of various ages should be included (Hathaway et al., 1986). The MAT is particularly useful in the diagnosis of disease caused by incidental, non-host-adapted serovars or acute disease caused by host-adapted serovars.

However, because of the frequent low or possibly negative MAT titres in animals recently infected with *L. hardjo*, making a diagnosis on the basis of a serological result from one animal is extremely difficult [17]. Ellis et al. [12] reported that there was no value in examining paired serum samples from individual cows after abortion because titres are either falling or static at the time of abortion.

For a diagnosis of leptospiral abortion in cattle, a reciprocal titre of 3000 was proposed by Elder et al [17] as the threshold for L. pomona but no similar critical figure was available for L. hardjo. For a herd diagnosis of leptospirosis due *to L. hardjo*, ten animals from each of the yearling, first calver, second calver and older age groups should be tested [21]. The main detriment of the MAT is low sensitivity because some cattle exhibit a low response to L. hardjo [14]. Study conducted by Ellis et al. [12] on 200 randomly selected cattle at abattoir indicated that 46.4% of renal carriers had antibody titres of less than 1/100 and 9.6% had no detectable MAT titre against *L. hardjo*.

Cross-reactions caused by exposure to leptospires of the same serogroup can occur, for example, infection by L. balcanica [26]and L. medanensis can produce false positive L. hardjo reactions. The MAT has the disadvantages that it is tedious and time consuming [15], and the use of live culture imposes a risk of human infection. Another disadvantage is the failure of the MAT to differentiate between titres after vaccination and those after natural infection, since the titres may be of similar magnitude [13, 22].

RESULTS

During months of May to November 2010 a studyon93petdogsinUrmia region(Iran)was conducted. The data obtained from 93dogsstudiedbytesting the MAT .87dogs (5.93%) without titres (healthy) and 6dogs (5.6%) qualify for the serum titers(1:100 and1:200) against the antigen of Leptospira (Table 1). In this study dogs with genders parameter was examined (Table 8). In other words, the seroprevalence of different serovars of Leptospira infection in the study is5.6% of populations that among them only one an

old mixed breed dogs has a history of vaccination titer of 100/1 (a false positive). The actual percentage of infection in pet dogs in Urmia regions is approximately5.4%. The predominant serovars were *icterohaemorrhagiae* (33.3%), *grippotyphosa* (33.3%), *hardjo* (33.3%) and *canicola* (16.7%) respectively. In other words, the most common serovars identified *grippotyphosa*, *icterohaemorrhagiae* and *canicola* were the same incidence that each in 2dogs (3.33%) had positive titers were observed. Also in one pet dog, two serovar *grippotyphosa* and *icterohaemorrhagia*e were observed at the same time.

Table 1: Seroprevalence of infection with different serotypes of Leptospira in dogs

	negative	**positive**
Abundance	87	6
Relative abundance	93.5	6.5

Table 2: Frequency of infection by serovar identified in domestic dogs

	Grippo	**Icter**	**Can**	**Hj**
Abundance	2	2	1	2
Relative abundance	33.3	33.3	16.7	33.3

Table 3: Frequency of age groups in the population of pet dogs

	1≥	**1≤3**	**3<**
Abundance	47	31	15
Relative abundance	50.6	33.3	16.1

Table 4: Frequency of infection in dogs of all ages

	1≥	**1≤3**	**3<**
Abundance	6	0	0
Relative abundance	100	0	0

Table 5: The effect of age on serum contaminants concentration and the Leptospira MAT titer

Infection * Age Cross tabulation

Count

		Age1			
		1.00	2.00	3.00	Total
Infection	.00	41	31	15	87
	1.00	6	0	0	6
Total		47	31	15	93

Chi-Square Tests

	Value	df	A symp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	6.277[a]	2	.043	.051		
Likelihood Ratio	8.595	2	.014	.028		
Fisher's Exact Test	5.005			.064		
Linear-by-Linear Association	4.979[b]	1	.026	.040	.014	.014
N of Valid Cases	93					

Table6: Frequency of Sex in dogs

	male	female
Frequency	44	49
Relative abundance	47.3	52.7

Table7: Gender-based prevalence study in dogs

	male	female
Abundance	1	5
Relative abundance	16.7	83.3

Table8: Sex effect on pollution levels and titers to Leptospira MAT

Infection * Sex Cross tabulation

Count

		Sex		
		1.00	2.00	Total
Infection	.00	43	44	87
	1.00	1	5	6
Total		44	49	93

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.416[a]	1	.120	.207	.128	
Continuity Correction[b]	1.281	1	.258			
Likelihood Ratio	2.654	1	.103	.207	.128	
Fisher's Exact Test				.207	.128	
Linear-by-Linear Association	2.390[c]	1	.122	.207	.128	.110
N of Valid Cases	93					

Based on Fisher's test with a confidence level of 95% (α=0.05) was determined that relationship between gender and contamination is no-significant (P>0.05). Gender effect is notsignificant on a MAT titer. In other words, based on the results of this study made no talent for leptospirosis in animals does not cause disease.

DISCUSSION

Central tribes in the western provinces of Iran on which the test was carried out on 400 patients, was determined that the48.5% of those tested had antibody against this disease and in women more than the men and most often the serovar were detected [16]. In another study which was performed by Zakeri et al in four different provinces of Iran, found that 26.5% of people and22% of dogs were infected and the most common species involved in the study *LeptospiraInterogans* in human and *Leptospira Volfi* in dogs have been reported [29].In this study, samples were taken from 93 pet dogs. Species leptospiral infection has been reported in the past and recently. For example, the first isolation of the organism from thedogs in Iranwas done by Jamshidiet al in 2008 [23]. But in the past years, investigation of dog's contamination was conducted by Rad et al [27]. Unfortunately the survey of 300samples from dog's high percentage of infection was reported. This survey showed that31%ofall dogs were found in the serum and 18.3% of those with acute symptoms of the disease were showed. Another study in 2009 has been reported by Khorrami et al, 31%ofurban and rural dogs were tested that infected with leptospirory [25]. A study on dogs in Thailand was undertaken, from 230 samples and a high contamination of stray dogs (about 83.5%) were reported as the most common organism involved is serovar Batavia [24]. However, in another study by Rojas et al on 525 samples taken in Dublin only 7% of dogs show the infections (Rojas, P. et al; 2010). In a similar study in South Africa on 530 dogs showed that only 25 cases of proven infection was the most common organism involved in Canicola [20].

AVKNOWLEGMENTS

The authors would like to thank Leptospirosis Reference Laboratory of Veterinary Faculty of Tehran University (Iran) for their collaboration and the staff of Islamic Azad University of Urmia (Iran)- Faculty of veterinary Medicine, for their high cooperation during the study. We also gratefully acknowledge T.Sabetpour for linguistic support and assistance and all the reviewers of this paper.

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CITATION OF THIS ARTICLE

Ali H, Ali G L, Amin H, Saman S G, Gholam R A, Reza T Z.Survey on Seroprevalence of *Leptospira* serotypes in household dogs using MAT method in Urmia, Iran. Bull. Env. Pharmacol. Life Sci., Vol 3 [Spl Issue V] 2014: 158-162