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REGULAR ARTICLES

Prevalence of *Leptospira* serogroup-specific antibodies in cattle associated with reproductive problems in endemic states of India

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Abstract

In this study, the seroprevalence and distribution of *Leptospira* in dairy cattle in endemic states of India were investigated in association with reproductive problems of the cattle. A total of 373 cattle serum samples from 45 farms in Maharashtra, Gujarat, Andhra Pradesh, Telangana, Karnataka, Tamil Nadu, Punjab, Haryana, Chhattisgarh, Sikkim and Uttarakhand states were collected from animals with a history of reproductive disorders like abortion, repeat breeding, anoestrus and endometritis, and also from apparently healthy animals. These samples were screened for *Leptospira* serogroup-specific antibodies by microscopic agglutination test (MAT) using a panel of 18 live reference serovar antigens. The seropositivity of 70.51% (263/373, 95% CI 0.65 to 0.75) was associated with reproductive problems ($\chi^2 = 55.71$, $p < 0.01$) and sampled states ($\chi^2 = 32.99$, $p < 0.01$) and independent of apparently healthy animals ($\chi^2 = 15.6$, $p > 0.10$) and age groups of cattle ($\chi^2 = 0.91$, $p > 0.10$). Further, the odds (risk-relation) of reproductive disorders was 5.29 compared to apparently healthy animals (0.25 odds). The frequency distribution of predominant serogroup-specific *Leptospira* antibodies were determined against the serovars: Hardjo (27.76%), Pyrogenes (18.63%), Canicola and Javanica (17.49%), Hebdomadis (17.11%), Shermani and Panama (16.73%), Djasiman (16.35%), Tarassovi, Grippotyphosa and Pomona (15.97%), Icterohaemorrhagiae (15.59%), Copenhageni (14.83%), Australis (13.69%), Kaup and Hurstbridge (10.65%), Bankinang (10.27%) and Bataviae (9.51%). In conclusion, dairy cattle have a role in maintaining important several serovars besides well-known Hardjo serovar in endemic states of India and warrant mitigating measures to reduce the incidence of cattle leptospirosis including need for an intensive surveillance programme, preventive vaccination and control strategies.

Keywords Leptospirosis · Cattle reproductive disorders · Serogroup antibody distribution · Seroprevalence · MAT

Introduction

Leptospirosis is a zoonotic disease with ubiquitous distribution, caused by infection with pathogenic *Leptospira* species. It is a rapidly emerging disease affecting the health of domestic animals and humans in most countries with tropical and sub-tropical climates (Vijayachari et al. 2008). Despite being so severe, this disease is neglected in most endemic countries in the world because of a lack of information and awareness about the extent of the problem. The disease affects a variety of domestic animals viz. cattle, buffalo, goats, sheep, horse and swine resulting in heavy economic losses to the farming community on account of reproductive problems (Srivastava 2008). The leptospirosis situation in India is a cause of concern, and it is endemic in all Southern states as well as in coastal states like Gujarat, Maharashtra, including Andaman and Nicobar Islands of India, where high prevalence was recorded both in animals and humans.

Leptospirosis in cattle and water buffaloes causes direct or indirect economic losses which include costs of abortion, early embryonic death, stillbirth, infertility, birth of weak

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calves, failure to thrive, mastitis, 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 more common in cattle and buffaloes. However, in subclinical infection, cattle show asymptomatic signs with chronic renal colonization of organisms and shedding in urine, infertility due to bacterial colonization in reproductive tract and occasionally abortion, whereas in clinical infection in calves, outbreaks of clinical diseases with haematuria and death could be observed some times. The presence of the complexity in the case of identification need for early diagnosis and lack of diagnostic facilities hinders diagnosis. Hence, leptospirosis is frequently under diagnosed because of the nonspecific symptoms and the difficulty of performing both culture and the international reference serological test, microscopic agglutination test (MAT) (WHO 2011; OIE 2013).

In India, a comprehensive study covering wider geographical locations on the prevalence of leptospirosis in cattle especially in dairy farms is lacking except few isolated location-specific reports (Balamurugan et al. 2016b). Generally, in organized or unorganized farms, the abortions of animals in various stages of gestation more specifically due to leptospirosis are over looked, and the studies on level of infection in animals in the farming sector throw light on the status of the disease per se, which in turn help in early diagnosis and subsequent prompt treatment, prevention and its control including zoonotic transmission to humans.

