

Comparison of *Leptospira interrogans* infection in the goats and sheep

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Abstract:

BACKGROUND: Most leptospiral infections in sheep and goat are asymptomatic but may result in high fever, abortion, still-birth, agalactiae. There is a different foraging behavior between sheep and goat that may cause the different prevalence of *Leptospira interrogans* infection in sheep and goats. **OBJECTIVES:** The purpose of the present study was to compare the prevalence of *L. interrogans* antibodies in sheep and goats. **METHODS:** Blood samples were taken from 246 sheep and 210 goats in 12 herds from 8 areas of Ahvaz where the animals were kept together. Sera were initially screened at dilution of 1:100 against 8 live serovars of *L. interrogans*: pomana, canicola, hardjo, ballom, icthrohaemorrhagiae, grippotyphosa, tarasovi and australis using the microscopic agglutination test (MAT). **RESULTS:** The prevalence of leptospiral infection was 8.53% in sheep and 10.95% in goats. The highest reacting leptospira in both species was *L.i. Pomona* with a reactor rate of 68.18% in sheep and 56% in goats, followed in descending order by icthrohaemorrhagiae (18.8%), canicula, hardjo and grippotyphosa (each of them 4.54%), in sheep and icthrohaemorrhagiae (28%), canicula (16%) in goats. Statistical analysis showed that there were no significant differences between sheep and goat ($P=0.428$). There were no significant differences among age groups in sheep ($p=0.301$) and goats ($p=0.363$), but there was a tendency in adults sheep and goats (≥ 3 years) to be more seropositive than young sheep and goats. Seroprevalence of leptospiral infection among various areas in sheep ($p=0.464$) and goats ($p=0.710$) was also not significantly different. **CONCLUSIONS:** It is concluded that there is no difference between sheep and goats in terms of leptospiral infection when they are kept together in the same herd and husbandry condition.

Introduction

Leptospirosis is a reemerging zoonotic disease of human and animals worldwide. It

is presumed to be the most widespread zoonosis in the world. The disease is caused by pathogenic species of spirochetes of the genus *Leptospira* (Samir et al., 2015). Among sheep

and goats, most of the outbreak goes unnoticed due to lack of proper clinical signs and they usually react asymptotically to the infection (OIE, 2000). The clinical manifestation of leptospires ranges from mild to severe life threatening disease with jaundice, renal failure or abortion during pregnancy (Shivaraj et al. 2009). Unfortunately, a definitive diagnosis of leptospirosis is difficult to make. Most diagnostic laboratories do not try to isolate leptospires because of their fragile nature, cost and complexity of the isolation media, and prolonged incubation period. Therefore, recognition of leptospiral infection has generally been based on serological evidence. Two tests have a role in veterinary diagnosis: the Microscopic Agglutination Test (MAT) and the Enzyme Linked Immunosorbent Assay (ELISA) (OIE, 2000)

Previous serological surveys of leptospiral infection in Ahvaz were carried out on cattle, horse and donkey (Hajikolaei et al., 2006, 2005a, 2005b) and two studies have been conducted to determine seroprevalence of leptospiral infection in goats and sheep, separately (Hajikolaei et al., 2007a, 2007b). A big difference between sheep and goats is their foraging behavior and diet selection. Goats are natural browsers, preferring to eat leaves, twigs, vines and shrubs. They are very agile and will stand on their hind legs to reach vegetation. Goats like to eat the tops of plants. Sheep are grazers, preferring to eat short, tender grasses and clover. Their dietary preference is forbs and they like to graze close to the soil surface. These differences between sheep and goats may be responsible for the difference in prevalence of leptospiral infection in sheep and goats (Bojkovski et al., 2014). As there has been no study on comparison of leptospiral infection in sheep and goats in similar region, this study was undertaken to compare the prevalence of *L. interrogans* antibodies in sheep and goats in different areas of Ahvaz, the center of Khuzestan province in the southwest of Iran.

Materials and Methods

Blood samples were taken from 246 sheep and 210 goats from 8 areas of Ahvaz, in the southwest of Iran, during May to July of 2015. None of these animals had been vaccinated against leptospires and there was no history of leptospirosis-related symptoms or sign of the disease at the time of sampling. According to dental formula, these goats and sheep were divided into 5 ages groups (≤ 1 , 1, 2, 3 and ≥ 4 years old). The number of samples of goats from area A to H were 40(A), 40(B), 40(C), 24(D), 14(E), 13(F), 16(G), 20(H) and those numbers of sheep were 40(A), 40(B), 40(C), 40(D), 20(E), 14(F), 21(G), 30(H), respectively.

