

Short paper

## Serosurvey of *Leptospira interrogans* Serovars *hardjo* and *pomona* in Cattle in Nineveh Province, Iraq

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### ABSTRACT

The purpose of the present study was to determine the serodiagnosis of anti-*Leptospira hardjo* and anti-*L. pomona* antibodies in cattle in Nineveh province, Iraq. For this purpose 96 sera were collected from adult native breed cattle 2 years old or over during an 7 month period from September 2012 to March 2013. Serodiagnosis of *Leptospira interrogans* serovars *hardjo* and *pomona* was carried out using a commercial ELISA. From a total of 96 sera, 6 (6.3%) were positive for *Leptospira hardjo* and only one animal (1 %) was seropositive to *L. pomona*. These data suggest that leptospirosis is present among cattle in the study area.

Keywords: Leptospirosis, Cattle, ELISA, Serodiagnosis, *Leptospira hardjo*.

## Irak' ta Nineveh Bölgesindeki Sığırlarda *Leptospira interrogans hardjo* ve *pomona* Serotiplerinin Serosürveyansı

### ÖZET

Bu çalışmada Irak'ta Nineveh bölgesindeki sığırlarda anti-*Leptospira hardjo* ve anti-*L. pomona* antikorlarının belirlenmesi amaçlandı. Bu amaçla 7 aylık bir süreçte, Eylül 2012 ile Mart 2013 arasında, 2 yaş ve üzeri 96 yerli sığır ırkında serum örnekleri toplandı. *Leptospira interrogans hardjo* ve *pomona* serotipleri ticari olarak elde edilen ELİZA test kitleri ile belirlendi. Doksanaltı serum örneğinin 6'sı (% 6.32) *Leptospira hardjo* yönünden, yalnızca 1'i (%1) *L. pomona* yönünden pozitif. Bu bulgular leptospirozis'in bu bölgedeki sığırlarda mevcut olduğunu göstermektedir.

Anahtar Kelimeler: ELISA, Leptospirosis, *Leptospira hardjo*, Serolojik tanı, Sığır

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## Introduction

Leptospirosis is a major problem worldwide, particularly in the tropics (World Health Organization 2003; Adler, 2010) Leptospiral servers that affect bovines more frequently are *Hardjo*, *Pomona*, *Canicola* and *Icterohaemorrhagiae*. Nowadays, serovar *Hardjo* is considered the most frequent and important serovar for bovines (Radostits *et al.*, 2007).

The clinical presentation of leptospirosis in cattle is variable. Bovine leptospirosis causes abortion, stillbirth or weak calves, infertility and a decrease in milk production (Faine *et al.* 2000 ; Victoriano *et al.* 2009).

Diagnosis of leptospiral infection in cattle is difficult because of low specificity, low sensitivity and vague interpretation of various diagnostic tests, plus the frequent absence of specific clinical signs—particularly in non-pregnant and non-lactating cows (Radostits *et al.*, 2007).

Laboratory routine diagnosis of bovine leptospirosis may be performed by use of serological methods and leptospires detection in urine and organs (Wagenaar *et al.*, 2000; Morgan *et al.*, 2007). The microscopic agglutination test (MAT) is considered the standard serologic test that is specific and provides useful epidemiologic data in the form of presumptive serogroups (Cole *et al.*, 1973). However, this assay is not suitable for routine laboratories since it is technically demanding, costly, and requires the maintenance of live, hazardous stock serovar cultures and also requires analyses of paired sera to verify the seroconversion which delays the diagnosis (Thiermann, 1984; Dassanayake *et al.* 2009). Enzyme-linked immunosorbent assay (ELISA) have been developed (Surujballi and Mallory 2004; Mariyar 2006) and several commercial test kits are available mostly using reactive *Leptospira* antigen obtained from pathogenic *L. hardjo* (Kavanagh *et al.*, 2002; Leonard *et al.*, 2004; Ryan, 2012). In the present study, researchers aimed to serodiagnosis of some pathogenic *Leptospira* spp (*L. hardjo* and *L. pomona*) in cattle from Nineveh province, Iraq.

