

Leptospiral abortions in Nili-Ravi buffaloes in Punjab state of India

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Leptospirosis is presumed to be the most widespread zoonosis in the world (WHO 1999). The causative agent, an obligate aerobic spirochaete, is frequently excreted through urine and contaminates the environment, thereby exposing human beings to a greater risk of infection (Scanziani *et al.* 1994). The states of Gujarat, Maharashtra, Tamil Nadu, Kerala and Andaman and Nicobar Islands are endemic in India and incidences in humans are perennially reported from these states during the monsoon season (Maskey *et al.* 2006). The organism has been detected in farm animals in many parts of the country (Ratnam 1994) and comprises more than 280 serovars, out of which about 10 serovars are commonly responsible for causation of disease. Leptospirosis, of late, has assumed considerable significance, as the disease is increasingly being involved in cases of abortions, repeat breeding and other reproductive problems (Srivastava and Kumar 2003).

We report an outbreak of leptospirosis in Nili-Ravi buffaloes in Nabha area of Punjab. Nabha lies in the south-east of Punjab state and constitutes a sub-division/tehsil of Patiala district. The true incidence of animal leptospirosis in this region apparently is not available in the literature. A spate of abortions/still births in Nili-Ravi buffaloes in the Nabha sub-campus of Central Institute for Research on Buffaloes was reported during November 2006. Aborting buffaloes were in the gestation periods ranging from 6 months to term. Investigations were conducted to ascertain the cause of abortions in buffaloes. Blood was collected following jugular phlebotomy from aborting and apparently healthy buffaloes. Serum was obtained after centrifugation at 4000 rpm, the samples being transported to the laboratory under refrigerated conditions within 12 h of sampling. These samples were then stored in the deep freezer at -2°C . Since majority of the abortions were late-term, immediate suspicion was focused on brucellosis, infectious bovine rhinotracheitis (IBR) and leptospirosis. The animals had a history of vaccination with *B. abortus* S19 vaccine. A total of 58 clinical samples

including 48 sera samples, 6 uterine discharge samples, 3 milk samples and 1 fetus sample were collected for carrying out investigations (Table 1). The samples were tested for brucellosis using cultural examination for isolation and an OIE prescribed assay, *i.e.* competitive ELISA at College Central Laboratory and Standard Tube Agglutination (SAT) test at Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Sciences, CCS HAU, Hisar. The samples were tested for IBR using ELISA Kit and for leptospirosis using immunocomb bovine *Leptospira hardjo* antibody test kit at this institute. Necropsy examination of the fetus was conducted at the Department of Veterinary Pathology, CCS HAU, Hisar.

Uterine discharge samples, milk samples and aborted fetus were subjected to isolation of *Brucella* spp. but no brucellae could be recovered using standard culture protocol. Twenty-eight sera samples including eight from bulls were subjected to SAT. All the 8 samples from bulls were negative for brucellosis while twenty samples showed a titre of 160 IU in SAT, which might have been due to the vaccination of animals with *B. abortus* S19 vaccine. Out of 48 sera samples (40 from females and 8 from males), examined by c-ELISA, 3 gave positive serological reactions, presumably due to recent vaccination with *B. abortus* S19 vaccine. Sera samples (28) examined for IBR were found negative by ELISA, however, ELISA based examination of 28 sera samples led to

Table 1. Examination of clinical samples for different diseases

Sample	N	Brucellosis	IBR	Leptospirosis	Remarks
Uterine discharge	6	-ve	ND	ND	
Milk	3	-ve	ND	-ve	
Aborted fetus	1	-ve	ND	ND	Positive for icterus in necropsy examination
Serum	48	-ve	20 ND, 28 -ve	13 +ve (6 ++, 7+) 20 ND 15 -ve	

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identification of 13 samples (65.00%) positive for *Leptospira hardjo*, out of which 6 were assigned ++ score while 7 were assigned + score. *Leptospira* could not be detected in the milk samples. PM examination of aborted fetus revealed splenomegaly with pale discoloration of liver. Tips of the hooves were also characteristically yellowish coloured indicative of jaundice. Histopathological examination revealed hepatitis associated with jaundice that further confirmed the diagnosis of abortions. Infected animals were advised treatment with streptomycin/oxytetracycline enrofloxacin along with exercising of other measures like control of rodent population at farm premises, proper drainage of wet places in the premises and avoiding the direct contact of veterinarians/handlers with urine of animals and contamination of feed/drinking water with the urine of infected animals in order to contain the infection at the animal farm.

