RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 5: 902-905 (May 2026)

Seroprevalence of Bovine Leptospirosis using Indirect ELISA

Shivangi Sharma[1], Devendra Gupta[1], Amita Tiwari[1], Ajit Pratap Singh[1], Pankaj Asati[1], Sanjay Shukla[1], Kush Shrivastava[1], Rahul Sharma[1], Sita Prasad Tiwari[1]

10.18805/IJAR.B-4951

ABSTRACT

Background: Leptospirosis is a zoonotic bacterial disease causing abortion, stillbirths and loss of milk production in livestock resulting in huge economic losses. Leptospirosis in cattle has been under-reported and under-diagnosed in India due to non-specific symptoms, complex laboratory tests, fastidious nature of bacterium and lack of proper diagnostic facilities. Scanty data pertaining to epidemiological status of leptospirosis is available in land locked states of India including Madhya Pradesh, thus to fulfil the gap, the current work was conducted to investigate seroprevalence of leptospirosis in cattle.

Methods: In the present study, cattle with a history of repeat breeding, abortion, haemogalactia and mastitis, were screened from various organized and unorganized farms of Jabalpur. A total of 300 blood samples were collected from suspected cattle and serum was separated. Enzyme linked Immune-sorbent assay was done as per the guidelines.

Result: The seroprevalence of leptospirosis among suspected cases was 48.67 per cent, it was significantly higher in organized sector in cattle with the history of abortion followed by repeat breeding. High seropositivity was reported in older cattle.

Key words: Cattle, ELISA, leptospirosis, Seroprevalence.

INTRODUCTION

Leptospirosis is known to be endemic in India since the early 20th century with most outbreaks reported from the coastal regions. The transmission cycle of leptospirosis involves the maintenance hosts, the carrier hosts, the environment and human beings. Cattle are known to be the carrier of leptospirosis and urinary shedding is very evident in cattle. Acute systematic infection in adult dairy cattle is reported as a cause of acute onset agalactia, called as milk drop syndrome (Ellis, 2015). In cattle, acute haemolytic syndrome of leptospirosis is characterized by fever, icterus, anaemia and haemoglobinuria including additional findings of anorexia, apathy, dehydration, dyspnoea, hyperpnea, bilirubinuria, mastitis and abortion. Chronic infections with leptospirosis can result in abortions, stillbirths and reduced fertility in ruminants (Pande *et al*., 2020).

Enzyme-Linked Immuno Sorbent Assay (ELISA) is one of the methods used for carrying out seroprevalence study and presumptive diagnosis and was predominantly designed to detect antibodies, referred to as indirect ELISA. It is more specific than MAT, though MAT is the gold standard serological test for leptospirosis. Proper understanding of the epidemiological features of these organisms is a critical step in designing interventions for diminishing the risk of the disease transmission Seroprevalence study contributes in understanding the disease's epidemiology in cattle.

MATERIALS AND METHODS

Place and duration of work

The proposed work was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur for a period of 12 months *i.e*. from January 2021 to December 2021.

[1]Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.

Corresponding Author: Shivangi Sharma, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur-482 001, Madhya Pradesh, India.
Email: spshivi094@gmail.com

How to cite this article: Sharma, S., Gupta, D., Tiwari, A., Singh, A.P., Asati, P., Shukla, S., Shrivastava, K., Sharma, R. and Tiwari, S.P. (2026). Seroprevalence of Bovine Leptospirosis using Indirect ELISA. *Indian Journal of Animal Research*. **60(5):** 902-905. doi: 10.18805/IJAR.B-4951.

Submitted: 08-06-2022 **Accepted:** 08-09-2022 **Online:** 16-09-2022

Location and features of the place

In Central Madhya Pradesh, Jabalpur is located at latitude 23°10 N to longitude 79°59 E. The city covers an area of 367sq km and lies at an altitude of 412 m above sea level. Jabalpur has a humid subtropical climate characteristic of north-central India. Summer season begins from March and last until June. May is the hottest month of the year with an average temperature exceeding 40°C (104°F). Summer season is followed by the southwest monsoon which extend from July to October. The average annual precipitation is nearly 1,386 mm (54.6 in).

