

# Serological and bacteriological study of leptospirosis in dairy herds and feedlot in Tehran suburbs

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## Abstract:

**BACKGROUND:** Leptospirosis is a worldwide zoonosis caused by *Leptospira interrogans*. Leptospirosis results in decreased milk production, abortion, stillbirth, infertility and mortality, which causes financial loss in the cattle industry. **OBJECTIVES:** The aim of this research was to perform a serological and bacteriological study of leptospirosis in 6 industrial dairy herds and 3 feedlots with previous records of leptospirosis in Tehran suburbs in 2011-2012. **METHODS:** For the purpose of this study, 408 blood samples from dairy cattle and 154 blood samples from feedlots were collected using sterile 10ml venoject vacutainers from tail vein. Two months later, 118 urine samples were collected from 20% of the two groups of serological negative and positive animals. All serum samples were serologically tested by microscopic agglutination test (MAT), a standard method for serological diagnosis of leptospirosis. The serum samples were tested for antibodies against five live antigens of *Leptospira interrogans* serovars: Pomona, Grippotyphosa, Hardjo, Ictero-haemorrhagiae and Canicola. Urine samples were used for bacteriological isolation of *Leptospira* spp. **RESULTS:** Serological results showed that 228 (40.6%) of animals had a positive reaction against one or more serovars. The most prevalent *Leptospira* serovars was Pomona 118 (40.3%) and the least prevalent was Canicola 4 (1.4%). The most prevalent titer was 1:100, and the highest titer was 1:1600. Also the most seropositive cases were observed in 3 to 4-year-old cows. Bacteriological results revealed that in 11 (9.3%) urine samples *Leptospira* spp. were isolated, all taken from one feedlot farm. According to the history taken from each farm, the main risk factors were the presence of rodents and low hygienic conditions of the farms. **CONCLUSIONS:** The results of this study revealed that cows could have a major role in maintaining Pomona, Grippotyphosa and Hardjo serovars; indeed, they are a potential zoonotic risk to slaughter house workers, meat inspectors, milkers and farmers.

## Introduction

Leptospirosis, a common zoonotic disease, affects most mammals throughout the world. Leptospirosis is known as a global public health

problem because of its high mortality and morbidity rates in different countries (Ahmed et al., 2006; World Health Organization, 2003). It leads to financial loss in the cattle industry as a result of decreased milk production, abortion, stillbirth, infertility and mortal-

ity (Levett, 2001). The first report of leptospirosis in Iran was published by Rafyi and Magami (1968) in which the prevalent serovars were Grippotyphosa, Pomona and Icterohaemorrhagia. Since then, the most prevalent *Leptospira* serovars reported in Iran include: Hardjo, Pomona, Grippotyphosa, Canicola and Icterohaemorrhagia (Abdollahpour, 2013). Published data, from 2004 to 2007, indicate that serovar Canicola was widely prevalent in the cattle population in some parts of Iran (Abdollahpour et al., 2004; Ebrahimi et al., 2004; Khaki et al., 2005; Haji hajikolaei et al., 2006). However, more recent studies show the variation of predominant serovars in different parts of Iran. In a bacteriological study, Maghami et al. (1977) isolated a *Leptospira interrogans* serovar Grippotyphosa from the kidney of a house mouse, *Mus musculus*. In 2002, *Leptospira* spp. was isolated from a calf in Karaj with MAT positive titer of 1:200 against serovar Pomona. This calf showed clinical symptoms of hemoglobinuria, jaundice and fever (Abdollahpour, 2002). *Leptospira interrogans* serovar Canicola was also isolated from urine of an infected five-year-old male cross-bred dog in Tehran (Jamshidi et al., 2008). According to the WHO guideline, MAT is considered a gold standard serologic method for serodiagnosis of leptospirosis (WHO, 2003). MAT is inexpensive, reliable and its sensitivity and accuracy are acceptable. In this method, serum samples are prepared in different dilutions, mixed with antigens and examined with dark field microscope. The lowest MAT titer, which is considered positive, is equal to or more than 1:100 (Radostits et al., 2007). The urine of infected animals or healthy carriers, which may contaminate soil, pasture, drinking water and food, is the main source of infection. Infection can be spread by the aborted foetus and uterine discharges. An infected foetus can carry the infection for up to 7 weeks after birth. The semen of an infected bull may carry leptospire. The transmission from such a bull to heifers by both coitus and artificial insemination has been observed. Field observations of herd outbreaks of *Leptospira Hardjo* infection have frequently implicated a bull as the source of introduced infection (Radostits et al., 2007). Diagnosis of leptospirosis is based on laboratory confirmations; because its clinical signs are nonspecific and may be mistaken with other febrile diseases (Vado-Solis et al., 2002). Laboratory

