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# Investigation on the prevalence of *Leptospira* serovar Hardjo in organized cattle dairy farms of India

V BALAMURUGAN[1], ANUSHA ALAMURI[2], S VEENA[3], K BHARATHKUMAR[4], S S PATIL[5], and G GOVINDARAJ[6]

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*ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru, Karnataka 560 064 India*

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Leptospirosis is the most widespread zoonosis in the world and is re-emerging as an important public health problem in India (Vijayachari *et al.* 2008). Leptospirosis in bovine causes direct or indirect economic losses, which include costs of abortion, stillbirth, infertility, failure to thrive, reduced productivity and decreased milk yield (Quinn *et al.* 1994) and associated veterinary costs in domestic and commercial livestock, with potential for malnutrition and impoverishment amongst individuals and communities dependent on animal sources of protein, especially in subsistence economies (Srivastava 2008). Leptospirosis can cause abortion as early as the fourth month, but abortion after 7 months is also more common in bovines. Presence of the complexity in case identification as well as the lack of diagnostic facility (especially early diagnosis must rely on an efficient reference laboratory settings) hinders in avoiding losses due to bovine leptospirosis in India. Leptospirosis is frequently under diagnosed because of the non-specific symptoms and the difficulty of performing both culture and the reference serological tests/asssys (OIE 2013). Microscopic agglutination test (MAT) is the gold standard reference serological test for leptospirosis (WHO 2011, OIE 2013). Despite its wide-spread usage and international recognition, it has a number of limitations. Diagnostic assays with high sensitivity and specificity will have an immense value in the initial screening of the farm to declare that it is free from leptospirosis. Hence, preliminary investigation was undertaken to know the status of *Leptospira* serovar Hardjo in organised cattle dairy farms of India by using *Leptospira* Bovine Hardjo ELISA kit to establish the seroprevalence.

Cattle serum samples (964) collected from animals in the organised cattle dairy farms in various areas of different states (Maharastra, Gurajat, Punjab, Tamil Nadu, Haryana, Telangana, Jharkhand, Chhattisgarh and Karnataka) in India during surveys 2015 were tested (Table 1). These samples were from randomly selected herds with history of repeated breeding, abortion, reproductive disorders, etc. including some apparently healthy animals. Collected serum samples were transported on ice to the ICAR-NIVEDI for testing and upon arrival the samples was stored at –20°C until further use.

All the serum samples were tested in *Leptospira* Bovine Hardjo kit for detection of *Leptospira* Hardjo serovar antibodies as per manufacturer's described protocols. Chi-square test was used as per standard statistical method (Snedecor *et al.* 1989) for testing the independence of seroprevalence of *Leptospira* across age groups and across the sample regions for statistical inference to determine significant difference. The estimation of apparent prevalence with 95% confidence interval and statistical data analysis were carried out using Excel Microsoft 2013 and Statistical Package for Social Sciences (SPSS) version 22.

Leptospirosis affects a variety of domestic animals resulting in heavy economic losses to the farming community on account of reproductive problems (Srivastava 2008). Out of total 964 sera tested, 122 samples were positive in ELISA indicating 12.67% prevalence of Hardjo serovar in dairy cattle farms. High seroprevalence (30.40%) was observed in Maharashtra while the least (3.7%) was observed in Punjab. Further, the results of chi-square test revealed that sero-prevalance is not independent across the states for the samples with the history of abortion/ reproductive/respiratory disorders ($\chi^2$, 11.64; $P<0.05$), abortion/reproductive ($\chi^2$, 49.11; $P<0.05$), abortion/ respiratory ($\chi^2$, 11.21; $P<0.05$) and apparently healthy animals ($\chi^2$, 37.65; $P<0.05$). Studies of bovine leptospirosis in different parts of the world indicated that serovars responsible for reproductive losses vary depending on which serovars are locally endemic/prevalent. The observed seroprevalence was more in animals with abortion and reproductive disorders. It is evident from the study that the seroprevalance of *Leptospira* in cattle across the disease history is not independent ($\chi^2$, 95.62; $P<0.01$) and seroprevalence is independent across the age ($\chi^2$, 0.58;

Present address: [1,5,6]Senior Scientist (balavirol@ gmail.com, sharanspin123@rediffmail.com, mggraj74@ gmail.com), [2]SRF (microbiologyjntua11@gmail.com), [3,4]Young Professional-1 (veena.jc@ gmail.com, bharathkumar.k111@ gmail.com).

Table 1. Details of the serum samples from different areas in various states of India and its results screened for leptospirosis

| State | No. of tested samples | Abortion/ Reproductive /Respiratory | Abortion/ Reproductive | Abortion/ Respiratory | Apparently healthy | Total no. of samples positive | Percentage positivity | Confidence interval at 95% |
|---|---|---|---|---|---|---|---|---|
| Maharashtra | 148 | 3(2) | 24(24) | 2(2) | 119(17) | 45 | 30.40 | 23.2-38.6 |
| Gujarat | 148 | 6(6) | 10(1) | 8(5) | 124(8) | 20 | 13.50 | 8.6-20.3 |
| Punjab | 188 | 2(0) | 0 | 4(4) | 182(3) | 07 | 3.70 | 1.6-7.8 |
| Tamil Nadu | 40 | 2(1) | 16(14) | 2(1) | 20(4) | 20 | 50.0 | 34.0-65.9 |
| Haryana | 112 | 3(1) | 4(2) | 3(0) | 10(2) | 05 | 4.46 | 1.7-10.6 |
| Telangana | 250 | 3(1) | 13(2) | 5(1) | 229(6) | 10 | 4.00 | 2.0-7.4 |
| Jharkhand | 10 | 0 | 6(1) | 0 | 4(0) | 01 | 10.00 | 0.5-45.8 |
| Karnataka | 30 | 0 | 7(4) | 0 | 23(1) | 05 | 16.66 | 6.3-35.4 |
| Chhattisgarh | 38 | 6(1) | 6(5) | 0 | 26(3) | 09 | 23.68 | 11.7-39.7 |
| Total | 964 | 25(12) | 86(53) | 24(13) | 737(44) | 122 | 12.65 | 10.66-14.96 |
| | $\chi^2$ value (Across disease history) | 95.62*** | | | | | | |
| | $\chi^2$ value (Across state) | 11.64** | 49.11** | 11.21** | 37.65** | | | |

**, Significant at 5% level of significance; ***, significant at 1% level of significance. Value in the parenthesis indicates the number of samples positive for leptospirosis in ELISA.

