

Seroepidemiology of canine *leptospirosis* in Ahvaz, Iran

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Abstract: The presence of dogs has been identified as a risk factor for human *Leptospirosis*. The aim of this study was the survey of the current state of leptospiral infection in dogs in Ahvaz. Sera from 100 rural dogs of villages around Ahvaz and 49 urban dogs referred to veterinary hospital of shahid Chamran University were detected for antibodies against serovars *canicola*, *icterohaemorrhagiae*, *grippotyphosa*, *ballum*, *hardjo* and *pomona* by microscopic agglutination test. Out of the 149 dogs investigated, 8(5.4%) were serologically positive against at least one serovar of leptospira. Titers $\geq 1:100$ were detected in 7 rural (7%) and one urban (2.04%) dogs. The predominant titers were directed to serovars *hardjo* (44.5%) followed by *ballum* (22.2%), *icterohaemorrhagiae* (22.2%) and *grippotyphosa* (11.1%). Mixed infection was detected only in one dog. Serovars *canicola* and *pomona* were uncommon in dogs from Ahvaz. As expected, a very low prevalence of infection was found in urban pet dogs but in contrast low prevalence of infection in rural shepherd and/or stray dogs with poor level hygiene was unexpected. However, the sources of infection in these dogs were not clear and this is the first report of infection with serovar *hardjo* from dogs in Iran.

Key words: Dog, *Leptospirosis*, serovars, zoonosis, Iran.

Introduction

Leptospirosis, a zoonotic disease of worldwide significance in many animals, is caused by infection with antigenically distinct serovars of *Leptospira interrogans sensu lato*. Serovars are maintained in nature by numerous subclinically infected wild and domestic reservoir hosts that serve as a potential source of infection and illness for humans and other incidental animal hosts (Greene *et al.*, 1998).

Diagnosis of *leptospirosis* is based on serologic findings or blood, cerebrospinal fluid and urine cultures in specific media. Most diagnostic laboratories do not attempt to isolate leptospire because of their fragile nature, the cost and complexity of the isolation media, and the prolonged

incubation period. Therefore, serology plays an important role in the recognition of leptospiral infection (Greene *et al.*, 1998). A wide variety of serological tests, which show varying degrees of serogroup and serovar specificity, have been described. Two tests have a role in veterinary diagnosis: the microscopic agglutination test (MAT) and the enzyme-linked immunosorbent assay (ELISA) (Faine, 1982). Dog is a good indicator of the distribution of different leptospiral serovars in its environment. It seems reasonable to practice occasionally serological investigations of these animals in order to detect possible changes in infecting leptospiral serovars. Consequently, adequate epizootological means could be taken (e. g. modification of the composition of a leptospiral vaccine for dogs).

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Table 1: Sex distribution in leptospiral antibody titers in rural and urban dogs from Ahvaz district by MAT at a serum dilution of 1:100.

| Sex | Rural dogs | | | Urban dogs | | |
|--------|------------|--------------|-----|------------|--------------|------|
| | No. tested | No. positive | % | No. tested | No. positive | % |
| Male | 70 | 5 | 7.1 | 33 | 0 | 0 |
| Female | 30 | 2 | 6.5 | 16 | 1 | 6 |
| Total | 100 | 7 | 7 | 49 | 1 | 2.04 |

Table 2: Age distribution of leptospiral antibody titers in dogs from Ahvaz district. * Significant difference between two various age groups.

| Age (Year) | Rural dogs | | | Urban dogs | | |
|------------|------------|--------------|------|------------|--------------|------|
| | No. tested | No. positive | % | No. tested | No. positive | % |
| <1 | 10 | 3 | 30* | 24 | 1 | 5 |
| 1-3 | 55 | 4 | 7.3* | 12 | 0 | 0 |
| 3-5 | 24 | 0 | 0 | 9 | 0 | 0 |
| >5 | 11 | 0 | 0 | 4 | 0 | 0 |
| Total | 100 | 7 | 7 | 49 | 1 | 2.04 |

For the first time canine *leptospirosis* in Iran was reported from Tehran (Abdollahpour, 1996). Sixteen years later, three surveys found evidence of infection in dog in Tehran and suburban areas (Zeinali *et al.*, 2003) and Mashhad (Kamrani and Sardari, 2003; Talebhkan Garoussi *et al.*, 2003). The aim of this study was determination of the prevalence of antibodies to *Leptospira interrogans*. This research is the first report of leptospiral infection in urban and rural dogs from Ahvaz.