The prevalence study is imperative in order to develop the appropriate long-term prevention and control strategies, including implementation of vaccination against diseases in the farm animals as well as to implement mitigating measures to reduce the incidence of the leptospirosis in animals and humans (Balamurugan et al. 2016b) as the number of *Leptospira* serovars and serogroups constantly increasing due to new strains isolation from animals, humans and the environment (Levett 2001). Generally, cattle are the maintenance host for serovar Hardjo, which consist of two serologically indistinguishable but genetically distinct species. Further, serovars causing infection in cattle have been classified into two groups: (a) those adapted to and maintained by other cattle (Hardjo) and (b) incidental infections caused by strains maintained by other domestic and free-living animals. For effective prevention of the leptospirosis in cattle and water buffalo dairy farms, vaccine should contain specific *Leptospira* serovars prevalent in that particular geographical area, in order to reduce or prevent the abortions and other reproductive disorders caused by *Leptospires* and eliminate the carrier status of animals by preventing the colonization of *Leptospires* in the kidney tubules, from which the spread of infection occurs through urine by contaminations. This can be achieved only through regular systematic yearly vaccination of farm animals with inactivated vaccine containing multiple major prevalent serovars to make the population immune against leptospirosis. This in turn reduces the zoonotic potential of disease in the farm workers and contact personnel besides preventing the periodical *Leptospira* abortions occurrence in the dairy farms. Hence, investigation was undertaken to know the status of *Leptospira* serogroup-specific antibody distribution and its prevalence in dairy cattle associated with reproductive disorders in endemic states of India.

Materials and methods

A total of 373 cattle serum samples from 45 dairy farms in various endemic areas of different states (Maharashtra, Gujarat, Punjab, Tamil Nadu, Haryana, Telangana, Jharkhand, Chhattisgarh and Karnataka, Andhra Pradesh, Sikkim, Himachal Pradesh and Uttarakhand) of India were collected by Veterinary Officers from animals with a history of reproductive disorders like abortion, repeat breeders, anoestrus, endometritis and also from apparently healthy animals with a status of non-vaccinated against leptospirosis during survey period of 2015–2016 (Table 1). These samples were from either randomly selected herd with history of reproductive disorders and collected by ICAR-NIVEDI teams or suspected samples from the farm submitted for either screening or diagnosis purpose with the same history. Collected serum samples were transported on ice to the ICAR-NIVEDI for testing, and upon arrival, the samples were stored at − 20 °C until further use.

All these samples were screened for *Leptospira* serogroup-specific antibodies by MAT at 1:100 dilution. Ellinghausen McCullough Johnson and Harris (EMJH) liquid medium was prepared and used for the propagation of *Leptospira* reference serovar cultures (Balamurugan et al. 2016a, 2017). MAT was performed as reported earlier (OIE 2013) using a panel of 18 live reference *Leptospira* serovar antigens (Australis, Bankinang, Canicola, Hardjo, Hebdomadis, Pyrogenes, Tarassovi, Icterohaemorrhagiae, Pomona, Shermani, Kaup, Grippotyphosa, Hurstbridge, Javanica, Panama, Djasiman, Copenhageni and Bataviae) obtained from WHO National Reference Laboratory, Indian Council of Medical Research (ICMR)-Regional Medical Research Centre (RMRC), Port Blair, and being maintained in *Leptospira* Laboratory, ICAR-NIVEDI, Bengaluru, for the presence of specific *Leptospira* antibodies as described earlier (Balamurugan et al. 2017), and a MAT titre of 1:100 is taken as positive reactor as per WHO/OIE manual for leptospirosis (WHO 2011; OIE 2013).

Table 1 Details of the cattle samples screened and its test results for leptospirosis

State	Total no. of samples	Total no. of farms	Samples with history of reproductive disorders	Apparently healthy samples	No. of samples reacted	Percentage positivity
Maharashtra	35	10	26	9	28	80.00
Gujarat	24	7	18	6	15	62.50
Punjab	6	5	4	2	3	50.00
Tamil Nadu	59	6	53	6	39	66.10
Haryana	8	5	7	1	6	75.00
Telangana	30	3	18	12	21	70.00
Jharkhand	10	1	6	4	5	50.00
Karnataka	59	3	55	4	26	44.07
Chhattisgarh	15	1	6	9	12	80.00
Andhra Pradesh	20	1	19	1	19	95.00
Sikkim	80	1	80	0	62	77.50
Himachal Pradesh	5	1	5	0	5	100.00
Uttarakhand	22	1	22	0	22	100.00
Total	373	45	319	54	263	70.51
χ^2 value			55.71*	15.6^{NS}	32.99*	