confirmations included culture of *Leptospira* spp, four fold increase in MAT titer and polymerase chain reaction (PCR). However, it should be pointed out that the culture of *Leptospira* from body fluids (blood or urine) is the most confirmative test (WHO, 2003). The objective of this study was to investigate the serological and bacteriological aspects of leptospirosis in 9 dairy and beef herds in Tehran suburbs, located in three central provinces of Iran, including Tehran, Alborz and Qazvin. The results of this study will update the present status of leptospirosis in central parts of Iran.

## Materials and Methods

**Study population:** Based on the literature review of leptospirosis in Iran, especially studies conducted in central parts of Iran, the most suspected herds with signs of leptospirosis were located. Finally 9 herds were selected, including six dairy and 3 beef farms in Tehran, Alborz and Qazvin. At the first stage, 562 blood samples were collected from all cows from October 2011 to March 2012. At the second stage, all sampled animals were divided in two groups of positive and negative MATs. Based on MAT results, 118 urine samples were collected from both MAT negative and positive groups.

**Serological procedures:** Blood samples were taken from tail vein using 10 ml glass tubes and were transferred to the *Leptospira* Research Laboratory of the Veterinary Research and Teaching Hospital of the Faculty of Veterinary Medicine, University of Tehran. Blood samples were centrifuged, and the serum was separated and transferred in 2 ml micro tubes, stored at -20°C for analysis. MAT was implemented on all serum samples, in accord with the standard method (WHO, 2003), using live 7-10 days antigens, representing the following *Leptospira interrogans* serovars: Pomona, Grippotyphosa, Hardjo, Icterohaemorrhagiae and Canicola. All serovars were grown in specific liquid mediums locally known as GRA-SINA, which is produced in *Leptospira* Research Laboratory in University of Tehran. Serial 2-fold dilution of each serum was prepared ranging from 1:50 to 1:1600; then, 10 µL of each diluted test sera was added to an equal volume of antigen suspension on a microscope slide. Following incubation at 30°C for 1.5 h, the slide was examined

under a dark-field microscope, using long working distance objectives at X100 or X200 magnifications. Agglutination was noted by observing clumps of leptospire. The lowest dilution for a serum to be considered significant was 1:100. The end point titer was the highest titer in which 50% agglutination occurred; the lowest titer that was considered positive was 1:100. The antigen that gave the highest titer was considered to be the infective serovar.

**Bacteriological procedure:** In order to take urine samples, cows were placed in individual boxes and their tails were bounded to reduce the risk of contamination. In order to minimize contamination of the urine samples, using a tissue the perineum area was washed, cleaned and dried. Then, by stroking (gentle tickling) of the perineum under the vulva, cows were stimulated to urinate. The same procedure was applied for the male animals, after cleaning the prepuce. Finally, a mid-stream urine sample was collected into sterile and dark-colored glass tubes. If no urine had been obtained after 3 to 5 minutes of stimulation, then the procedure would have stopped and 0.5 to 1 mg/kg of Furosemid would have been injected intra muscularly. It has to be noted that for this injection the animal should not be pregnant. Urine samples were transferred immediately to a clean room which was prepared in advance, then about 20  $\mu$ l of each urine sample inoculated into a semisolid GRA-SINA medium enriched with 2% pooled rabbit serum and 200  $\mu$ g/mL of 5-Fluoracil. All culture procedures were performed using sterile pipettes, next to the flame. Cultured test tubes were placed in a dark place and transferred to the *Leptospira* Research Laboratory and incubated at 29°C incubator. In order to check the growth of *Leptospira* spp., all cultured test tubes were examined at 7-day intervals by dark-field (DF) microscopy for 3 months.