Table 2. Age-wise prevalence of *Leptospira* Hardjo serovar in dairy herd in various states of India by statistical analysis

| Age of cattle (years) | Total no. of samples screened | No. of positive samples | Percentage positivity | Confidence interval at 95% |
|---|---|---|---|---|
| <2 years | 252 | 34 | 15.6 | 11.1-21.2 |
| 2 to 5 years | 418 | 49 | 13.2 | 10.0-17.3 |
| >5 years | 294 | 39 | 15.3 | 11.2-20.4 |
| $\chi^2$ value | $0.58^{ns}$ | | | |

ns, non-significant.

P>0.10). Besides being an important cause of bovine abortion, reduced fertility and agalactia, serovar Hardjo also poses a potential zoonotic threat to humans who are exposed to infected cattle. On screening of 135 serum samples with history of abortion, 78 samples showed positivity in ELISA for Hardjo serovar indicating 57.7% of animals with history of abortion and reproductive disorders harbouring *Leptospira* organism. Generally, cattle are the maintenance host for serovar Hardjo, which consist of 2 serologically indistinguishable but genetically distinct species. Further, serovars causing infection in cattle have been classified into 2 groups: those adapted to and maintained by other cattle (serovar Hardjo), and incidental infections caused by strains maintained by other domestic and free-living animals.

Antibodies against different serovars were reported from Karnataka (4.6%), Andaman (24.2%), Tamil Nadu {51.4%–87% (in endemic form also reported by (Natarajaseenivasan *et al.* 2011)} and Andhra Pradesh (10.5%) {cattle,12.2%; buffalo,11.1%) mainly to Patoc, Icterohaemorrhagiae by Srivastava (2008)}, Maharashtra (7.3%) and Uttar Pradesh (4–8%) (Srivastava and Kumar 2003), Odisha (42.5%) (Balamurugan *et al.* 2013), Gujarat (12.8%) (Patel *et al.* 2014) during different surveys. Earlier investigations conducted in India since 1995 have revealed that the seroprevalence of leptospirosis in various states has been 5.4% in buffaloes, 7.5% in cattle, 12.5% in sheep, 14.6% in horses and 15.9% in dogs (Srivastava 2008). As it is a well-known fact that Hardjo serovar is the common one, dairy cattle have a role as a natural host of serovars Hardjo, Pomona, and Grippotyphosa (Leonard *et al.* 2004). The present study also showed the similar results of prevalence of Hardjo serovar in organised dairy cattle farms. Significant percentage of animals that are actively infected with *Leptospira* sp. serovar are shedding leptospires and have antibody titres >100 against serovar Hardjo are considered to be seropositive (Balamurugan *et al*. 2016). Infection with host-adapted serovars produced subclinical infection with apparently healthy animals serving as chronic carriers and persistent shedders of the organism through their urine, body fluid or tissue (Balamurugan *et al.* 2013, 2016). They pose a risk and source of infection to livestock owners, farm workers, occupational workers, etc. Leptospirosis seroprevalence was higher in some farms which might be due to introduction of unscreened new animals into farm or intensive management practices or apparently healthy seropositive animals may be shedding *Leptospira* and they serve as source of infection to other animals and humans, since vaccination against leptospirosis in cattle is not practiced in India and higher percentage positivity in all age groups indicate natural circulation of *Leptospira* in the population.

In conclusion, significant prevalence of *Leptospira* Hardjo serovar in the organised dairy farms of India proves the endemicity of this serovar in dairy cattle. The presence of this agent in the dairy farm which may be a potential zoonotic risk to animal hadlers, milkers, and other domestic species in the farm. This study also determines the need of an intensive control and surveillance of the disease burden

in animals and humans in close proximity to each other to combat or reduce zoonotic infection.

## SUMMARY

The investigation was carried out to know prevalence of *Leptospira* serovar Hardjo in organised cattle dairy farms of India by using the commercial *Leptospira* Bovine Hardjo ELISA kit. Cattle serum samples (964) collected from different organized farm in various states, viz. Maharastra, Gurajat, Punjab, Tamil Nadu, Haryana, Telangana, Jharkhand, Chhattisgarh and Karnataka, were tested. The overall seroprevalence of 12.7% was observed with high prevalence of 30.4% in Maharashtra and least with 3.7% in Punjab. The results revealed that seroprevalance is not independent across the states for the samples with the history of abortion/reproductive/respiratory disorders ($\chi^2$, 11.64), abortion/reproductive ($\chi^2$, 49.11), abortion/respiratory ($\chi^2$, 11.21) and apparently healthy animals ($\chi^2$, 37.65). However, across the disease history revealed that the seroprevalence is not independent ($\chi^2$, 95.62) and seroprevalence is independent across the age ($\chi^2$, 0.58). This study supported that cattle have a role in maintaining a well-known reservoir for *Leptospira* Hardjo serovar and warrants an intensive control and surveillance programme for reducing leptospirosis in organised cattle dairy farms in India.

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