Materials and Methods

Blood samples were taken from 100 rural dogs (70 male, 30 female) in four villages around Ahvaz city and 49 urban dogs (33 male, 16 female) that referred to Small Animal Clinic of Veterinary Faculty of Shahid Chamran University in Ahvaz from June 2004 to March 2005. These dogs were selected among the non-vaccinated dogs against canine *leptospirosis* and there was no history of *leptospirosis*-related symptoms or signs of *leptospirosis* at the time of sampling. These dogs were 3 months to 12 years old. All of the urban dogs were among different pure or mixed breeds but rural

dogs were from mongrels.

Five ml of blood was collected from the cephalic or saphenous vein of each dog. The blood samples were allowed to clot and were centrifuged for 10 min at 2500g. Serum samples were marked and were stored at -20°C until they were examined through Research Laboratory of *Leptospirosis* of the University of Tehran located in the Educational and Research Hospital of Mard Abad (Karaj).

The serum samples were tested for antibodies to six live antigens of *Leptospira interrogans* (serovars *canicola*, *grippityphosa*, *hardjo*, *pomona*, *icterohaemorrhagiae* and *ballum*) using the MAT. This test was performed by the standard method, recommended by WHO (Faine, 1982). Sera were screened at a serum dilution of 1:100 and greater up to 1:800 dilutions against antigens. Results were considered positive when 50% or more of agglutination of leptospire at the test serum dilution of 1:100 or greater was found (Faine, 1982).

Results were analyzed by fisher's exact test to determine seroepidemiologic status of canine *leptospirosis* in order to find out the relationship between natural positive cases and some important factors such as age, sex, and environmental conditions of animals.

Results

The leptospiral sero-prevalence in dogs was 5.4% (8/149). Out of 100 rural and 49 urban dogs tested, 7(7%) and 1(2.04%) were positive respectively for at least one leptospiral antibody (Table 1). All of the sero-positive animals had titer (1:100). There was no significant difference between rural and urban dogs in reactors to leptospire ($p>0.05$). Also statistically significant differences were not found between sero-positive dogs from various villages ($p>0.05$).

The predominant titers were directed to serovars *hardjo* 4 (44.5%), followed in descending order by *ballum* 2 (22.2%), *icterohaemorrhagiae* 2 (22.2%), and *grippityphosa* 1 (11.1%). Mixed infection was detected only in one dog (*hardjo* and *icterohaemorrhagiae*). Serovars *canicola* and *pomona* was uncommon in dogs in this study. In one out of the 49 dogs referred by private owners to the Clinic of Veterinary Faculty of Ahvaz,



there was antibody against serovar *hardjo*.

Although the prevalence of leptospiral antibody titers in male rural and female urban dogs was greater than others, there were no statistically significant differences between male and female dogs ($p > 0.05$) (Table 1).

Sero-prevalence of leptospiral antibodies in rural and urban dogs based on age-grouped is shown in table 2. Results show that only the dogs less than 3 years old had antibodies against *leptospirosis*. The only statistically significant difference was found between rural dogs less than 1 year old and greater than 3 years old ($p \leq 0.05$).

Discussion

Leptospirosis is an emerging infectious disease that occurs in dogs in urban and rural environments. This is the first serological study of *leptospirosis* in Ahvaz, Iran, in order to determine the predominant serovars of leptospira in rural and urban dogs' population in this region of country. MAT is considered to be a sensitive and specific serological test for diagnosis of *leptospirosis* and is called as standard serologic means (Greene *et al.*, 1998; Rentko *et al.*, 1992).