**Data analysis:** All data obtained from serological and bacteriological methods were analyzed using SPSS, version 16 software. Chi-square and Fisher's exact tests were used to detect significant differences among sex, MAT and culture results. A p value of  $\leq 0.05$  was considered statistically significant.

## Results

The results of this study indicated that 228 (40.6%)

Table 1. Prevalence of MAT reaction with one or more serovars in 228 positive samples.

Number of serovar	Number of MAT positive(%)
One	171(75%)
Two	49(21.5%)
Three	7(3.1%)
Four	1(0.4%)
Total	228(100%)

cows had a positive reaction against one or more serovars of *Leptospira interrogans* (Table 1). Fifty seven samples (25%) showed serological reaction with more than one serovar. one sample (0.4%) showed serological reaction with four serovars, and seven samples (3.1%) showed reaction with three serovars. Forty nine samples (21.5%) showed serological reaction with two serovars. one hundred and seventy one of the positive samples (75%) showed serological reaction with one serovar (Table 1). The most prevalent *Leptospira* serovars were Pomona, Grippotyphosa and Hardjo with 40.3%, 29% and 26.6% respectively. The least prevalent *Leptospira* serovars were Icterohaemorrhagiae and Canicola with 2.7% and 1.4% respectively (Table 2). The most frequent titer level was 1:100, and the least frequent titer was 1:1600 with 77.8% and 0.7% respectively for all serovars (Figure 1). Regarding the age groups, the result of the present study proved that the most seropositive cases 64 (48.1%) were observed in 3 to 4-year-old cows (table 3).

Bacteriological culture of 118 urine samples showed that *Leptospira* spp. was isolated from 11 (9.3%) urine samples of calves, using GRA-SINA semi-solid medium. All positive cultures belonged to a feedlot farm in which many of the calves showed clinical form of acute leptospirosis around the time of sampling. Despite all hygienic criteria applied for collecting urine samples about 30% of the cultured test tubes showed contamination with other bacteria, and therefore they were discarded. In this research, there was a statistically significant difference between sex and MAT results ( $p=0.001$ ) (See table 4). Also, there was a statistically significant difference between sex and culture results ( $p=0.0005$ ) (See table 5). Moreover, the difference between MAT and culture results was statistically significant ( $p=0.0005$ ). In addition, the results of this research demonstrated that the most prevalent *leptospira* serovar in the cattle

Table 2. Frequency distribution of positive MAT in 562 serum samples of 9 industrial dairy cattle and feedlot in Tehran suburbs by serovars and titers (Titers  $\geq 1:100$  were considered positive).

Serovar	Titer					Total positive (%)
	1:100	1:200	1:400	1:800	1:1600	
Pomona	99	15	2	2	0	118(40.3%)
Grippotyphosa	66	7	6	6	0	85(29%)
Hardjo	53	6	9	8	2	78(26.6%)
Icterohaemorrhagiae	6	2	0	0	0	8(2.7%)
Canicola	4	0	0	0	0	4(1.4%)
Total	228	30	17	16	2	293

Table 3. Distribution of the MAT positive samples in 5 age groups of dairy cattle and feedlot in Tehran suburbs.

