

Seroprevalence of *Leptospiral* infection in goats of Ahvaz

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Abstract: In a serological survey to investigate the prevalence of *leptospiral* infection in goats in Ahvaz, blood samples were taken from 172 female goats. They were initially screened at serum dilution of 1: 100 against six live antigens of *leptospira* interorgans serovar *pomona*, *canicola*, *hardjo*, *ballum*, *icterohemorrhagiae*, and *grippotyphosa* using the microscopic agglutination test (MAT) and samples were considered positive, if 50% or more of agglutination of leptospires in a dilution of 1: 100 or greater was found. Sera with positive results were titrated against reacting antigens in serial twofold dilution from 1:100 to 1:1600. Antibodies against one or more serovars were detected in 18 (10.46%) sera at dilution \geq 1: 100. Antibodies against more than one serovar were found in 5 (27.77%) positive sera. Antibodies were most frequent to serovar *grippotyphosa* (39.13%) followed by in descending, *canicola* (26.80%), *pomona* (21.72%), *icterohemorrhagiae* (8.69%) and *hardjo* (4.34%).

Key words: *Leptospirosis*, goat, Ahvaz.

Introduction

Leptospirosis is a common global zoonotic disease of man and in all farm animals species specially in sub - tropical and tropical regions of the world. Most *leptospiral* infections in sheep and goat are asymptomatic but may result in high fever, abortion, stillbirth, agalactiae and prenatal death. Affected lambs and kids may manifest fever, jaundice and hemoglobinuria, which may also result in death (1, 4, 16).

Unfortunately, a definitive diagnosis of leptospirosis is difficult to make. Most of diagnostic laboratories do not attempt to isolate leptospires because of their fragile nature, cost and complexity of the isolation media, and prolonged incubation period (5, 16). Therefore, recognition of *leptospiral* infection has been based generally on serological

evidence. A wide variety of serological tests, which show varying degrees of serogroups and serovar specificity, have been described. 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) (14).

Previous serological surveys of *leptospiral* infection in Ahvaz were carried out on cattle, buffalo, horse and donkey (8, 9, 10). These surveys indicated that *leptospiral* infection is common in these animals. Because, there was no evidence of the study on *leptospiral* infection in goat in Ahvaz, this study was carried out to determine the prevalence of leptospiral antibodies in goat and compare with other farm animals.

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Materials and Methods

Blood samples were taken from 172 female goats from 5 suburbs of Ahvaz, Southwest of Iran, during January to March of 2003. None of these animals had been vaccinated against leptospires. According to dental formula, these goats were divided into 5 age groups (≤ 1 , 1, 2, 3 and ≥ 4 years old). The numbers of samples from suburb one to five were 52, 36, 25, 15, and 44, respectively. At the time of blood collection all of the examined animals appeared healthy and presented no clinical sign suggestive of leptospirosis. Ten ml of blood was collected from the jugular vein of each goat. The blood samples were allowed to clot and were centrifuged for 10 min at 2500g. After centrifugation, the serum was removed and stored at -20°C until ready for test.

The sera were tested for antibodies to six live antigens of leptospira interrogans (L. interrogans serovar *pomona*, *canicola*, *hardjo*, *ballum*, *icterohemorrhagiae*, *grippotyphosa*) using the microscopic agglutination test (MAT), in Leptospiral Research Laboratory in Faculty of Veterinary Medicine, University of Tehran. According to the methods of OIE, sera were initially screened at a dilution of 1:100 against these antigens. At first, serum dilution of 1:50 was performed and a volume of each antigen, equal to the diluted serum volume, was added to each well of microtitration plates, making the final serum dilution 1:100. The microtitration plates were incubated at 29°C for 2 h. The plates were examined by dark-field microscopy. Results were considered positive when 50% or more of agglutination of leptospires were found (OIE, 2000). Sera with positive result were titrated against reacting antigens in serial twofold dilution from 1:100 to 1:1600.

Obtained results were analyzed statistically using chi-square and Fisher's-exact test with confidence level 95%.