The blood samples were allowed to clot and were centrifuged for 8 min at 3000g. After centrifugation, the sera were removed and stored at -20°C until ready for test. The sera were tested for antibodies to 8 live antigens of *leptospira interrogans* (*L. interrogans* serovars pomona, canicola, hardjo, ballum, icterohemorrhagiae, grippityphosa, tarasovi, australis) 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 serovar, equal to the diluted serum volume, was added to each well of microtitrations plates, making the final serum dilution 1:100. The microtitrations plates were incubated at 29°C for 2h. The plates were examined by dark-field microscopy. Results were considered positive when 50% or more of agglutination of leptospires was found (OIE, 2000). Sera with positive result were titrated against reacting antigens in serial two-fold dilution from 1:100 to 1:800.

Statistical analysis was achieved using Chi-Square and Fisher's exact test which aimed to

Table 1. The Results of MAT on sheep and goats in some areas of Ahvaz-Iran.

Animals	No. positive (%)	No. negative (%)	No. exam
Sheep	21 (8.53)	225(91.47)	246
Goats	23 (10.95)	187(89.05)	210

detect differences between all variables.

Results

Out of 246 sheep and 210 goats tested, 21 (8.53%) and 23 (10.95%), were seropositive and antibodies against one or more serovars were detected (Table 1). One goat (4.76%) and two sheep (8.69%) were positive for more than one serovar. Significant difference ($p=0.428$) between the sheep and goats as reactors to leptospire were not found. The highest number of reactors in sheep (68.2%) and goats (56%) was due to *L.introgans* serovar pomona, followed in descending order by icterohaemorrhagiae (18.2%), hardjo (4.5%), canicola (4.5%), and grypothyphosa (4.5%) in sheep and icterohaemorrhagiae (28%), and canicola (16%), in goats (Table 2). With the exception of one sample that had a titer level of 200, the other samples had a titer level of 100.

There was no significant difference among age groups in sheep ($p=0.301$) and goats ($p=0.363$), but there was a tendency in adult sheep and goats (≥ 3 years) to be more seropositive than the younger animals (Table 3 and 4). Distribution of leptospiral infection in sheep ($p=0.464$) and goats ($p=0.710$) among various areas was also not significantly different (Table 5 and 6). In area F, none of the examined sheep and goats have shown antibodies against various serovars of *L.interrogans* (Table 5, 6).

Discussion

Leptospirosis is an infectious zoonotic disease caused by different serotypes of the leptospire in any geographical area and infor-

mation about the serotypes in one region may help the epidemiology and pathogenesis of this bacteria. Cattle are maintenance host for many serotypes of this bacteria. Sheep and goats are not naturally maintenance hosts for some of the serotypes such as pomona or hardjo and are likely to have infection of relatively short duration and produce severe pathologic effects. However, persistent leptospiruria and high seroprevalence rates of infection in sheep and goat where no contact with cattle have occurred suggest that sheep and goat may be a maintenance host for some serovars. This could complicate control of the infection in cattle, sheep and goat. Infected sheep and goat are a potential zoonotic risk to humans such as abattoir workers, sheep and goat farmers and shearers which previously had not been considered (Radostits et al., 2007). Long-term survival of pathogenic leptospire outside the host requires a warm, moist environment with a near natural pH (Miller et al., 1991). According to different foraging behavior in sheep and goats it is suspected that leptospiral infection in these animals has substantial differences but in this study there were no significant differences of the seroprevalence infection between sheep and goats in similar regions and situations. However, it may be due to the fact that neither of them like to get their feet wet and both prefer upland grazing to lowland that results in less exposure to leptospire (Bokovski et al., 2014). We found that the seroprevalence of leptospiral infection in goat and sheep in Ahvaz was 10.95% and 8.53%, respectively. The prevalence of leptospiral infection in goats and sheep from other countries based on serological survey has been reported to be 1.2%, 12.3%, 13.1, 14.3%, 16.8%, 32%, 40%, 42%, 42.1%, 55.2% and 70% in France, Italy, Nigeria, Bolivia, Greece, Croix, Belize, Australia, Egypt, India and New Zealand (Agunloye, 2002; Ciceroni et al., 1997; Flint et al., 1988; Maronpot and Barsoum, 1972; Sratnam, 1992; Trap and Gaumont, 1983) and

Table 2. The Prevalence of leptospiral antibody titer to different serovars in sheep and goats in some areas of Ahvaz-Iran. G-grypothyphosa, P-ponnona, I-icterohaemorrhagiae, C-canicola, H-hardjo, B-ballum, T- tarasovi, A-australis.

Animals	G	P	I	C	H	B	T	A
Sheep	1 (4.5%)	15 (68.2%)	4 (18.2%)	1 (4.5%)	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)
Goats	0 (0%)	14 (56%)	7 (28%)	4(16%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 3. Age distribution in leptospiral seroprevalence of sheep in some areas of Ahvaz-Iran.