Age groups	MAT positive (%)	MAT negative (%)	Total
6 - 12 months	19(29.2)	46(70.8)	65
1-2 years	38(34.5)	72(65.5)	110
2-3 years	48(40.7)	70(59.3)	118
3-4 years	64(48.1)	69(51.9)	133
> 4 years	59(43.4)	77(56.6)	136
Total	228(40.6)	334(59.4)	562

Table 5. Distribution of Leptospiral isolation in cattle by farming system. (\*) All sampled animals were female. (#) All sampled animals were male.

Sex	Culture isolated results		Total
	Number of positive (%)	Number of negative (%)	
dairy(*)	0(0.0%)	89(100%)	89
feedlot(#)	11(38%)	18(62%)	29
Total	11(9.3%)	107(90.7%)	118

of the aforementioned provinces was Pomona and the least prevalent leptospira serovar was Canicola (Table 2).

### Discussion

Leptospirosis is a zoonosis of worldwide distribution, caused by *Leptospira interrogans*. It is one of the well-known causes of bovine reproductive losses such as abortion, infertility, stillbirth, birth of weak calves, weight loss and decreased milk production. In some areas of central regions of Iran, including the three provinces in which the present study was conducted, the climate conditions and density of dairy and beef farms is suitable for

Table 4. Distribution of Leptospiral infection in cattle by farming system. (\*) All sampled animals were female. (#) All sampled animals were male.

Sex	MAT antibodies results		Total
	Number of MAT positive (%)	Number of MAT negative (%)	
Dairy(*)	187(44.5%)	233(55.5%)	420
Feedlot(#)	41(28.9%)	101(71.1%)	142
Total	228(40.6%)	334(59.4%)	562

maintaining *Leptospira* spp. This study was conducted to investigate the seroprevalence of leptospirosis and urinary shedding in dairy cattle and feedlot. The results of this study showed that the seroprevalence of leptospirosis in cattles in Tehran suburbs was 40.6% in 9 industrial herds. The results of the present study showed that prevalence of leptospirosis is quite high comparing with the study carried out 5 years ago in the same region, the result of which was 14.5% (Sakhaee et al., 2007). The reported results of seroprevalence of leptospiral infection in cattles are different from country to country. In Portugal, 15.3% of cattles reacted to one or more serovar of *Leptospira interrogans*; the most prevalent was *Leptospira intrrogans* serovar Hardjo (Rocha, 1988). In Turkey, the seroprevalence of leptospiral antibodies in cattles was 44.8% and the most prevalent serovar was *Leptospira introgans* Grippotyphosa (Ozdemir and Erol, 2002). In another study in 2005 by Ozkan and Vildan in Turkey, seroprevalence of leptospirosis was found to be 57.8%, and Grippotyphosa was the predominant serovar. However, in 2009 a study conducted by Gumussoy et al. 25.4% of cattle were found seropositive in which the most prevalent serovar was Hardjo. In Malaysia, a tropical country with high

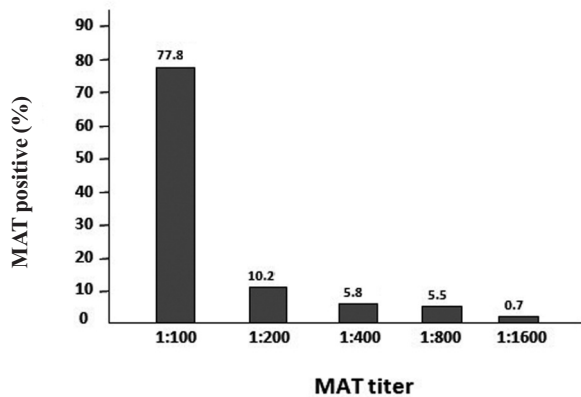


Figure 1. Seroprevalence of Leptospirosis by MAT titers in Tehran suburbs in 9 herds.

annual rainfall in which leptospirosis seems to be an endemic infection, 40.5% of cattle were found to have MAT positive reaction, and the most prevalent serovar was Hardjo (Bahaman et al., 1987).