Experimental animals

For the present study, 6755 cattle were screened from organized and unorganized farms of Jabalpur. Out of 6755 cattle, 300 bio-samples were collected from cattle with a history of repeat breeding, abortion, haemogalactia and mastitis.

Collection of blood samples

Five ml of blood was collected aseptically from the jugular vein of properly restrained cattle in clot-activated vacutainers. They were kept in an upright position at room temperature for 2 hours for serum separation. The straw-coloured serum was then poured into 1.5 ml sterile cryovials and aliquoted for future use. Samples were transported to the laboratory and stored at -20°C till further use.

Seroprevalence-Indirect ELISA

The Vet Check bovine *Leptospira* indirect Elisa kit which detects specific antibodies (Ab) directed against *Leptospira interrogans* in cattle. Indirect ELISA was performed strictly as per the standard protocol.

Material provided in kit

Bovine Leptospira antigen coated micro wells (12×8 wells), positive control, negative control, wash buffer (10×), sample diluent buffer (1×), protein G HRP conjugate (12 ml), chromogen solution (12 ml), stop solution (12 ml of 1 M H_2SO_4). Additional materials used for the test includes accurate adjustable micro-pipettes and disposable pipette tips, deionized/distilled water, micro plate washing system, micro plate reader, timer, test tube for serum dilution, graduated cylinder and vortex.

Test procedure

Test samples were diluted in sample diluent buffer separately in 1:100 dilutions. Both serum and diluent buffer were mixed thoroughly. Then 100 µl of ready-to-use controls/diluted sera was dispensed into their respective wells. The micro titre plate was covered with aluminium foil and incubated at 37°C for one hour. After one hour of incubation, the plates were taken out and washed four times with 300 µl diluted wash buffer provided with the kit. Wash buffer residues were completely removed by tapping the plate on a towel or tissue paper. The 100 µl of the conjugate solution was added to each well and incubated at 37°C for one hour. The plates were taken out and washed four times with 300 µl diluted wash buffer provided with the kit. Wash buffer residues were completely removed by tapping the plate on a towel or tissue paper. Then 100 µl of chromogen solution was added to each well. The plates were incubated at room temperature for 10-15 minutes in the dark until the colour developed. The reaction was stopped after the colour development, by adding 100 µl of stop solution provided with the kit in each well. The OD values of the micro titre plate was taken with an ELISA reader at 450 nm and results were interpreted.

Statistical analysis

Significance of prevalence was calculated by chi square test.

RESULTS AND DISCUSSION

The screening of cattle for the presence of clinical signs pertaining to leptospirosis was done in present study. Out of 6755 cattle screened, a total of 300 samples were suspected on the basis of clinical signs and 146 cattle were found positive for leptospirosis. Thus the overall seroprevalence was found to be significantly high *i.e*. 2.16 per cent (Table 1).

The seroprevalence of leptospirosis among suspected cases was 48.67 per cent. Among 300 samples, 177 were from organized farming systems and 123 were from unorganized farms. The seroprevalence of organized sector was significantly higher (53.67 per cent) than unorganized sector (41.46 per cent). In clinically ailing cattle, the significantly higher sero-prevalence (53.30 per cent) was reported in cattle with the history of abortion followed by repeat breeding (46.48 per cent). In the cases of hemogalactia/mastitis and still death the seroprevalence was 26.92 per cent and 16.67 per cent, respectively. The seropositivity was significantly higher *i.e*. 55.41 per cent (87 out of 157 cattle) in the cattle of age above 6 years followed by 47.22 per cent (51 out of 108) in 4-6 years of age and least *i.e*. 22.85 per cent (8 out of 35) in the cattle below 4 years of age. The breed wise seroprevalence study of leptospirosis in cattle revealed a significantly higher prevalence in exotic and crossbred cattle as compared to indigenous breeds (Table 1).