The overall leptospiral seroprevalence was 5.04%. Leptospiral antibody titers in client-owned dogs from Tehran (Zeinali *et al.*, 2003) and stray and herding dogs of Mashhad (Kamrani and Sardari, 2003; Talebhkan Garoussi *et al.*, 2003) were reported 31%, 14.38% and 41.6% respectively, which are greater than this study. Ambient temperatures between 0°C and 25°C favor the survival and replication of leptospores, whereas freezing markedly decreases survival (Greene *et al.*, 1998), therefore clinical *leptospirosis* is rarely seen in Switzerland due to the climatic condition (Steffen and Widmer, 2000) whereas in Ahvaz geographic zone that temperature rises up to 50°C in summer, hot weather and dryness of soil decrease the survival of leptospores. The temperature requirement for maximal leptospiral survival may explain the apparent differences of leptospiral seroprevalences in these parts of country. In Tehran, the higher prevalence may stem from the use of current

vaccination in dogs, whereas in Mashhad, it may be due to the greater exposure of stray dogs in rural and suburban environments or contacting of herding dogs with the urine of cattle (Kamrani and Sardari, 2003; Talebhkan Garoussi *et al.*, 2003; Zeinali *et al.*, 2003).

In a review of international surveys of more than 12,000 dogs, the highest seroprevalences were detected in South America and Asia (Ryu, 1976), whereas negative results were obtained for some countries including Iran. Thus it appears from the present work and several other surveys that the epidemiology of canine *leptospirosis* in Iran has changed like other parts of the world (e.g. Australia, South Africa, and Ethiopia) (Moch *et al.*, 1975; Myburgh *et al.*, 1993; Watson *et al.*, 1976).

Although serologic surveys may provide an estimate of the exposure rate for dogs, it does not provide information regarding how many dogs are actively shedding leptospores and posing a potential zoonotic risk. Results of several studies suggest that dog can be seronegative and clinically normal just still actively shed leptospores (Harkin *et al.*, 2003). Despite of low prevalence of seroreactivity, the presence of antibodies against *leptospirosis* in dogs is the main public health concern because the close contact between dogs and man provides the link between a reservoir in the environment and susceptible humans.

Among the six serovars that were used in the present study, *hardjo*, *ballum*, *icterohaemorrhagiae* and *grippityphosa* serovars were the most prevalent in Ahvaz. *Leptospirosis* is a zoonotic disease caused by antigenically distinct serovars of leptospira interrogans, of which at least eight are of importance for dogs in the world. But traditionally, serovars *canicola* and *icterohaemorrhagiae* are considered to be the most significant serovars in dogs worldwide (Greene *et al.*, 1998). According to the introduction of a bivalent vaccine for protection of dogs against *leptospirosis* due to serovars *canicola* and *icterohaemorrhagiae*, the incidence of disease attributed to these serovars has decreased in the world (Ward *et al.*, 2002). On the contrary, cases caused by infection with other serovars have increased (Murphy *et al.*, 1958; Nielson *et al.*, 1991). The



recognized primary reservoir hosts for serovars infecting dogs include the dog (*canicola*), vole (*grippotyphosa*), rat (*icterohaemorrhagiae*), cow (*hardjo*) and pigs and cow (*pomona*) (Greene *et al.*, 1998). It suggests that the dog population of the Ahvaz district may have been exposed to one of these reservoirs (especially farm animals and rodents) or to environmental contamination of the urine of these species, located in farms or recreational areas.

Serovars *canicola* and *pomona* were uncommon in dogs from Ahvaz. Also because low seroprevalences against only four serovars of leptospira have been found, their epidemiology is relatively simple when compared with the tropical environments.