## Material and Method

The present study was performed in Nineveh province of Iraq comprising 96 blood samples were withdrawn from adult native breed cattle 2 year old or over during September 2012 to March 2013. Cows were brought from different farms as individual cases to the Veterinary Teaching Hospital, College of Veterinary Medicine ,University of Mosul, which showed at least one of the clinical manifestations compatible with for leptospirosis, as jaundice, haemoglobinuria, abortion or mastitis, during sample collection or some months before they were enrolled in this study. The blood was allowed to clot, the serum was harvested after centrifugation at 1500g for 10 minutes and kept at -20°C until use .

Nineveh province is located in the north of Iraq . The climate of the Nineveh province is hot, dry at summer and cool rainy at winter.

A commercial indirect ELISA kit for detection of antibodies against *L. Leptospira interrogans* serovar *hardjo* and *L. interrogans* serovar *pomona* in serum was used , the kit has been supplied from Bovichek Lepto kit, Biovet, Canada. All sera were tested according to the manufacturer's instructions, then read the optical densities in the microwells using a micro plate reader at a wavelength of 450 nm. ELISA optical density (O D) reading were transformed to serum / positive percentage ( S /

P) according to a specific equation cited by manufacturer.

## Results

The results of study showed that the total percentage of seropositive of *Leptospira* spp. antibodies was 7.3 (mean 7 seropositive out of 96 sera). Six ( 6.3%) of 96 sera were found positive for *Leptospira interrogans* serovar *hardjo* and only one (1 %) of 96 sera were seropositive to *L. interrogans* serovar *pomona* (Figure 1).

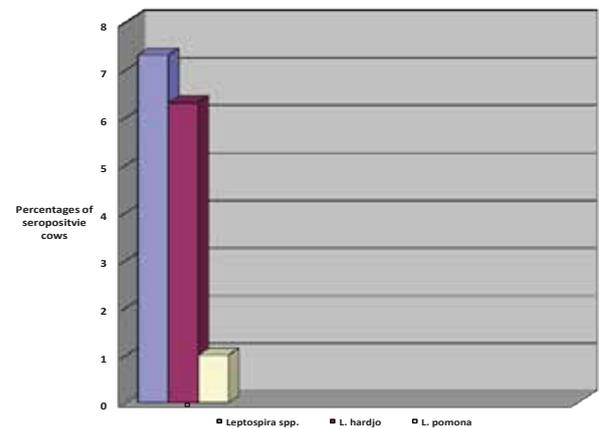


Figure 1. Percentages of seropositivity *Leptospira* spp. in cattle in Mosul , Iraq.

Şekil 1. Irakta Mosul'daki sığırlarda *Leptospira* spp. seropozitif yüzdeleri.

## Discussion

This is the first report indicating serodiagnosis of Leptospirosis in cattle in the Nineveh province, Iraq. In this study, antibodies against *L. interrogans* serovar *hardjo* and *L. interrogans* serovar *pomona* were detected 6 out of 96 sera and 1 of 96 sera respectively. Leptospirosis is one of the most important zoonotic disease spreading throughout the world with numerous reservoir hosts (World Health Organization 2003; Adler, 2010).

Despite the fact that Nineveh province is one of the major livestock husbandry centers in Iraq, there is no published data on the epidemiology of bovine leptospirosis in this province.

There are only one report in cattle in Baghdad. In this study the seroprevalence to one or more serovars of *L.interrogans* was detected as 57.3 % among in cattle. MAT was the only test that had been used for serological survey of leptospiral infection and in these study the highest prevalence was obtained against for *hardjo* serovar (Al-Badrawi *et al.*, 2010).