Leptospirosis is an apparently emerging as a significant public health problem in developing countries which remains under diagnosed due to non-specific symptoms in the majority infection cases and also absence of advanced laboratory facilities. The most frequent sources of infection are urine contaminated water through which leptospires enter into the host body is through intact skin. Humid, wet and damp environment favours the transmission of leptospires. Till date there is no published data of seroprevalence of leptospirosis in Madhya Pradesh since most of the outbreaks are recorded in coastal states of India. Thus the study was planned to know the sero-epidemiological status of leptospirosis. A high seroprevalence in cattle (47.06 per cent, 24/51) was recorded from Valsad, Gujrat by Patel (2014) and (42.15 per cent, 180/427) from Andaman and Nicobar by Jai Sunder *et al.* (2018) are in agreement with the present study.

In buffaloes high seroprevalence *i.e*. 54.14 per cent was reported in Gujarat (Balakrishnan *et al.,* 2011), 26.66 per cent in Andaman and Nicobar Islands (Varma *et al*., 2001) and 88.8 per cent (125/111) in Chennai (Selvaraj *et al*., 2010). A sero-survey among animal populations of Andaman and Nicobar Islands (Sharma *et al.,* 2006) showed that about 40 per cent of the cows and 26 per cent of the bulls were seropositive. In contrary to this, less prevalence in cow was reported by Mandal *et al.* (2008) in West Bengal which was 16.84 percent. The high sero-prevalence in Jabalpur might be due to the location of organized dairy farms which are situated nearby to water bodies leading to favourable conditions for growth of leptospires and easy transmission.

Reproductive signs seen in cattle could be due to the localization of leptospires in reproductive tract/uterus.

Table 1: Seroprevalence of bovine leptospirosis using indirect ELISA.

	Suspected cases	Positive cases	Seroprevalence (%)
Overall seroprevalence of leptospirosis in cattle			
6755	300	146	2.16
χ^2 = 208.406, p<0.001 (Highly significant)			
Sector wise seroprevalence of leptospirosis in suspected cattle			
Organized	177	95	53.67
Unorganized	123	51	41.46
Total	300	146	48.67
χ^2 = 13.26; p<0.001 (Significant)			
Symptom wise seroprevalence of leptospirosis in clinically ailing cattle			
Abortion	197	105	53.30
Repeat breeding	71	33	46.48
Haemogalactia/Mastitis	26	07	26.92
Still death	06	01	16.67
χ^2 = 105.83 p<0.001 (Significant)			
Age wise seroprevalence of leptospirosis in cattle			
< 4 years	35	8	22.85
4-6 years	108	51	47.22
> 6 years	157	87	55.41
χ^2 = 64.28; p<0.001 (Significant)			
Breed wise seroprevalence of leptospirosis in cattle			
Indigenous	119	45	37.81
Exotic and cross bred	181	101	55.80
χ^2 = 21.47; p<0.001 (Significant)			

Role of leptospira infection in abortions and reproductive disorders is well established by earlier workers including our earlier studies (Poonacha *et al.,* 1993; Gangadhar *et al.,* 2008). Similar findings in clinically ailing seropositive cattle history of mastitis/ agalactia was recorded in maximum number of cases (24.00 per cent, 6/25) followed by abortion (20.00 per cent, 3/15) and repeat breeding (11.76 per cent, 2/17) by Patel (2014).

Balakrishnan *et al.* (2011) reported the similar findings that the risk of leptospirosis was more in the cattle of age group above seven years (55.88 per cent), followed by four to seven years (51.28 per cent) and below seven years (28.42 per cent). Thus, it was concluded that higher age groups (above 4 years) in cattle favour the occurrence of leptospirosis and supported the observations of earlier workers (Agrawal *et al*., 2005; Balakrishnan *et al.,* 2011). The older cattle are maximum prone for diseases due to senility and immune exhaustion along with the lodging of leptospira in kidney and reproductive system.

The high seropositivity in exotic and cross bred cattle may be attributed to their higher susceptibility to disease and poor disease resistance The findings of present study are similar to those reported by Balakrishnan *et al.* (2011) who reported the risk of leptospirosis was highest in exotic pure breed, followed by indigenous pure breeds and cross bred cattle. Varma *et al.* (2001) and Nagarajan (2005), also reported more seropositivity among cross bred cattle than indigenous cattle.

