Original Research Article

https://doi.org/10.20546/ijcmas.2017.611.156

Seroprevalence of *Leptospira hardjo* in Cattle of Gujarat, India

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**Abstract**

The aim of this study was to determine prevalence of *Leptospira interrogans* serovar hardjo in the define area. To serve the purpose a total 398 serum samples were collected from different age group, breed and sex of cattle. Out of 398, some of cattle (101) showed history of abortion, mastitis/agalactia/oligolactia, repeat breeder and fever. These samples were screened by I-ELISA kit which detect antibody directed against *L. hardjo*. The distribution of serovar hardjo was significantly differed between different districts (Navsari, Tapi, Valsad, Surat) of South Gujarat. The highest seroprevalence of *L. hardjo* was found in Valsad district (21.56 %) followed by Surat (6.89 %), Tapi (3.30 %) and Navsari (3.04 %). However, there was statistically insignificant difference was observed in distribution of serovar hardjo between different breeds, age and sex of cattle.

**Keywords**

I-ELISA, Leptospirosis, *L. hardjo*, Seroprevalence, Weather data.

**Introduction**

Leptospirosis is a zoonotic bacterial disease with global distribution. It is caused by any one or more than one serovars of about 260 serovars belonging to 23 serogroups (serotypes) of pathogenic species *Leptospira interrogans* (Adler and Pena Moctezuma, 2010).

Globally a number of serovars are recognized but only a limited numbers are usually endemic to a particular region (Angeliki, 2010). *Leptospira* is associated with infertility, early embryonic death and agalactia/oligolactia/mastitis. Leptosiral serovar hardjo, pomona and grippotyphosa are implicated in bovine abortion leads to heavy economic losses of dairy farmers.

Among different leptospiral serovar, hardjo serovar is considered the most frequent and important serovar (Radostitis et al., 2007). So, in present study researcher aimed to know prevalence of this specific serovar in cattle of Gujarat, India.
Materials and Methods

Collection of blood/serum samples

A total of 398 blood/serum samples were collected randomly from clinically ailing (cattle = 101) and apparently healthy (cattle = 297) cattle of both sex reared in villages of various districts (Navsari, Surat, Tapi, Valsad) of South Gujarat. Whole blood samples were collected from jugular vein directly in sterile 9.0 ml plain vacutainers. To obtain serum whole blood was kept in slanting position in 9.0 ml plain vacutainers until serum was extracted out of the whole blood. The 9.0 ml plain vacutainers were centrifuged at 7000 rpm for 10 minutes. The clear straw coloured serum was collected into 1.5 ml sterile cryo vials and kept at -20°C until use.

I-ELISA Test

A commercial indirect ELISA kit (M/S: The Cypress, Ref.VB066/ 480 tests/kit) was used for detection of antibodies against *Leptospira interrogans* serovar hardjo (*L. hardjo*) in serum. Indirect Elisa was performed strictly as per the protocol outlined in the user’s manual supplied with the kit. Then read the optical densities in the microwells using a micro plate reader at a wavelength of 450 nm within 15 minutes of stopping the colour development. ELISA optical density (OD) readings were transformed to serum/positive percentage (PP) according to specific equation cited by manufacture.

Statistical analysis

Chi-square test was used at 95 % confidence interval according to WEB AGRI STAT PACKAGE software developed by Jangam and Wadekar, ICAR research complex, Goa for statistical analysis of data (Jangam and Wadekar, 2012).

Results and Discussion

In the present study, distribution of serovar hardjo between different districts of South Gujarat was statistically significant (Table 1). A total of 398 samples tested from South Gujarat, 23 (5.77 %) were found to be positive against hardjo specific antibody (Figure 1). In Valsad district seroprevalence of *L. hardjo* was found highest (21.56 %) followed by Surat (6.89 %), Tapi (3.30 %) and Navsari (3.04 %). There was statistically insignificant difference was observed in distribution of serovar Hardjo between different breeds, age and sex of cattle (Table 2). Serovar hardjo infecting cattle shown history of abortion, mastitis/agalactia/oligolactia, repeat breeder and fever (Table 3).

In the present study seroprevalence of serovar hardjo was found to be 5.77 per cent and supported the findings of Savalia, 2001 and Balakrishnan *et al.,* (2011) who too reported serovar hardjo was most prevalent in Gujarat. In the same line serovar hardjo also predominated in different states of India, such as Uttaranchal (Agrawal *et al.,* 2005) and West Bengal (Mandal *et al.,* 2008) among cattle. Moreover, in some of the countries also serovar hardjo was found to be most prevalent i.e. in Arizona, USA (Songer *et al.,* 1983), Malaysia (Bahamani *et al.,* 1987; El Jalili, 2008), Turkish (Kocabiyyik and Cetin, 2004), Iraq (Al-Badrawi *et al.,* 2010) and Brazil (Mineiro *et al.,* 2011), however in few of the countries like in Mexico serovar tarassovi (Cárdenas-Marrufo *et al.,* 2011) and in Iran serovar canicola (Bahari *et al.,* 2011), icthyoaemorhagiae (Sakhaee *et al.,* 2007) were most prevalent. So prevalence of leptospiral serovars are varies from country to country and depends upon weather condition, rainfall, humidity, presence of carrier animals and soil components (Himani *et al.,* 2013). Highest seroprevalence in Valsad attributed to
location (temperate zone) of district and comparatively higher rainfall as compare to other district of South Gujarat (Table 4). In the present study we could not found significant difference in distribution of hardjo serovar in different exotic, indigenous and cross breed of cattle.