This study demonstrated that leptospiral infection was more common in rural dogs than urban ones. As expected, a very low prevalence of infection was found in client-owned dogs (2.04%) but in contrast low prevalence of infection in rural dogs (7%) with unsanitary quarter was unexpected. Dogs in various villages were not at greater risk of *leptospirosis*. Altogether all of villages had similar environmental variables such as mean annual rainfall, temperature, humidity, dryness, livestock population, drainage and proximity to stagnant water.

Based on results of this study between the sexes, the male presented higher index of positivity though the difference was not statistically significant. In dogs, a predisposition for leptospiral infection in males has been previously suggested (Hartman, 1984; Moch *et al.*, 1975; Rubel *et al.*, 1997). The higher prevalence of infection in males seems to be related to the habit of sniffing the genital and licking recently voided urine (Scanziani *et al.*, 2002). Although the seropositive urban dog was female, the epidemiological source of infection and root of contamination of this dog are not so clear.

In accordance with serologic study of canine *leptospirosis* in Tehran, dogs less than 1 year old were at significantly greater risk than dogs more than 3 years old (Zeinali *et al.*, 2003). Other previous studies showed that leptospiral seroprevalence in older animals are more common than in puppies (Rubel *et al.*, 1997; Ward *et al.*, 2004) but the present study could not repudiate or confirm this trend.

The trend of seroprevalence of canine *leptospirosis* in Ahvaz and suburban areas is increasing and more investigations are needed to be conducted in this regard in order to clarify the epidemiological picture of *leptospirosis* in Iran.

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بررسی سرواپیدمیولوژیکی لپتوسپیروز در سگ‌های اهواز

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چکیده

سگ‌ها به عنوان یکی از عوامل خطر در آلودگی انسان به شمار می‌روند. هدف از این مطالعه بررسی شیوع آلودگی به لپتوسپیروز در سگ‌های شهر ستان اهواز بود. در این مطالعه سرم ۱۰۰ قلاده از سگ‌های روستاهای اطراف شهر اهواز و ۴۹ قلاده سگ‌های شهری ارجاعی به بیمارستان دامپزشکی دانشگاه شهید چمران اهواز توسط آزمایش آگلوتیناسیون میکروسکوپی بر علیه پادتن‌های سروواری کانیکولا، پومونا، ایکتر و هموراژی، هار جو، گریپوتایفوزا و بالوم مورد بررسی قرار گرفتند. در کل هشت قلاده از ۱۴۹ سگ مورد مطالعه ۴/۵ (درصد) از لحاظ سروولوژی دارای تیترو سیمی مثبت، حداقل به یک سروواری لپتوسپیروز بودند. هفت قلاده از سگ‌های روستایی ۷ (درصد) و یک قلاده از سگ‌های شهری ۲/۴ (درصد) تیترو سیمی برابر یا بیشتر از ۱۰۰:۱ داشتند. سروواری‌های غالب به ترتیب لپتوسپیروز ۵/۴۴ (درصد)، بالوم ۲/۲۲ (درصد)، ایکتر و هموراژی ۲/۲۲ (درصد) و گریپوتایفوزا ۱/۱۱ (درصد) بودند. تیترو آنتی بادی علیه بیش از یک سروواری تنها در یک مورد از سگ‌ها دیده شد. سروواری‌های کانیکولا و پومونا در سگ‌های اهواز مشاهده نشدند. اگرچه پائین بودن میزان آلودگی در سگ‌های شهری قابل انتظار بود، اما برعکس میزان کم آلودگی در سگ‌های نگهبان و یا ولگرد روستایی آن هم با وضعیت بهداشتی پائین غیر منتظره بود. به هر حال منشأ عفونت در این سگ‌ها مشخص نبود و این اولین گزارش آلودگی سگ‌ها با سروواری هار جو در ایران می‌باشد.

واژه‌های کلیدی: سگ، لپتوسپیروز، سروواری، بیماری مشترک، ایران.

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