Cattle are susceptible to a wide variety of leptospires, but serovar *hardjo* (genotypes *Hardjobovis* and *Hardjoprajitno*) is the most common leptospire for which cattle can become permanent carriers (Bolin , 2003). *L. interrogans* serovars *pomona*, as accidental hosts, may result in acute disease and abortion (Miller *et al.*,1991; Peregrine *et al.*, 2006).

Serological surveys indicate wide spread exposure to *L.serovar hardjo* in cattle as in Ireland (Ryan, 2012), United Kingdom (Pritchard ,1986), Portugal (Rochat, 1998), Nigeria (Ezeh, 1989), Tanzania (Machang , 1997), West Malaysia (Bahaman , 1987) , Turkey ( Kocabiyik and Cetin, 2004), and United States (Larson *et al.*, 2007).

The variability in the prevalence among reports may be attributed to environmental differences between geographical areas and topographical reasons. Herd management may affect the overall seroprevalence of the disease and the distribution of serovars, and the prevalence is generally higher in dairy than beef cattle (Faine *et al.* 2000), and the higher prevalence of Hardjo was found in our study could be explained by the fact that the cattle had close contact with the reservoirs of this serovar (Radostits *et al.* 2007).

In our study serodiagnosis of bovine leptospirosis based on the results of the indirect ELISA test. This diagnosis of leptospirosis in the live animal may be achieved by detection of antibodies using MAT. ELISA was sensitive and could detect antibodies to multiple pathogenic *Leptospira* serovars. It is a good assay for bovine leptospirosis screening (El Jalii, 2008). The ELISA has some advantages over the MAT. It is relatively sensitive and specific and semiautomated, it uses killed antigens, and the results may be interpreted objectively (Surujballi and Mallory 2004).

In conclusion, serovar hardjo infections were determined to be more common leptospiral infections in cattle in Nineveh provinces, Iraq.

