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Full Length Research Paper

Serological and bacteriological study of leptospirosis in slaughtered cattle in north of Iran (Rasht)

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A serological and bacteriological study was performed in Guilan industrial slaughter house, in Rasht, North of Iran in 2009. To investigate the seroprevalence of leptospirosis in slaughtered cattle in Guilan, 59 and 39 random serum samples were collected from cows and bulls, respectively. None of the cattles was vaccinated against leptospirosis. Also urine samples were collected from all of the blood-sampled cattle and cultured. All serum samples were serologically tested by microscopic agglutination test (MAT), as a standard method for serological diagnosis of leptospirosis. The serum samples were tested for antibodies against eight live antigens of *Leptospira interrogans* serovars: Australis, Autumnalis, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona and Sejroe. The lowest dilution that each serum was considered positive was 1:100. The results of this study showed that 37 (37.8%) animals had a positive reaction against one or more serovars. The most prevalent *Leptospira* serovars was Pomona (49.0%). One leptospiral organism was isolated from 98 urine samples of cows and bulls. The results of this study indicates that leptospiral infection is magnified in cattle in Rasht, and cattle have a major role in maintaining Pomona serovar; indeed they are a potential zoonotic risk to slaughter house workers, meat inspectors, milkers and farmers.

Key words: Iran, Rasht, serology, bacteriology, cattle, leptospirosis.

INTRODUCTION

Leptospirosis is known as a global public health problem because of its increased mortality and morbidity in different countries (Ahmed et al., 2006; World Health Organization, 2001). Leptospirosis causes economic loss to the cattle industry from decreased milk production, abortion, stillbirth, infertility and mortality. Diagnosis of leptospirosis is based on laboratory confirmation because its clinical signs are nonspecific and may be mistaken with other febrile diseases (Vado-Solis et al., 2002).The culture of *Leptospira* from body fluids (blood or urine) is the most confirmative test. The carrier cows secrete leptospires in their urine without clinical signs of disease because of the tendency of bacteria to accumulate in kidneys. Therefore, they have an important role in the epidemiology of disease (Ellis et al., 1986; Waitkins, 1986). The earliest recognised report of leptospirosis in Iran is published by Rafyi and Magami (1968). Since then the most prevalent *Leptospira* serovars reported in Iran includes: Hardjo, Pomona, Grippotyphosa, Canicola and Icterohaemorrhagia (Abdollahpour, 2009). As North of Iran, especially Guilan province, has a humid temperate climate with plenty of annual rainfall, is a suitable environment for maintaining of leptospira (Abdollahpour et al., 2009). The objective of this study was to investigate the seroprevalence of leptospirosis and urinary shedding in cattle population in Guilan industrial slaughter house.

MATERIALS AND METHODS

Study population

For this study, a total of 98 samples were randomly collected from

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Table 1. Distribution of leptospiral infection in cattle stratified by sex.

Sex	MAT reaction results		Total
	Number of positive	Number of negative	
Cow	25 (42.4%)	34 (57.6%)	59 (100%)
Bull	12 (30.8%)	27 (69.2%)	39 (100%)
Total	37	61	98

non-vaccinated cows (n=59) and bulls (n=39) of Guilan industrial slaughter house in Rasht, North of Iran, in May and August, 2009.

Seruma samples

Blood samples were collected from the jugular vein and were transferred to the Leptospira Research Laboratory of the faculty of Veterinary Medicine, University of Tehran, in 10 ml evacuated glass tubes. Serum was separated by centrifugation and stored in 2 ml cryotubes at -20°C until using. Microscopic agglutination test (MAT) was implemented on all of the serum samples, according to the methods of WHO (2003). MAT was performed using live antigens, which were grown in liquid Ellinghausen McCullough Johnson Harris (EMJH) medium for 7 to 10 days. The following serovars were used in this study: *Leptospira interrogans* serovars: Australis, Autumnalis, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona and Sejroe. Serial dilution of test serum were prepared ranging from 1:50 to 1:800 and 10 µl of diluted test sera added to an equal volume of antigen suspension on a microscope slide. Following incubation at 28 - 30°C for 1.5 h, the slide was examined under a dark-field microscope, using a long working distance objective at x100 or x200 magnification. Agglutination was noted by observing clumps of leptospires. The lowest dilution that each serum was considered significant was 1:50. The end point titre was the highest titre in which 50% agglutination occurred, so that the lowest titre that was considered as positive was 1:100.

Urine samples

Urine samples were obtained about ten minutes after slaughter. Urinary bladders were carefully removed from the carcasses and they were flamed on the surface using a red-hot blade, then approximately 5 ml aliquots of urine were collected from each animal by aspiration using sterile disposable syringes. Urine samples were transferred to a location near the slaughter room immediately and 20 µl of each urine sample inoculated into EMJH medium enriched with rabbit serum. 5-fluorouracil added to medium at a concentration of 200 µg/ml, to inhibit the growth of contaminants. Then, cultures were incubated at 30°C and examined at 7-day intervals by dark-field (DF) microscopy for 24 weeks as recommended by WHO (2003).

Data analysis

The results of the MAT and culture methods were analyzed using Statistical Package for Social Sciences, version 16. Chi-square and Fisher's exact tests were used to detect significant differences among sex, MAT and culture results. A p value ≤ 0.05 was considered statistically significant.