NS non-significance

*Significance at 1% level

Chi-square test was used for testing the independence of seropositivity of leptospirosis across age groups and across the sampled regions (Snedecor and Cochran 1989). The null hypothesis (H_0) is independent of seropositivity with specific disease history, age groups and study regions (vs H_1 dependent). Further, odds (risk-relation), risk of disease history status and apparently healthy status associated with seropositivity was compared statistically with β estimate, at 95% confidence interval (CI), with p value (< 0.05). The estimation of apparent prevalence with 95% CI and statistical data analysis were carried out using Microsoft Office Excel 2013 and SPSS version 22 (IBM), India.

Results

Out of total 373 sera tested, 263 samples found to be reactive in MAT representing 70.51% seropositivity (263/373, 95% CI 0.65 to 0.75) in dairy cattle, of which 20 samples from apparently healthy animal and 243 samples from cattle associated with reproductive problems showed reactivity with different serovars. The details of samples screened and its test results are presented in Table 1. It is evident from the pooled chi-square analysis that the seroprevalence of leptospirosis in cattle across the disease history is significantly dependent ($\chi^2 = 55.71$, $p < 0.01$) and sampled states ($\chi^2 = 32.99$, $p < 0.01$). However, seropositivity is independent across the age groups of cattle ($\chi^2 = 0.91$, $p > 0.10$) (Table 2) and apparently healthy animals ($\chi^2 = 15.6$, $p > 0.10$) (Table 1). Out of total 263 reactors in MAT, 186 samples showed reaction with multiple serovars representing 70.72% prevalence. The cross-reactivity observed between the different serovars tested is shown in Tables 3 and 4. Further, the odds (risk-relation) of reproductive disorders was 5.29 compared to apparently healthy animals (0.25 odds) (Table 5). The frequency distribution of predominant serogroup-specific *Leptospira* antibodies was determined against the serovars—Hardjo (27.76%), Pyrogenes (18.63%), Canicola and Javanica (17.49%),

Table 2 Age-wise prevalence of *Leptospira* serogroup-specific antibodies in cattle

Age of cattle (years)	Total No. of samples screened	No. of reactive samples	Percentage positivity	Confidence interval at 95%
< 2 years	89	64	71.91	62.5–81.3
2 to 5 years	125	91	72.80	65.0–80.6
> 5 years	159	108	67.92	60.7–75.2
Total	373	263	70.51	65.9–75.1
	χ^2 value	$0.91^{\text{Non-significance } (p > 0.10)}$		

Table 3 Cross-reactivity amongst different *Leptospira* serovars in cattle

Serovars	Aus	Ban	Can	Had	Heb	Ict	Pyr	Tar	Pom	She	Kau	Gri	Hus	Jav	Pan	Dja	Cop	Bat
Aus	–	0	11	12	11	10	3	7	9	9	9	1	5	6	7	8	6	5
Ban	–	–	2	4	1	4	6	3	3	3	2	4	3	7	6	2	1	0
Can	–	–	–	8	10	11	9	17	12	14	10	8	5	6	9	10	4	8
Had	–	–	–	–	12	9	10	9	10	9	5	10	6	6	9	8	9	6
Heb	–	–	–	–	–	11	14	16	8	12	14	5	4	8	5	16	5	6
Ict	–	–	–	–	–	–	2	12	13	12	9	4	5	5	3	8	4	9
Pyr	–	–	–	–	–	–	–	11	7	12	9	6	7	8	11	9	11	9
Tar	–	–	–	–	–	–	–	–	11	16	12	3	5	3	12	11	4	12
Pom	–	–	–	–	–	–	–	–	–	12	5	3	3	4	9	9	2	9
She	–	–	–	–	–	–	–	–	–	–	16	6	8	8	15	14	2	14
Kau	–	–	–	–	–	–	–	–	–	–	–	5	7	6	6	9	2	6
Gri	–	–	–	–	–	–	–	–	–	–	–	–	8	7	4	5	3	1
Hus	–	–	–	–	–	–	–	–	–	–	–	–	–	11	12	0	6	3
Jav	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11	7	9	2
Pan	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	6	10
Dja	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6	6
Cop	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
Bat	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