Age (year)	No. positive (%)	No. negative (%)	Total (%)
<2	0 (0)	10 (100)	10 (4.06)
2	1 (2.7)	36 (97.29)	37 (15.04)
3	8 (13.33)	52 (86.66)	60 (24.39)
≥4	12 (8.63)	127 (91.37)	139 (56.50)
Total	21 (8.53)	225 (91.47)	246

Table 4. Age distribution in leptospiral seroprevalence of goat in some areas of Ahvaz-Iran.

Age (year)	No. positive (%)	No. negative (%)	Total(%)
<2	3 (5.36)	56 (94.64)	59 (28.09)
2	3 (13.04)	20 (86.96)	23 (10.95)
3	5 (13.51)	32 (86.49)	37 (17.61)
≥4	12 (13.18)	79 (86.82)	91 (43.33)
Total	23 (10.95)	187 (89.04)	210

14.3%, 19.7%, 4.2%, 60.4%, 6.1% and 16.8% in Bolivia, Argentina, Egypt, India, Italy and Greece, respectively (Ciceroni et al., 1997; Draghi et al., 1984; Maronpot and Barsonm, 1972; Stratname et al., 1992; Ciceroni et al., 2000 and Burriel et al., 2002).

There are some reports of leptospiral infection in goat and sheep from Iran (Hajikolaei et al., 2007a; 2007b; Hassanpour et al., 2012; Zainali et al., 1997; Ramin, AG and Azizadeh, F., 2013). According to these reports, the seroprevalence of leptospiral infection in goat was 27.5%, 10.46% and 13.3% in Ahvaz, Uremia and Khoy, respectively and in sheep 14.9% and 19.3% respectively in Ahvaz and Uremia. The results of these reports confirm that prevalence of leptospiral infection in goat and sheep is different from region to region or country to country. These differences may be the consequence of environmental factors and control efforts (Maleki et al., 2014 and Milller et al., 1991). The results of this study showed that the serological infection rate in goat and sheep in Ahvaz is relatively high and

consequently the preventive methods must be applied to stop the spread of disease and its transmission to the human and other farm animals and the important role of goat and sheep on the epidemiology of the infection must be emphasized. The predominant leptospire serovars in serological reaction varies somewhat among countries. For example, *L. interrogans* serovars poi and pommona in Bolivia (Ciceroni et al., 1997), wolffi, pomona and ballum in Argentina (Draghi et al, 1984), pomona in India (Manickavel et al., 1991), autumnalis in Egypt (Maronpot and Baarsoum, 1972) and pomona in Malaysia (Bahaman et al., 1987) were the predominant serovars in sheep and automnalis, pomona, automnalis, poi, bratislava and ictrhemorrhagiae were the common serovars in goats in Egypt, Nigeria, India, Bolivia, Italy and France, respectively (Agunloye, 2002; Cerri, 2003; Ciceroni et al., 1997; Maronpot and Barsoum, 1972 and Trap and Gaumont, 1983).

In this study, *L. interrogans* serovars pomona and icterohaemorrhagiae were detected as the

Table 5. Distribution of leptospiral infection in sheep from various areas of Ahvaz-Iran.

Area	No. positive (%)	No. negative (%)	Total
A	2 (5)	38 (95)	40
B	6 (15)	34 (85)	40
C	3 (7.5)	37 (92.5)	40
D	3 (7.5)	37 (92.5)	40
E	1 (5)	19 (95)	20
F	0 (0)	14 (100)	14
G	2 (9.09)	20 (90.9)	22
H	4 (13.33)	26 (86.67)	30
Total	21	225	246

Table 6. Distribution of leptospiral infection in goats from various areas of Ahvaz-Iran.

Area	No. positive (%)	No. negative (%)	Total
A	4 (10)	36 (90)	40
B	7 (17.5)	33 (82.5)	40
C	3 (7.5)	37 (92.5)	40
D	4 (16.66)	20 (83.33)	24
E	1 (6.66)	14 (93.33)	15
F	0 (0)	14 (100)	14
G	2 (11.76)	15 (88.23)	17
H	2 (10)	18 (90)	20
Total	23	187	210

most prevalent serovars with 68.18%, 18.8% and 56%, 28% in sheep and goats, respectively. In previous studies in Tehran, Tabriz, Ahvaz and Khorramabad, the predominant serovars in cattle were Pomona; Pomona; grippotyphosa and Pomona; canicola and grippotyphosa, respectively, respectively (Hajikolaie et al., 2007; Hassanpour et al., 2012; Maleki et al., 2013). It is probable that this serovar may be adapted to and maintained by these farm animals in Ahvaz. There is a need for further investigation on clinical cases of leptospirosis to determine whether this serovar is the main cause of leptospirosis in this region.