RESULTS

This study indicated that 25 (42.4%) cows and 12 (30.8%) bulls had a positive reaction against one or more serovars of *L. interrogans* (Table 1). There were positive seroreactions against all serovars except for serovar Icterohaemorrhagiae. The most prevalent Leptospira serovars were Pomona, Canicola and Grippotyphosa. The least prevalent Leptospira serovars were Australis, Autumnalis and Sejroe (Table 2). Ten samples (27.2%) showed serological reaction with more than one serovar. One sample (2.7%) showed serological reaction with four serovars: Canicola, Grippotyphosa, Hardjo and Pomona, and two samples (5.4%) showed reaction with three serovars: Canicola, Grippotyphosa and Pomona. Seven samples (18.9%) showed serological reaction with two serovars: Grippotyphosa and Pomona (two samples), Hardjo and Sejroe (one sample), Canicola and Pomona (two samples), Canicola and Hardjo (one sample), Canicola and Grippotyphosa (one sample) (Table 3). The majority of titre levels were 1:100 for all serovars except serovar Icterohaemorrhagiae and the frequency of 1:100, 1:200 and 1:400 were 74.5, 25.5 and 0.0%, respectively (Table 1). In the present investigation, there was no statistically significant difference between sex and MAT results (p = 0.246).

Leptospires were demonstrated in one out of 98 urine cultures (1%) in EMJH medium. The isolate was obtained from a bull that had clinical signs of leptospirosis such as icterus and hemoglobinuria therefore, the carcase was removed. In this study there was no statistically significant difference between sex and culture results (p = 0.216).

Table 2. Serum antibody titres for serovars of *L. interrogans* in 98 cattle sampled at the Guilan industrial slaughter house in Rasht, Iran by MAT* (Titres ≥ 1:100 were considered seropositive).

Serovar	Total		
	1:100	1:200	seropositive
Australis	1	0	1 (2.0%)
Autumnalis	1	0	1 (2.0%)
Canicola	5	4	9 (17.6%)
Grippotyphosa	8	1	9 (17.6%)
Hardjo	3	2	5 (9.8%)
Icterohaemorrhagiae	0	0	0
Pomona	19	6	25 (49.0%)
Sejroe	1	0	1 (2.0%)

*Microscopic agglutination test.

Table 3. Incidence of MAT* reaction with one or more antigens in 37 positive reactors.

Number of antigen	Number of positive
One	27 (73.0%)
Two	7 (18.9%)
Three	2 (5.4%)
Four	1 (2.7%)
Total	37 (100%)

*Microscopic agglutination test.

DISCUSSION

Leptospirosis is a zoonosis of worldwide distribution, caused by *L. interrogans*. It is a well known causes of bovine reproductive losses such as abortion, infertility, stillbirth, birth of weak calves, decreased milk production. North of Iran (Rasht) has a humid temperature climate with plenty of annual rainfall, where is suitable for maintaining of Leptospira spp. Therefore, this study was conducted to investigate the seroprevalence of leptospirosis and urinary shedding in cattle that referred to Guilan industrial Abattoir in Rasht during May and August, 2009. The results of this study showed that the seroprevalence of leptospirosis in cattle in North of Iran was 37.8%. The reported results of seroprevalence of leptospiral infection in cattle are different from country to country. In Portugal, 15.3% of cattle reacted to one or more serovar of *L. interrogans* (Rocha, 1988). According to the report of Ozdemir and Erol (2000), the prevalence of leptospiral infection in cattle and sheep in Turkey was 44.77 and 8%, respectively. In Malaysia, 40.5% of cattle reacted to one or more serovar of *L. interrogans* (Bahaman et al., 1987). In Turkey, 25.42% of cattle reacted to one or more serovar of *L. interrogans* (Gumussoy et al., 2009). The Results of this study also showed that Pomona was the most predominant serovar in cattle in North of Iran. On the other hand, in a previous study in North of Iran (Guilan province), the predominant serovar was Canicola in cattle (Abdollahpour et al., 2009). These results showed that the predominant serovar varied in a period of time and in different situations. In Ahvaz (Southwestern Iran) the predominant serovar in cattle was Grippotyphosa (Hajj et al., 2005). The predominant serovar in sheep in Ahvaz was Pomona (Hajj et al., 2007). In Tehran suburb, the predominant serovar in cattle was Icterohaemorrhagiae (Sakhaee et al., 2007). The reported results of the predominant serovar of leptospiral infection in cattle are variable in different parts of Iran. This confirms the need for regional study for leptospirosis, because host-parasite relationship may change depending on the ecology of the region. Pomona is a common serotype in cattle and pigs, and these animals are considered to be the main reservoir for the mentioned serotype.

In the present study, 27% of seropositive cattle had antibodies against more than one serovar. This may be related to mixed serovar infection or cross-reactivity among serovars. In this study, the high prevalence of infection and dominant titre of 1:100 indicate that leptospiral infection in cattle in North of Iran is endemic and occurs mostly in subclinical form. In this study, one leptospiral organism was isolated. It would be interesting to follow up the isolated leptospira, that is characterize the isolate both genetically and serologically, as well as performing serological studies with the cattle sera using this local strain as antigen in the MAT.

In conclusion, this study revealed that infection with *L. interrogans* is common in cattle of North of Iran and is threaten to the public health and there is a potential zoonotic risk to slaughter house workers, meat inspectors, milkers and farmers.

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