The majority of cross-reactive serovars are shown in Table 4

Aus Australis, *Ban* Bankinang, *Can* Canicola, *Had* Hardjo, *Heb* Hebdomadis, *Ict* Icterohaemorrhagiae, *Pyr* Pyrogenes, *Tar* Tarassovi, *Pom* Pomona, *She* Shermani, *Kau* Kaup, *Gri* Grippotyphosa, *Hus* Hurstbridge, *Jav* Javanica, *Pan* Panama, *Dja* Djasiman, *Cop* Copenhageni, *Bat* Bataviae. The majority of cross-reactive serovars are shown in separate Table 4

Hebdomadis (17.11%), Shermani and Panama (16.73%), Djasiman (16.35%), Tarassovi, Grippotyphosa and Pomona (15.97%), Icterohaemorrhagiae (15.59%), Copenhageni (14.83%), Australis (13.69%), Kaup and Hurstbridge (10.65%), Bankinang (10.27%) and Bataviae (9.51%)—and are summarized in Table 6. Distribution of reactive pathogenic serovars in different states is presented in Table 7. Further, the number of seropositive samples to intermediate and other *Leptospira* spp. serovars tested in MAT is summarized in Table 8. State-wise coverage of the positive reactive samples with selected panel of serovars was tabulated (Table 9).

Discussion

It is evident from the analysis that the seroprevalence across the disease history is significantly dependent with odds, indicating that animal with history of reproductive disorders has more probability of having leptospirosis than in apparently healthy animals. Besides being an important cause of cattle abortion, reduced fertility and agalactia, serovar Hardjo also poses a potential zoonotic threat to humans who are exposed to infected cattle. Significant percentage of animals that are actively infected with *Leptospira* spp. serovar are shedding

Table 4 Major selected reacted serovars versus cross-reacted serovars

Major selected reacted serovars	Cross-reacted serovars
Hardjo	Australis, Hebdomadis, Pyrogenes, Pomona, Grippotyphosa
Pyrogenes	Hebdomadis, Hardjo, Shermani, Tarassovi, Panama, Copenhageni
Canicola	Tarassovi, Shermani, Pomona, Australis, Icterohaemorrhagiae, Hebdomadis
Javanica	Hurstbridge, Panama, Copenhageni, Pyrogenes, Hebdomadis, Shermani
Hebdomadis	Tarassovi, Djasiman, Kaup, Hardjo, Shermani, Icterohaemorrhagiae, Canicola
Pomona	Icterohaemorrhagiae, Canicola, Shermani, Tarassovi, Hardjo
Icterohaemorrhagiae	Pomona, Shermani, Tarassovi, Hebdomadis, Canicola, Australis, Hardjo, Kaup
Total cross-reacted serovars	Australis, Grippotyphosa, Shermani, Tarassovi, Panama, Copenhageni, Hurstbridge, Djasiman, Kaup

Table 5 Odds ratio test of seropositive reaction versus history of disease status

Test compared with different groups of animals	Odds ratio	Confidence interval		*p* value
		Lower limit	Upper limit	
Reproductive disorders	5.296	2.882	9.731	< 0.001
Apparently healthy	0.246	0.136	0.446	< 0.001

Leptospires and have antibody titres ≥ 100 against serovar Hardjo that are considered to be seropositive (Balamurugan et al. 2016a, 2017). Infection with host-adapted serovars has been reported to produce 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, 2016a), which may act as source and pose a potential risk to livestock owners, farm workers, occupational workers, other domestic species in the farm, etc. (Balamurugan et al. 2013). It was found that seroprevalence was higher in some farms which might be due to exposure of infection from newly introduced unscreened animals into the farm or intensive farm management practices, or apparently healthy seropositive status animals may be shedding *Leptospirae* and that may serve as source of infection to others, since vaccination against leptospirosis in cattle is not regularly practiced in India and higher percentage positivity in all age groups indicates natural circulation of *Leptospira* in the farms.

Further, the results are dependent with the history of disease status for the sampled regions, and the studies of cattle leptospirosis in different parts of the globe also indicated that serovars responsible for reproductive losses vary depending on which serovars are locally endemic. Seropositivity in apparently healthy animals also indicates that animals were exposed or harbouring *Leptospira* organism, irrespective of the history. Further, seropositivity is independent across the age group of cattle indicating that leptospirosis affects all the age group of animals. The most prevalent predominant *Leptospira* antibodies amongst the reacted samples were against serovars Hardjo, Pyrogenes, Canicola, Javanica, Hebdomadis, Pomona, Icterohaemorrhagiae, etc. The observed majority of the cross-reactivity was between these aforesaid major reacted serovars with other serovars Australis, Grippotyphosa, Shermani, Tarassovi, Panama, Copenhageni, Hurstbridge, Djasiman, Kaup, etc. Further, on analysis of frequency distribution of major serovars, Hardjo showed the highest positive reactions followed by Pyrogenes; Canicola and Javanica; Hebdomadis; Shermani and Panama; Djasiman, Tarassovi, Grippotyphosa and Pomona; Icterohaemorrhagiae; Copenhageni; Australis; Kaup and Hurstbridge; Bankinang; and Bataviae. Out of 186 multiple serovars reacted sera, 168 samples have reacted with