Results

Antibodies against one or more serovars were detected in 18 (10.46%) goats. The highest number of reactors was due to *grippotyphosa* (39.3%), followed in descending order by *canicola* (26.08%), *pomona*

(21.72%), *icterohemorrhagiae* (8.64%), and *hardjo* (4.34%). None of the sera were reacted to *ballum*. The majority of titre levels were between 1:200 to 1:400 for all serovars and the frequency of 1:100, 1:200, 1:400 and 1:800 was 13.04%, 34.78%, 34.78% and 17.39%, respectively.

Antibodies against more than one serovars were found in 5 (27.77%) sera, so that mixed infection of *pomona* and *grippotyphosa*, *canicola* and *grippotyphosa*, *hardjo* and *icterohemorrhagiae* were seen in 2, 2 and one positive sera, respectively.

There is significant difference in age distribution of leptospiral infection in seropositive goats ($p < 0.05$). There is a tendency in adult goats to be more seropositive than younger ones.

Statistical analysis showed there is not an area dependent antibody response to leptospire. Distribution of seropositive goats in various suburbs was no significant difference but suburb two and suburb four had highest and lowest number of seropositive goats.

Discussion

Antibodies against one or more serovars were detected in 18 (10.46%) goats. The prevalence of leptospiral infection in goats from another countries based on serological survey has been reported to be 1.2%, 55.2%, 42.1%, 70%, 13.1% and 19.7% in France, India, Egypt, New Zealand, Nigeria, and Bolivia, respectively (1, 3, 7, 12, 17, 18). There is only one documented report of leptospiral infection in goats from Iran. This study was carried out on goats from Uremia by Zainali *et al.*, (1997) that reported 27.5% of goats from this area had leptospiral antibodies (19). These reported results confirm that prevalence of leptospiral infection in goat is different between countries. These differences may be the consequence of environmental factors and control efforts. It seems the prevalence of leptospiral infection in Ahvaz is lower than of some countries specially in contrast with uremia. Since, at present, there is no specific control strategy against leptospirosis in Ahvaz, this can not therefore be attributed to control efforts. On the other hand, environmental factors have been shown in influence



Tablea: Distribution of serovar specific antileptospiral antibodies and theirs titration in seropositive goats.

	1: 100 (%)	1: 200 (%)	1: 400 (%)	1: 800 (%)	Total (%)
<i>Grippotyphosa</i>	0(0)	4 (17.39)	4 (17.39)	1 (4.35)	9 (39.13)
<i>Canicola</i>	1(4.34)	3 (13.02)	1 (4.34)	1 (4.34)	6 (26.08)
<i>Pomona</i>	1(4.34)	0 (0)	2 (8.69)	2 (8.69)	5 (21.37)
<i>Icterohemorrhagiae</i>	1(4.34)	0 (0)	1 (4.34)	0 (0)	2 (8.69)
<i>Hardjo</i>	0(0)	1 (4.34)	0 (0)	0 (0)	1 (4.34)
Total	3 (13.04)	8 (34.78)	8 (34.78)	4 (17.3)	23 (100)

Tableb: Distribution of leptospiral infection in age groups of goats.

age (year)	No. positive (%)	No. Negative (%)	Total
< 1	1 (7.14)	13 (92.86)	14
1	0(0)	22 (100)	22
2	2 (5.12)	37 (94.88)	39
3	9 (23.07)	30 (76.93)	39
≥4	6 (10.34)	52 (89.66)	58
Total	18 (10.46)	154 (89.54)	172

Tablec: Distribution of leptospiral infection in goats from various suburbs of Ahvaz.