In the contrast to this Balakrishnan et al., (2011) noted that exotic pure breeds are more susceptible followed by indigenous pure breeds and cross breeds with different leptospiral serovar infection. Further, Agrawal et al., (2005) found hardjobovis serovar only in indigenous breeds of cattle.

**Table 1** Seroprevalence of leptospirosis in different district of South Gujarat among cattle using ELISA

| Attributes            | Districts          | Total |
|-----------------------|--------------------|-------|
|                       | Navsari | Valsad | Tapi | Surat |       |
| No. of Tested         | 197     | 51     | 121  | 29    | 398   |
| No. of Positive       | 06      | 11     | 04   | 02    | 23    |
| Percent Positive      | 3.04    | 21.56  | 3.30 | 6.89  | 5.77  |
| $\chi^2$=7.82 *(P<0.05) |

Note: NS-Non significant at P < 0.05 * - Significant at P < 0.05

**Table 2** Seroprevalence of leptospirosis in different district, breed, sex and age of cattle

| Attributes            | No. of Tested | No. of Positive | Percent Positive |
|-----------------------|---------------|-----------------|------------------|
| Districts             |               |                 |                  |
| Navsari               | 197           | 06              | 3.04             |
| Valsad                | 51            | 11              | 21.56            |
| Tapi                  | 121           | 04              | 3.30             |
| Surat                 | 29            | 02              | 6.89             |
| Total                 | 398           | 23              | 5.77             |
| $\chi^2$= 11.07 $^{NS}$ (P<0.05) |

**Breed wise**

| Breed     | No. of Tested | No. of Positive | Percent Positive |
|-----------|---------------|-----------------|------------------|
| Holstein  | 65            | 03              | 4.61             |
| Friesian  |               |                 |                  |
| Jersey    | 26            | 02              | 7.69             |
| Gir       | 36            | 03              | 8.33             |
| HF Cross  | 242           | 14              | 5.78             |
| Jersey Cross | 16    | 01              | 6.25             |
| Gir Cross | 13            | 00              | 00               |
| Total     | 398           | 23              | 5.77             |
| $\chi^2$ = 3.84 $^{NS}$ (P<0.05) |

**Sex wise**

| Sex   | No. of Tested | No. of Positive | Percent Positive |
|-------|---------------|-----------------|------------------|
| Male  | 35            | 01              | 2.85             |
| Female| 363           | 22              | 6.06             |
| Total | 398           | 23              | 5.77             |
| $\chi^2$ = 5.99 $^{NS}$ (P<0.05) |

**Age wise**

| Age       | No. of Tested | No. of Positive | Percent Positive |
|-----------|---------------|-----------------|------------------|
| <1 year   | 35            | 00              | 00.00            |
| 1-4 years | 124           | 08              | 6.45             |
| >4 years  | 239           | 15              | 6.27             |
| Total     | 398           | 23              | 5.77             |
| $\chi^2$ = 5.99 $^{NS}$ (P<0.05) |

Note: NS-Non significant at P < 0.05 * - Significant at P < 0.05
Table 4 Monthly average of weather data of sample collection period from June, 2012 to May, 2013 of four districts (Navsari, Surat, Tapi and Valsad)