The incidence of leptospirosis is highly variable—it may range from a sporadic case to more than 69% in some buffalo herds (Pathak and Sharma 1988). In buffaloes, various serovars of *L. interrogans* were incriminated for abortion and other reproductive disorders (Khan and Khan 1988, Xu *et al.* 1983). *L. hardjo* is also recognized as a cause of bovine mastitis and premature calving as reported from a number of countries (Ellis *et al.* 1982). Such incidents have, however, been poorly reported and ill defined in buffaloes (Chaudhry *et al.* 1996). Barring one report from Oberoi and Kwatra (1982), literature appears to be scanty on leptospiral abortions in and around Punjab. *Leptospira* spp. incriminated in their report included *L. canicola*, *L. wolfii* and *L. australis*. Srivastava and Kumar (2003) carried out a comprehensive seroprevalence of leptospirosis in 4,994 sera from various animals. They screened 443 samples of buffaloes from Andhra Pradesh with a seropositivity of 2.7% and 19 buffaloes from Tamil Nadu with no seropositivity at all. Serovars identified in AP belonged to *L. hardjo*, *L. icterohemorrhagiae*, *L. australis*, *L. autumnalis*. Their study, however, did not include samples of buffalo from Punjab or Haryana state. *L. hardjo* encountered in our investigation in the Punjab region probably constitutes first report of this infection in Nili-Ravi buffaloes. Following table enlists the prevalence of leptospirosis in different regions across India.

The ecological habitat is directly associated with the occurrence of disease in all species of animal life, their interaction with each other, and the resultant contamination of the ecosystem. High prevalence of leptospira has been recorded in agricultural areas where domestic animals share a geographical area with a wild animal population (Diesch and Ellinghausen 1975, Amaddeo *et al.* 1991). The extent to which infection is transmitted depends on many factors, including climate, population density, and the degree of contact between maintenance and accidental hosts. The incidence is significantly higher in warm climate countries than in temperate regions due to longer survival of leptospirae

Table 2. Prevalence of leptospirosis in India

State	Author(s)
Across different states	Srivastava <i>et al.</i> (1983, 1990)
Andaman and Nicobar islands	Varma <i>et al.</i> (2001)
AP	Ramani Pushpa and Punyakumari (2005), Mrunalini and Ramasastry (2000)
Gujarat, Maharashtra, Tamil Nadu, Kerala, and Andaman and Nicobar Islands	Prabhudas <i>et al.</i> (2008) Maskey <i>et al.</i> (2006)
Punjab	Oberoi and Kwatra (1982)
Karnataka	Rajasekhar and Nanjiah (1971)
NE Hill region	Ghosh (1991)
Orissa	Biswal <i>et al.</i> (2000)
Tamil Nadu	Balakrishnan <i>et al.</i> (2008)
Uttar Pradesh, Uttarakhand and Tamil Nadu	Adinarayanan <i>et al.</i> (1960) Mariya <i>et al.</i> (2007)
Uttarakhand	Agrawal <i>et al.</i> (2005)
West Bengal	Debnath <i>et al.</i> (2005)

in the environment in warm, humid conditions. Patiala, predominantly a rural district of Punjab, forms a part of the Indo-gangetic plain and the environmental conditions make this area a favourable bed for persistence of leptospirae outside and inside host animals. The cases of abortion and still births were reported from sixth month to term. This is in agreement with Ellis *et al.* (1982), who reported that with *hardjo* infection, abortion has been diagnosed at all stages from the fourth month through to term. Contamination of the wallowing tank/pond may result in contact of the contaminated water with the vaginal, conjunctival, and oronasal mucous membranes which might contribute to spread of infection. We based our diagnosis on a combination of clinical history and laboratory results. It is true that water buffalo working in paddy fields, bathing in the waterholes and drinking sewage water, have ample opportunities of exposure and, if infected, of spreading the disease organisms. If water buffalo are susceptible to infection with leptospira they would be a serious source of infection not only to other animals but to man, thus creating an important public health problem. Veterinarians are at risk for leptospirosis and should take measures to decrease potential exposure to infectious agents in general (Whitney *et al.* 2009). Epidemiological studies on this disease should continue with a view to monitor the disease trend as well as to evolve control measures. A knowledge of the prevalent serovars and their maintenance hosts is essential to understanding the epidemiology of the disease in any region.

SUMMARY

Leptospirosis is an anthroponozoonosis with a ubiquitous distribution. Several states in India are endemic for leptospirosis with abortions, repeat breeding and reproductive

problems being common sequelae of this infection in farm animals. Investigations were carried out in Nabha sub-division of Patiala district in Punjab state to ascertain the cause of abortions in Nili-Ravi buffaloes in the gestation periods ranging from 6 months to term. Thirteen out of 28 sera samples (65.00%) were found positive for *L. hardjo* antibodies in ELISA. *L. hardjo* encountered in the investigation in the Punjab region probably constitutes first report of this infection in Nili-Ravi buffaloes.