Suburb	No. Positive (%)	No. Negative (%)	Total
1	4 (7.69)	48 (92.31)	52
2	5 (13.88)	31 (86.12)	36
3	2 (8.00)	23 (92.00)	25
4	1 (6.66)	14 (93.34)	15
5	6 (13.63)	38 (86.37)	44
Total	18 (10.46)	154 (89.54)	172

of development of leptospiral infection in animal and human beings. Long term survival of pathogenic leptospire outside the host requires a warm, moist environment with pH that is near natural (13). According to climate situations in Ahvaz it has been expected that leptospiral infection in goat was nearly similar to a previous reports in cattle, buffalo, horse and donkey in Ahvaz. Because, in previous serological surveys the prevalence of leptospiral infection was 53.79%, 58.73%, 27.88% and 40% in cattle, buffalo, horse and donkey, respectively (8, 9, 10). It may be due to the behaviors of the goat, which is differing with other animals. This animal habitually tries to use the head branches of grass and stay in muddy and swampy grounds less than others that it results to less expose to leptospire.

The predominant leptospire serovars in serological reaction varies some what between countries. For example: automnalis, pomona, automnalis, poi, bratislava and icterohemorrhagiae

were the common serovars in goat in Egypt, Nigeria, India, Bolivia, Italy, and France, respectively (1, 2, 3, 12, 18). In addition, one serovar may be predominant in a country but none of the animal reacted with this serovar in another ones. This emphasizes the need for regional surveys for leptospirosis, since host-parasite relationship may change depending on the ecology of the region. *Grippotyphosa* was present in 39.13% of seropositive goats in this study making it the most prevalent of all serovars for which we tested. On the other hand, *grippotyphosa* was the predominant serovar in cattle, horse and donkey in Ahvaz (9, 10). It is probable that this serovar may be adapted to and maintained by these farm animals in Ahvaz. There is need for further investigation on clinical cases of leptospirosis to determine whether this serovar is main cause of leptospirosis in this area.

Percentage of seropositive for more than one serovar was 27.77% in seropositive goats. In serological tests for leptospirosis such as MAT, the



results often indicate infection with more than one serovar (6, 9, 10, 11). This may be the result of mixed serovar infection but the existence of cross reactivity in the MAT between the serovars is well known and can be excluded from this interpretation.

In conclusion, the results of this study indicate that *leptospiral infection* is endemic in Ahvaz and that various serovars concur in the etiology of leptospirosis. Because of the importance of *Leptospira interrogans* as an abortifacient agent in goat, it will be considered as one of the possible causes of abortion in goat in Ahvaz.

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مطالعه سرولوژیکی عفونت لیتوسپیری در بز در اهواز

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به منظور بررسی سرمی عفونت لیتوسپیری در بز در اهواز، نمونه خون از ۱۷۲ راس بز ماده اخذ گردید. سرمها بعد از جداسازی در دمای ۲۰- درجه سانتیگراد نگهداری شدند. نمونه ها با استفاده از ۶ آنتی ژن زنده لیتوسپیرا اینتر و گانس سروارهای پومونا، گریپو تیغوزا، کانیکولا، هار جو، ایکتر و همور اژی و بالوم وبا استفاده از روش آگلوتیناسیون میکروسکوپی جهت جستجوی آنتی بادی علیه این سروارها مورد آزمایش قرار گرفتند. نمونه هایی که در آنها بیس از ۵۰ در صد لیتوسپیراها در رقت ۱:۱۰۰ یا بیشتر آگلوتینه می شدند، مثبت در نظر گرفته شدند. سپس از نمونه های مثبت رقت های مختلف، تا ۱:۱۶۰۰ تهیه و مورد آزمایش قرار می گرفتند. از این تعداد (۴۶/۱۰ درصد) ۱۸ نمونه به یک یا چند سروار در رقت های مساوی یا بیشتر از ۱:۱۰۰ واکنش مثبت نشان دادند. در (۲۲/۷۷ درصد) ۵ نمونه آنتی بادی علیه بیش از یک سروار مشاهده شد. از نظر فراوانی گریپو تیغوزا ۳۹/۱۳ درصد دارای بیشترین و بعد از آن به ترتیب کانیکولا (۲۶/۸۰ درصد)، پومونا (۲۱/۷۲ درصد)، ایکتر و همور اژی (۸/۶۹ درصد) و هار جو (۴/۳۴ درصد) قرار داشتند.

واژه های کلیدی: لیتوسپیروزیس، بز، اهواز.