|       | Navsari |          | Rainfall (mm) |          | Rainfall (mm) |          | Rainfall (mm) |          | Rainfall (mm) |
|-------|---------|----------|---------------|----------|---------------|----------|---------------|----------|---------------|
|       | RH (%)  | Temp. (°C)|               | RH (%)  | Temp. (°C) | Rainfall (mm) | RH (%)  | Temp. (°C) | Rainfall (mm) | RH (%)  | Temp. (°C) | Rainfall (mm) |
| Jun-12| 84.4    | 30.2     | 155.0         | 77.1    | 30.8        | 86.0     | 64.50         | 26.9     | 8.00          | 79.7    | 29.8     | 139.4         |
| Jul-12| 92.1    | 28.5     | 271.0         | 85.7    | 29.2        | 185.6    | 79.00         | 25.6     | 262           | 92.9    | 28.0     | 577.6         |
| Aug-12| 92.4    | 27.8     | 200.0         | 83.7    | 28.6        | 127.6    | 86.20         | 26.0     | 275           | 94.1    | 27.4     | 348.1         |
| Sep-12| 93.0    | 27.4     | 618.0         | 87.5    | 27.5        | 376.2    | 87.50         | 25.3     | 240           | 94.0    | 27.2     | 512.9         |
| Oct-12| 80.0    | 28.5     | 12.0          | 74.8    | 27.3        | 0.0      | 80.00         | 25.9     | 11.00         | 84.1    | 27.9     | 19.2          |
| Nov-12| 72.8    | 24.7     | 0.0           | 69.4    | 26.7        | 0.0      | 74.50         | 25.7     | 0.0           | 44.8    | 12.4     | 0.0           |
| Dec-12| 71.9    | 24.1     | 0.0           | 70.1    | 24.2        | 0.0      | 60.90         | 22.7     | 0.0           | 89.7    | 22.6     | 0.0           |
| Jan-13| 81.6    | 21.0     | 0.0           | 68.3    | 22.8        | 0.0      | 68.60         | 19.3     | 0.0           | 89.3    | 19.9     | 0.0           |
| Feb-13| 71.5    | 23.1     | 0.0           | 57.5    | 23.5        | 0.0      | 62.30         | 24.2     | 0.0           | 81.0    | 21.8     | 0.0           |
| Mar-13| 72.9    | 26.1     | 0.0           | 66.5    | 27.0        | 0.0      | 65.65         | 25.3     | 0.0           | 78.1    | 25.4     | 0.0           |
| Apr-13| 84.2    | 28.4     | 0.1           | 83.2    | 28.8        | 0.0      | 62.05         | 25.6     | 0.0           | 79.5    | 27.3     | 13.1          |
| May-13| 84.3    | 30.8     | 0.4           | 84.3    | 30.9        | 0.0      | 61.75         | 25.4     | 0.0           | 75.3    | 29.8     | 0.0           |
| Average| 81.76  | 26.78    | 251.20        | 75.68   | 27.28       | 155.08   | 71.08         | 24.81    | 159.20        | 81.88   | 24.94    | 319.44        |

RH*: Relative Humidity Temp**: Temperature
**Table 3** Prevalence of *Leptospira* serovar *hardjo* in clinically ailing and apparently healthy cattle

| Sr. No. | Particulars                      | Total cases | Seropositive cases |
|---------|----------------------------------|-------------|--------------------|
| 1.      | Clinically ailing animals        | 101         | 9 (8.91%)          |
|         | a. Mastitis/Agalactia/Oligolactia| 25          | 3 (12.00%)         |
|         | b. Abortion                      | 15          | 3 (20.00%)         |
|         | c. Repet breeder                 | 17          | 1 (5.88%)          |
|         | d. Fever                         | 27          | 2 (7.40%)          |
|         | e. Anorexia                       | 17          | 0                  |
| 2.      | Apparently healthy               | 297         | 14 (4.71%)         |
|         | **Total (1.to 2.)**              | **398**     | **23 (5.77%)**     |

**Fig. 1** ELISA module showing positive and negative reactions for *Leptospira* antibodies Well A1 as S : Substrate blank, Well B1 and B2 as NC: Negative control, Well C1 and C2 as PC : Positive control, Rest all wells: Field serum samples

Sex-wise seroprevalence did not differ significantly (P≤0.05) in the study. This could possibly be due to number of sample tested from male and female cattle and supported the observations of Shafighi *et al.*, (2010) who too did not observed any sex bias in respect of seropositivity against different leptospiral serovar. Age-wise seroprevalence also did not revealed significant difference between different age group of cattle. In the contrast of present findings Health and Johnson (1994) concluded that proportion of seropositivity of serovar hardjo increase with age of cattle.

Serovar hardjo is considered as host adapted serovar of cattle though in this study it was
also reported in clinically ailing cattle having history of abortion, mastitis/agalactia/oligolactia, repeat breeder and fever in different percent combination and supported the findings of Momtazand Moshkelani (2012) who too reported *L. hardjo* is a major pathogen causing bovine abortion in Iran. Further, Ellis *et al.*, (1986) observed full range of clinical signs including abortion, mummification, still birth, premature birth/weak calf birth and also full term birth of live apparently healthy calves in experimental study of leptospiral serovar hardjo in cattle. So in *L. hardjo* infection, cattle remain apparently healthy or a range of clinical signs of reproductive/systemic involvement in different combination.

**Acknowledgements**

Authors are thankful to the Dean and Principle, Vanbandhu College of Veterinary Sciences and A.H., Navsari Agricultural University, Eru cross road, Navsari, Gujarat, India for providing the necessary facilities to carry out this work.

**Conflict of interest statement**

Authors declare that they have no conflict of interest.

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How to cite this article:
Patel, J.M., M.C. Prasad, P.D. Vihol, I.H. Kalyani, M.G. Prajapati, H.C. Parmar, R.D. Varia and Patel, K.M. 2017. Seroprevalence of Leptospira hardjo in Cattle of Gujarat, India. Int.J.Curr.Microbiol.App.Sci. 6(11): 1304-1310. doi: https://doi.org/10.20546/ijcmas.2017.611.156