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 antigenetically 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 leptospires 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).
For the first time canine leptospirosis in Iran was reported from Tehran (Abdollahpour, 1996). Sixteen years later, three surveys found evidence of infection in dogs 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, grippotyphosa, 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 leptospires 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 urban and rural dogs in reactors to leptospires (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 grippotyphosa 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 \textit{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 \textit{leptospirosis}. The only statistically significant difference was found between rural dogs less than 1 year old and greater than 3 years old (p<0.05).

**Discussion**

\textit{Leptospirosis} is a re-emerging infectious disease that occurs in dogs in urban and rural environments. This is the first serological study of \textit{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 \textit{leptospirosis} and is called as standard serologic means (Greene \textit{et al}., 1998; Rentko \textit{et al}., 1992).

The overall leptospiral seroprevalence was 5.04%. Leptospiral antibody titers in client-owned dogs from Tehran (Zeinali \textit{et al}., 2003) and stray and herding dogs of Mashhad (Kamrani and Sardari, 2003; Talebhhkan Garoussi \textit{et al}., 2003) were reported 31%, 14.38% and 41.6% respectively, which are greater than this study. Ambient temperatures between 0ºC and 25 ºC favor the survival and replication of leptospires, whereas freezing markedly decreases survival (Greene \textit{et al}., 1998), therefore clinical \textit{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 50ºC in summer, hot weather and dryness of soil decrease the survival of leptospires. 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; Talebhhkan Garoussi \textit{et al}., 2003; Zeinali \textit{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 \textit{leptospirosis} in Iran has changed like other parts of the world (e.g. Australia, South Africa, and Ethiopia) (Moch \textit{et al}., 1975; Myburgh \textit{et al}., 1993; Watson \textit{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 leptospires and posing a potential zoonotic risk. Results of several studies suggest that dog can be seronegative and clinically normal just still actively shed leptospires (Harkin \textit{et al}., 2003). Despite of low prevalence of seroreactivity, the presence of antibodies against \textit{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, \textit{hardjo}, \textit{ballum}, \textit{icterohaemorrhagiae} and \textit{grippotyphosa} serovars were the most prevalent in Ahvaz. \textit{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 \textit{canicola} and \textit{icterohaemorrhagiae} are considered to be the most significant serovars in dogs worldwide (Greene \textit{et al}., 1998). According to the introduction of a bivalent vaccine for protection of dogs against \textit{leptospirosis} due to serovars \textit{canicola} and \textit{icterohaemorrhagiae}, the incidence of disease attributed to these serovars has decreased in the world (Ward \textit{et al}., 2002). On the contrary, cases caused by infection with other serovars have increased (Murphy \textit{et al}., 1958; Nielson \textit{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 unexpectable. 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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**References**

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بررسی سروپایدیمیولوژیکی لیپوسیپرور در سگ‌های اهواز

چکیده
سگ‌هایی با نشان‌های اختلال در سطح اندام جریان خون در اهلی‌گیری که مورد مطالعه قرار گرفتند به شکلی‌های شدید چربی اهواز و اهلی‌گیری سگ‌های شهربازی و دیگر شکل‌های اهلی‌گیری سگ‌های سهولتگر، بنا بر روی نتایج مطالعه که در این مقاله ذکر گردیده است، اثرات مثبتی به شکلی که در واقع می‌تواند به راحتی از تفاوت‌های فیزیولوژیکی بین سگ‌های سوپیدیمیولوژیکی روند نشان دهنده بهبود در سطح اندام جریان خون در سگ‌های اهلی‌گیری و سگ‌های با پایداری در انتقال خون و واکنش به عوامل افزایشی بهبود نشان می‌دهد.

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