In a serological study of cattle in Tehran suburbs in 2001, it was shown that 46.8% samples, taken from 39 herds, had a positive reaction against one or more serovars. The most prevalent *Leptospira* serovar was Canicola (59 samples) and then Ballum (37 samples) and Grippotyphosa (36 samples). The less prevalent *Leptospira* serovar was Hardjo (7 samples). None of the samples had seropositive reaction against serovar Icterohaemorrhagiae (Abdollahpour et al., 2004). The study conducted by Sakhaee et al. (2007), in Tehran suburbs, showed that the predominant serovar was Icterohaemorrhagiae in cattle. However, the results of the present study showed that Pomona was the most predominant serovar and Canicola was the least prevalent serovar in dairy cattle and feedlot. Also in the present study, 25% of seropositive cattle showed antibodies against more than one serovar. This may be related to mixed infection or cross reaction in the MAT. In this study, the high prevalence of infection and dominant titer of 1:100 may indicate that *leptospira infection* in cattle in Tehran suburbs is endemic and occurs mostly in a subclinical form.

A serological study in Guilan province of Iran in 2004 showed that 53 (25.8%) samples had a positive reaction against one or more serovars, in which the most prevalent *Leptospira* serovar was Canicola with 24 (11.7%) samples, and the least prevalent *Leptospira* serovars was Icterohaemorrhagiae with 1 (0.5%) sample (Abdollahpour et al., 2009). However, one year

later Shafighi et al. in 2010 reported that the most prevalent serovar in slaughtered cattle in Guilan was Pomona. Moreover, in Tehran suburbs, Pomona was found to be the predominant serovar (Abdollahpour et al., 2012). Both of the results are consistent with the findings of our study. The results revealed that the predominant serovar could be changed not only in one region, but also in one species.

It was illustrated in the review of literature that the predominant serovar of leptospiral infection is variable in different parts of Iran, and the main reason is Iran's different climatic conditions. In southwest of Iran (Ahvaz) the predominant serovar in cattle was Grippotyphosa (Haji Hajikolaei et al., 2005), whereas the predominant serovar in sheep was Pomona (Haji Hajikolaei et al., 2007). The findings substantiated the need for surveillance regional study for leptospirosis, because host-adapted serovars may change based on the ecology of a region. Considering that dogs are playing as the maintenance host for Canicola serovar and this serovar has been reported to be the predominant in cattle population in some regions (Abdollahpour et al., 2004; Ebrahimi et al., 2004; Khaki et al., 2005; Haji hajikolaei et al., 2006), vaccination of dogs against leptospirosis can play an important role in the control of leptospirosis in cows.

The results of bacteriological culture of 118 urine samples in the present study showed that 11 *Leptospira* organisms were isolated using the semisolid GRA-SINA enriched medium. All these isolates belonged to one feedlot farm in which many of the calves exhibited clinical symptoms of leptospirosis including: anorexia, hemoglobinuria, jaundice and fever. It has to be mentioned that we collected 35 blood samples from this farm most of which showed a high MAT titer against *Leptospira interrogans* serovar Grippotyphosa. No more *Leptospira* were isolated from the other 8 farms, which may indicate the higher concentration of leptospires in urine of calves with the acute form of leptospirosis in comparison with the chronic form of the disease in other farms, which did not show evident clinical symptoms of leptospirosis.

However, isolation of *Leptospira* spp. from 11 animals was a great success as we know the fastidious nature of this organism. Until now, the researchers were not able to serotype these isolates; hence, the next important step would be to identify the serotype of each isolate.

In conclusion, the findings of this study revealed that leptospirosis is still an important infection in cattle populations of Tehran suburbs, and because of its public health hazard it should be considered as a potential zoonotic risk to human; especially those whose jobs are in the category of high risk for leptospirosis.

