

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

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### ABSTRACT

#### Keywords

I-ELISA,  
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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.

### 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 andagalactia/oligolactia/mastitis. Leptospiral 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 (Radostits *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 (Bahaman *et al.*, 1987; El Jalii, 2008), Turkish (Kocabiyik 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), ictrohaemorrhagiae (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 hardjovovis 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	
<b>No. of Tested</b>	197	51	121	29	398
<b>No. of Positive</b>	06	11	04	02	23
<b>Percent Positive</b>	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
<b>Districts</b>			
Navsari	197	06	3.04
Valsad	51	11	21.56
Tapi	121	04	3.30
Surat	29	02	6.89
<b>Total</b>	<b>398</b>	<b>23</b>	<b>5.77</b>
$\chi^2 = 7.82$ *(P<0.05)			
<b>Breed wise</b>			
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
<b>Total</b>	<b>398</b>	<b>23</b>	<b>5.77</b>
$\chi^2 = 11.07$ <sup>NS</sup> (P<0.05)			
<b>Sex wise</b>			
Male	35	01	2.85
Female	363	22	6.06
<b>Total</b>	<b>398</b>	<b>23</b>	<b>5.77</b>
$\chi^2 = 3.84$ <sup>NS</sup> (P<0.05)			
<b>Age wise</b>			
<1 year	35	00	00.00
1-4 years	124	08	6.45
>4 years	239	15	6.27
<b>Total</b>	<b>398</b>	<b>23</b>	<b>5.77</b>
$\chi^2 = 5.99$ <sup>NS</sup> (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)

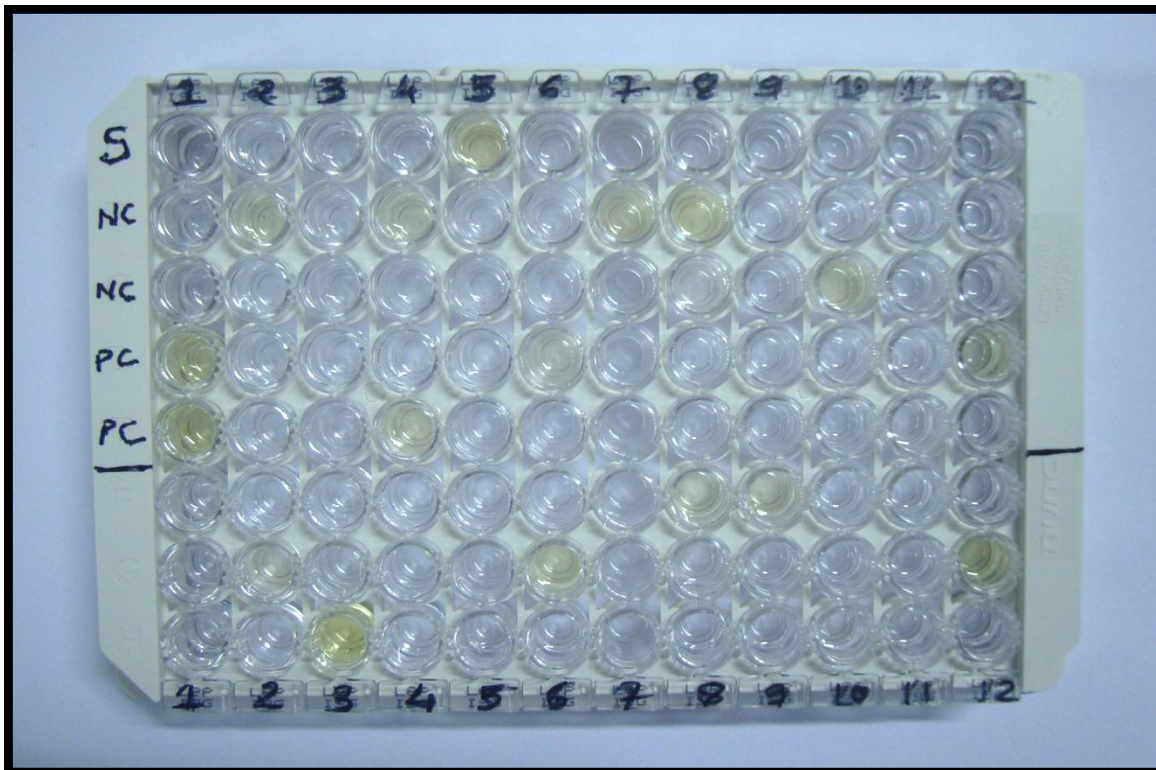
	Navsari			Surat			Tapi			Valsad		
	RH (%)	Temp. (°C)	Rain fall (mm)	RH (%)	Temp. (°C)	Rain fall (mm)	RH (%)	Temp. (°C)	Rain fall (mm)	RH (%)	Temp. (°C)	Rain fall (mm)
<b>Jun-12</b>	84.4	30.2	155.0	77.1	30.8	86.0	64.50	26.9	8.00	79.7	29.8	139.4
<b>Jul-12</b>	92.1	28.5	271.0	85.7	29.2	185.6	79.00	25.6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

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%)
	<b>Total (1.to 2.)</b>	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 ( $P \leq 0.05$ ) 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.

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### Conflict of interest statement

Authors declare that they have no conflict of interest.

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