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### References

- Al-Badrawi TYG, Habasha FG, Sultan SH (2010). Serological study of Leptospirosis in cattle, sheep and goats in Baghdad Province. Al-Anbar Journal Veterinary Science, 3,78-82.
- Adler B, Moctezuma A (2010). Leptospira and leptospirosis. Veterinary Microbiology, 140, 287–296.
- Bahaman AR, IBRAHIM AL, ADAM H (1987). Serological prevalence of leptospiral infection in domestic animals in West Malaysia. Epidemiology and Infection, 99, 379-392
- Bolin CA. (2003). Leptospirosis. In: Fowler ME, Miller RE. Zoo and wild animal medicine. St. Louis, Missouri: WB Saunders, 699–702.
- Cole JR, Sulzer CR, Pursell AR (1973). Improved microtechnique for the leptospiral microscopic agglutination test. Applied Microbiology, 25, 976–980.
- Ezeh AO, Addo PB, Adesiyun AA, Bello CSS, Makinde A (1989). Serological prevalence of bovine leptospirosis in Plateau State, Nigeria. Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux, 42,505-508.
- Dassanayake DLB, Wimalaratna H, Agampodi SB, Liyanapathirana VC, Piyarathna TACL, Goonapienuwala BL (2009). Evaluation of surveillance case definition in the diagnosis of leptospirosis, using the Microscopic Agglutination Test: a validation study. BMC Infectious Diseases, 9:48.
- El Jalii IM (2008). Comparison between ELISA and the Microscopic Agglutination a test for the Diagnosis of Bovine Leptospirosis. Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux, 61 (2), 73-75.
- Faine S, Adler B, Bolin C, Perolat P (2000). *Leptospira* and Leptospirosis, 2nd Ed., Med. Sci., Melbourne, Australia.
- Kavanagh OV, Skibinska A, Mackie DP, Montgomery JM, Logan EF, Ellis WA (2002). In Bovine leptospirosis: Validation of an ELISA to detect antibodies to *Leptospira borgpetersenii* serovar hardjo. Research in Veterinary Science, 72:26-26
- Kocabiyik A L, Cetin C (2004). Bovine leptospirosis in south Marmara region of Turkey : A serological survey. Revue de Médecine Vétérinaire, 155, 12, 606-608
- Larson RL, Maas J, Richey E, Bolin, C (2007). Herd Prevalence and Risk Factors of Leptospira Infection in Beef Cow/calf Operations in the United States: *Leptospira borgpetersenii* Serovar Hardjo. Bovine Practitioner, 41, 15-23.
- Leonard N, Mee JF, Snijders S, Mackie D (2004). Prevalence of antibodies to *Leptospira interrogans* serovar hardjo in bulk tank milk from unvaccinated Irish dairy herds. Irish Veterinary Journal, 57, 226-231.
- Miller DA, Wilson MA, Beran GW (1991). Survey to estimate prevalence of *Leptospira interrogans* infection in mature cattle in the United States. American Journal of Veterinary Research, 52,1761–1765.
- Machang URS, Mgdde G, Mpanduji D (1997). Leptospirosis in animals and humans in selected areas of Tanzania. Belgian Journal of Zoology, 127, 97-104.
- Mariyar CP, Kumar AA, Thangapandian E, Amuthar R, Srivastava SK (2006). Evaluation of a recombinant LipL41 antigen of *Leptospira interrogans* serovar canicola in ELISA for serodiagnosis of bovine leptospirosis. Comparative Immunology, Microbiology & Infectious Diseases., 29,269-277.
- Morgan LH, Mark AW, Tammy MA, Corrie LT, David AM (2007). Comparative serological study of *Leptospira* serovar hardjo genotypes for use in the microscopic agglutination test. Journal of Veterinary Diagnostic Investigation, 19, 84–87.
- Peregrine AS, Martin SW, Hopwood DA (2006). *Neospora caninum* and *Leptospira* serovar serostatus in dairy cattle in Ontario. Canadian Veterinary Journal, 47, 467–470.
- Pritchard DG (1986). National situation of leptospirosis in the United Kingdom. In: W.A. ELLIS and T.W.A. LITTLE (ed.) Present state of leptospirosis diagnosis and control. Martinus Nijhoff, Dordrecht, The Netherlands, pp 221-233
- Radostits OM, Gay CC, Hinchcliff KW, Constable PD. (2007). In: Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, sheep, Pigs and Goats. Saunders (Elsevier), Edinburgh, pp. 1094-1110.
- Rochat T (1998). A review of leptospirosis in farm animals in Portugal. Revue scientifique et technique, 17, 699-712.
- Ryan EG, Leonard N, Luke OG, Michael LD, Simon JM (2012). Herd-level risk factors associated with *Leptospira* Hardjo seroprevalence in Beef/Suckler herds in the Republic of Ireland. Irish Veterinary Journal, 65, 6
- Surujballi OM, Mallory M (2004). An indirect enzyme linked immunosorbent assay for the detection of bovine antibodies to multiple *Leptospira* serovars. The Canadian Journal of Veterinary Research, 68,1–6.
- Thiermann AB (1984). Leptospirosis: current developments and trends. Journal of the American Veterinary Medical Association, 184, 722-725.
- Victoriano AF, Smythe LD, Gloriani-Barzaga N, Cavinta LL, Kasai T, Limpakarnjanarat K, Ong BL, Gongal G, Hall J, Coulombe CA, Yanagihara Y, Yoshida S, Adler B (2009). Leptospirosis in the Asia Pacific region. BMC Infectious Diseases, 9,147.
- Wagenaar J, Zuerner RL, Alt D, Bolin CA. (2000). Comparison of polymerase chain reaction assays with bacteriologic culture, immunofluorescence, and nucleic acid hybridization for detection of *Leptospira borgpetersenii* serovar hardjo in urine of cattle. American Journal of Veterinary Research, 61, 316–320.
- World Health Organization. (2003). Human Leptospirosis: Guidance for Diagnosis Surveillance and Control. Geneva: World Health Organization, :109, ISBN 92-4-154589-5.