CONCLUSION

It can be concluded from the present study that the cattle showing the reproductive signs were found to be seropositive for leptospirosis. Since it is a disease of public health importance, a comprehensive and good understanding of the prevalence of the disease and its epidemiological characteristics is an essential prerequisite for evolving an effective control measures.

ACKNOWLEDGEMENT

Authors would like to acknowledge Environment Planning and Coordination Organization (EPCO), Bhopal for Chief Minister's Scholarship on Climate Change.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

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):** 109-113.

Balakrishnan, G., Roy, G.P., Govindarajan, R., Ramaswamy, V. and Manohar, B.M. (2011). Seroepidemiological studies on leptospirosis among bovines in organized farm. *International Journal for Agro Veterinary and Medical Sciences.* **5(6):** 511-519.

Ellis, W.A. (2015). Animal leptospirosis. *Current Topics in Microbiology and Immunology*. **387:** 99-137.

Gangadhar, N.L., Prabhudas, K., Bhushan, S., Sulthana, M., Barbuddhe, S.B. and Rhaman, H. (2008). Leptospira infection in animals and humans: A potential public health risk in India. *Revue Scientifique et Technique O.I.E*. **27(3):** 885-892.

Jai Sunder, Sujatha, T., Kundu, A. and Kundu, M.S. (2018). Carrier status and seroprevalence of leptospirosis in cattle of South Andaman. *Indian Journal of Animal Research*. **52:** 140-143. doi: 10.18805/ijar.B-3186.

Mandal, S., Joardar, S.N., Chakraborty, D. and Sardar, N. (2008). Seroepidemiological study of bovine leptospirosis in West Bengal. *Indian Journal of Comparative Microbiology Immunology and Infectious Diseases*. **29(1 and 2):** 42-44.

Nagarajan, M. (2005). Seroepidemiological Studies on Leptospiral infections in dairy cows in selected districts of Tamil Nadu. M.V.Sc. Thesis Submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai.

Pande, D., Khan, W., Chaudhari, S., Shinde, S., Patil, A., Likhite, A. and Allai, R. (2020). Study of leptospira Infection in buffaloes through molecular and bacteriological techniques. *Indian Journal of Animal Research*. **54:** 1024-1028. doi: 10.18805/ijar.B-3860.

Patel, J.M., Vihol, P.D., Prasd, M.C., Kalyani, I.H., Raval, J.K., Patel, K.M., Thirumalesh, S.R.A. and Balamurugan, V. (2014). Seroepidemiological pattern of leptospirosis in bovine of South Gujarat, India. *Veterinary World*. **7(11):** 999-1003.

Poonacha, K.B., Donahue, J.M., Giles, R.C., Hong, C.B., Petrites-murphy, M.B., Smith, B.J., Swerczek, T.W., Tramont, R.R. and Tuttle, P.A. (1993). Leptospires in equine fetuses, stillborn foals and placentas. *Veterinary Pathology.* **30(4):** 362-369

Selvaraj, J., Murali Manohar, B., Govindarajan, R., Jayakumar, V., Meenambigai, T.V. and Balachandran, C. (2010). Seroprevalence of leptospirosis in she- buffaloes (*Bos bubalis*) at slaughter in Chennai, India. Short Communication. *Buffalo Bulletin*. **29(2):** 95-98.

Sharma, S., Vijayachari, P., Sugunan, A., Nataraja, A., Seenivasan, K. and Sehgal, S. (2006). Seroprevalence of Leptospirosis among high risk population of Andaman Islands, India. *American Journal of Tropical Medicine and Hygiene.* **74:** 278-283.

Varma, A., Rai, R.B., Balakrishnan, P., Gupta, A. and Naveen, K.A. (2001). Seroprevalence of leptospirosis in animas of Andaman and Nicobar Islands. *Indian Veterinary Journal*. **78:** 936-937.