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## مطالعه سروباکتریولوژیک لپتوسپیروز در گاو داری های شیری و پرواری اطراف تهران

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## چکیده

**زمینه مطالعه:** لپتوسپیروز یک بیماری مشترک بین انسان و دام با گسترش جهانی است که توسط باکتری لپتوسپیرواگونه‌های بیماری‌زا ایجاد می‌گردد. این بیماری سبب خسارات اقتصادی فراوانی نظیر کاهش تولید شیر، سقط جنین، مرده‌زایی، عدم باروری و مرگ و میر در صنعت دامداری می‌شود. **هدف:** انجام مطالعات سرم‌شناسی و باکتری‌شناسی لپتوسپیروز در شش گاوداری صنعتی شیری و سه گاوداری پرواری (دارای سابقه ثبت شده لپتوسپیروز) در اطراف استان تهران در طی سال‌های ۱۳۹۰-۱۳۹۱ بود. **روش کار:** به همین منظور، تعداد ۴۰۸ نمونه خون از گاوهای شیری و ۱۵۴ نمونه خون از گاوهای پرواری از طریق ورید دم با استفاده و نوجکت اخذ گردید. دو ماه بعد، تعداد ۱۱۸ نمونه ادرار از ۲۰ دام‌هایی که در هر دو گروه دارای واکنش مثبت و منفی سرولوژیک بودند اخذ گردید. تمامی نمونه‌های سرمی با آزمایش میکروآگوتیناسیون (MAT)، به عنوان یک روش استاندارد WHO جهت تشخیص سرولوژیک لپتوسپیروز، مورد بررسی قرار گرفتند. در این مطالعه نمونه‌های سرمی برای تعیین عیار آنتی‌بادی بر علیه پنج سروتیپ زنده لپتوسپیروا شامل: پومونا، گریپوتایفوزا، ایکتروهموراژیه، هارجو و کنیکولا مورد آزمایش قرار گرفتند. نمونه‌های ادرار نیز جهت جداسازی باکتریایی لپتوسپیروا مورد استفاده قرار گرفت. **نتایج:** نتایج آزمایشات سرم‌شناسی نشان داد که تعداد ۲۲۸ نمونه (۴۰/۶٪) بر علیه یک یا تعداد بیشتری سروتیپ لپتوسپیروا واکنش مثبت داشتند. فراوانترین سروتیپ لپتوسپیروا، پومونا با ۱۱۸ مورد (۴۰/۳٪) و کمترین آن کنیکولا با ۴ مورد (۱/۴٪) بودند. شایع‌ترین تیتراژ سرمی ۱:۱۰۰ و بالاترین تیتراژ سرمی ۱:۱۶۰۰ بود. همچنین بیشترین موارد مثبت سرولوژیک در گروه سنی ۳ تا ۴ ساله مشاهده گردید. نتایج آزمایشات باکتری‌شناسی مبتنی بر جداسازی اجرام لپتوسپیروا از ۱۱ (۹/۳٪) نمونه ادرار بود که همگی متعلق به یک گاوداری پرواری بودند. **نتیجه‌گیری نهایی:** با توجه به اطلاعات ثبت شده از هر گاوداری، ریسک فاکتورهای اصلی برای بروز لپتوسپیروز شامل حضور جوندگان و موش‌ها و فقدان شرایط بهداشتی مطلوب در دامداری‌ها بودند. نتایج تحقیق حاضر بیانگر این نکته است که گاوها می‌توانند نقش مهمی در حفظ و بقا سروتیپ‌های لپتوسپیروا پومونا، گریپوتایفوزا و هارجو ایفا نموده و در واقع خطری بالقوه برای انتقال بیماری به کارکنان کشتارگاه‌ها، بازرسان گوشت، شیردوشان و دامداران محسوب می‌گردند.

واژه‌های کلیدی: لپتوسپیروز، سرولوژی، باکتریولوژی، میکروآگوتیناسیون، گاو

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