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REFERENCES

- Adinarayanan N, Jain N C, Chandiramani N K and Hajela S K. 1960. Studies on leptospirosis among bovines in India. I. A preliminary report on the occurrence based on serological evidence. *Indian Veterinary Journal* **37**: 251–54.
- Agrawal R, Kumar M, Kumar M and Srivastava S K. 2005. Epidemiological pattern of leptospirosis in livestock of Uttaranchal state. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **26**(2): 109–13.
- Amadeo D, Autorina GL and Aleandri M. 1991. Epidemiological studies on leptospirosis in cattle, sheep and buffaloes in the Lazio region. *ATTA dalla Federazione Meditterrenea Sanita e produzione Ruminanti* **1**: 263–67.
- Balakrishnan G, Govindarajan R, Meenambigai T V, Jayakumar V and Murali Manohar B. 2008. Seroprevalence of animal leptospirosis in certain parts of Tamil Nadu. *Indian Veterinary Journal* **85**: 227–28.
- Biswal B C, Kar B C, Rathnam S, Suresh Babu L and Natarajasreenivasan K. 2000. Evidence of antibodies to leptospira among farm cattle and farm workers in Orissa state. *Indian Veterinary Journal* **77**: 622–23.
- Chaudhry J I, Khan M A, Akhtar T, Khan A G and Chaudhry M S. 1996. Seroprevalence of leptospirosis in buffaloes. *Buffalo Journal* **1**: 65–71.
- Debnath C, Bakshi S, Pramanik A K and Biswas M K. 2005. Serological study of leptospirosis among different animals in and around Kolkata. *Indian Journal of Animal Sciences* **75**(11): 1283–85.
- Diesch SL and Ellinghausen H C Jr. 1975. *The Leptospirosis: Diseases Transmitted from Animals to Man*. 6th edn, pp. 436–62. Charles C. Thomas, Springfield.
- Ellis W A, O'Brien J J and Neil S D. 1982. Bovine leptospirosis; microbiological and serological findings in aborted fetuses. *Veterinary Record* **110**: 147–50.
- Ellis W A. 1986. Effects of leptospirosis on bovine reproduction. *Current therapy in theriogenology*. Vol. II. *Diagnosis, Treatment and Prevention of Reproductive Diseases in Small and Large Animals*. (Ed.) Morrow D A. W B Saunders Company.
- Ghosh S S. 1989. Sero-incidence of leptospirosis in organized cattle farms in North-Eastern hill region. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **10**(1): 48–50.
- Khan M A and Khan M S. 1988. Serological response against *Leptospira interrogans* in the aborting buffaloes. *Proceedings of Second World Buffalo Congress*. pp. 352, December 12–16, New Delhi, India.
- Mariya R, Srivastava S K and Thangapadian E. 2007. Seroprevalence of leptospiral antibodies in bovines. *Indian Veterinary Journal* **84**: 547–48.
- Maskey M, Shastri JS, Saraswathi K, Surpam R and Vaidya N. 2006. Leptospirosis in Mumbai: Post deluge outbreak 2005. *Indian Journal of Medical Microbiology* **24**(4): 337–38.
- Mrunalini N and Ramasastry P. 2000. A note on seroprevalence of subclinical leptospirosis in organized farms in Andhra Pradesh. *Indian Veterinary Journal* **77**: 713–14.
- Oberoi M S and Kwatra M S. 1982. Sero-detection of bovine abortions at organized farms in and around Punjab. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **3**(2): 101–02.
- Pathak N N and Sharma M C. 1988. Buffalo health management. *Proceedings of Second World Buffalo Congress*. pp. 156–84, December 12–16, New Delhi, India.
- Prabhudas K, Gangadhar N L, Mukunda P and Ahmed K. 2008. Incidence of *Leptospira* infection due to heavy rains: A study in Gujarat state. *Indian Journal of Animal Sciences* **78**(4): 360–61.
- Rajasekhar M and Nanjiah R D. Animal leptospirosis in Mysore state-A serological study. *Indian Veterinary Journal* **48**: 1087–95.
- Ramani Pushpa R N and Punyakumari B. 2005. Seroprevalence of leptospirosis in domestic animals. *Indian Veterinary Journal* **82**: 670–71.
- Ratnam S. 1994. Leptospirosis. An Indian perspective. *Indian Journal of Medical Microbiology* **12**: 228–39.
- Scazziani E, Origgi F, Giusti AM, Iacchia G, Vasio A, Pirovano G, Searpa P and Tagliabue S. 2002. Serological survey of leptospiral infection in Kennelled dogs in Italy. *Journal of Small Animal Practice* **43**: 154–57.
- Srivastava S K and Kumar A A. 2003. Seroprevalence of leptospirosis in animals and human beings in various regions of the country. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **24**(2): 155–59.
- Srivastava S K, Singh S P and Srivastava N C. 1983. Seroprevalence of leptospirosis in animals and man in India. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases* **4**: 243.
- Srivastava S K, Verma R and Harbola P C. 1990. Seroprevalence of leptospirosis in animals and man in India. *Indian Journal of Animal Sciences* **60**: 1439–41.
- Varma A, Rai R B, Balakrishnan P, Gupta A and Naveen K A. 2001. Seroprevalence of leptospirosis in animals of Andaman & Nicobar islands. *Indian Veterinary Journal* **78**: 936–37.
- Whitney EAS, Ailes E, Myers LM, Saliki JT and Berkelman, RL. (2009). Prevalence of and Risk Factors for Serum Antibodies against *Leptospira* Serovars in US Veterinarians. *Journal of the American Veterinary Medical Association* **234**(7): 938–44.
- World Health Organization. 1999. Leptospirosis worldwide 1999. *Weekly Epidemiological Record* **74**: 237–42.
- Xu J T, Pian R L and Mao J S. 1983. A survey of leptospiral abortions in buffaloes. *Chinese Journal of Veterinary Medicine* **9**: 19–20.