Percentage of seropositive for more than one serovar was 8.6% and 4.76% in seropositive goats and sheep, respectively. In serological tests for leptospirosis such as MAT, the results often indicate infection with more than one serovar (Egan and Yearly, 1989; Hajikolaie et al., 2005, Hataway et al., 1981). This may be the result of mixed serovar infection but the exist-

tence of cross reactivity in the MAT among the serovars is well known and can be excluded from this interpretation. The high prevalence of infection and dominant titre of 1:100 reveal that leptospiral infection in goats and sheep in Ahvaz (in the southwest of Iran) is endemic and occurs mostly in subclinical form. There were no significant differences among the ages and areas groups in sheep and goats, but there was a tendency in adult sheep and goats (≥ 3 years) to be more seropositive than young sheep and goats that is in agreement with the other studies (Hassanpour et al., 2008 and 2012 and Maleki et al., 2014).

These results confirm that leptospiral infection may exist in the goat and sheep population in Ahvaz area and the presence of antibodies in the absence of infection indicates the exposure to the organism and must be acknowledged. In addition, these results confirm that the majority of leptospiral infections is asymptomatic. Because of the importance of leptospira inter-

rogans as an abortifacient agent in goat and sheep, it will be considered as one of the possible causes of abortion in goat and sheep in Ahvaz, southwestern Iran.

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مقایسه آلودگی به لپتوسپیرواينتروگانس در گوسفند و بز

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چکیده

زمینه مطالعه: اکثر آلودگی‌های ناشی از لپتوسپیرواينتروگانس در گوسفند و بز بدون علامت هستند اما ممکن است علائمی مانند تب، سقط جنین، مرده‌زایی و قطع تولید شیر را بدنبال داشته باشد. تفاوت رفتار تغذیه‌ای گوسفند و بز ممکن است باعث اختلاف در میزان فراوانی آلودگی به لپتوسپیرواينتروگانس در آنها شود. **هدف:** هدف از این مطالعه مقایسه شیوع آنتی بادی‌های ضد گونه لپتوسپیرواينتروگانس در گوسفند و بز می‌باشد. **روش کار:** به منظور مقایسه میزان شیوع سرولوژیکی لپتوسپیرواينتروگانس در گوسفند و بز، از تعداد ۲۴۶ راس گوسفند و ۲۱۰ راس بز از ۱۲ گله و از ۸ منطقه اهواز نمونه‌های خون اخذ گردید. نمونه‌های سرم با آزمایش آگلوتیناسیون میکروسکوپی (MAT) و با استفاده از سروتیپ‌های پومونا، کانیکولا، بالوم، ایکتروهموراژی، گریپوتیفوزا، تاراسوی و آسترالیس ارزیابی شدند. **نتایج:** شیوع سرمی آلودگی به لپتوسپیرواينتروگانس در گوسفند ۸/۵۳٪ و در بز ۱۰/۹۵٪ بود. بالاترین فراوانی سرمی در هر دو گونه مربوط به سروتیپ پومونا به میزان ۶۸/۱۸٪ در گوسفند و ۵۶٪ در بز و بعد از آن به ترتیب ایکتروهموراژی (۱۸/۸٪)، کانیکولا، هارگو و گریپوتیفوزا (هر کدام ۴/۵٪) در گوسفند و ایکتروهموراژی (۲۸٪) و کانیکولا (۱۶٪) در بز قرار داشتند. بررسی‌های آماری نشان داد که از نظر فراوانی آلودگی سرمی بین گوسفند و بز ($p=0/428$) و نیز بین گروه‌های سنی در گوسفند ($p=0/310$) و بز ($p=0/363$) اختلافی وجود ندارد. هرچند، یک گرایش به درصد آلودگی بیشتر در حیوانات بالغ (بالای سه سال سن) در مقایسه با جوان‌ترها مشاهده گردید. توزیع فراوانی آلودگی به لپتوسپیرواينتروگانس در گوسفند ($p=0/466$) و بز ($p=0/710$) هیچ اختلاف آماری در بین مناطق مختلف را نشان نمی‌دهد. **نتیجه‌گیری نهایی:** از نتایج این مطالعه چنین نتیجه‌گیری می‌شود که اختلافی در درصد آلودگی به لپتوسپیرواينتروگانس در بین گوسفند و بز که در یک گله و تحت شرایط مدیریتی یکسان نگهداری می‌شوند، وجود ندارد.

واژه‌های کلیدی: بز، لپتوسپیروزیس، شیوع سرمی، گوسفند

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