Table 6 Frequency distribution of reacted pathogenic serovars (descending order)

Serovars	Overall samples reactivity	Percentage reactivity (%)	Animals with reproductive disorders	Percentage reactivity (%)	Apparently healthy animals	Percentage reactivity (%)
Hardjo	73	27.76	65	26.74	8	40
Pyrogenes	49	18.63	48	19.75	1	5
Canicola	46	17.49	44	18.11	2	10
Javanica	46	17.49	42	17.28	4	20
Hebdomadis	45	17.11	42	17.28	3	15
Shermani	44	16.73	44	18.11	0	0
Panama	44	16.73	39	16.05	5	25
Djasiman	43	16.35	42	17.28	1	5
Tarassovi	42	15.97	38	15.64	4	20
Pomona	42	15.97	42	17.28	0	0
Grippotyphosa	42	15.97	37	15.23	5	25
Icterohaemorrhagiae	41	15.59	39	16.05	2	10
Copenhageni	39	14.83	37	15.23	2	10
Australis	36	13.69	34	13.99	2	10
Kaup	28	10.65	28	11.52	0	0
Hurstbridge	28	10.65	24	9.88	4	20
Bankinang	27	10.27	24	9.88	3	15
Bataviae	25	9.51	25	10.29	0	0

Table 7 State-wise distribution of reacted pathogenic serovars in cattle

State	Reacted serovars in the panel of microscopic agglutination test
Maharashtra	Copenhageni, Tarassovi, Panama, Djasiman, Autumnalis, Pyrogenes, Javanica, Canicola, Grippotyphosa, Australis, Hardjo, Shermani
Gujarat	Pyrogenes, Hurstbridge, Javanica, Copenhageni, Hardjo, Hebdomadis, Australis, Grippotyphosa, Icterohaemorrhagiae
Punjab	Australis, Bankinang, Hardjo, Icterohaemorrhagiae, Hurstbridge, Javanica, Copenhageni, Grippotyphosa
Tamil Nadu	Hardjo, Hurstbridge, Shermani, Canicola, Bankinang, Australis, Tarassovi, Pyrogenes, Panama, Djasiman
Haryana	Hurstbridge, Panama, Hardjo, Hebdomadis, Australis, Bankinang, Tarassovi, Djasiman, Copenhageni
Telangana	Javanica, Panama, Hebdomadis, Hardjo, Hurstbridge, Bankinang, Icterohaemorrhagiae, Copenhageni, Australis, Tarassovi
Jharkhand	Hebdomadis, Pyrogenes, Tarassovi, Pomona, Icterohaemorrhagiae, Djasiman
Karnataka	Hardjo, Hebdomadis, Panama, Copenhageni, Pomona, Javanica, Icterohaemorrhagiae, Djasiman, Pyrogenes
Chhattisgarh	Hardjo, Icterohaemorrhagiae, Canicola, Hurstbridge, Shermani, Australis, Kaup, Javanica, Tarassovi, Djasiman, Copenhageni, Bataviae
Andhra Pradesh	Shermani, Bataviae, Canicola, Javanica, Pyrogenes, Australis, Hebdomadis, Icterohaemorrhagiae, Pomona, Bankinang, Hardjo
Sikkim	Tarassovi, Canicola, Shermani, Hebdomadis, Icterohaemorrhagiae, Bataviae, Pomona, Pyrogenes, Kaup, Javanica, Djasiman
Himachal Pradesh	Bataviae, Shermani, Pyrogenes, Canicola, Javanica, Panama, Hebdomadis, Tarassovi, Djasiman
Uttarakhand	Icterohaemorrhagiae, Canicola, Hurstbridge, Shermani, Australis, Kaup, Javanica, Tarassovi, Panama, Djasiman, Autumnalis
Prevalent serogroup-specific antibodies in dairy farms	Australis, Autumnalis, Canicola, Sejroe, Hebdomadis, Pyrogenes, Tarassovi, Pomona, Shermani, Hurstbridge, Icterohaemorrhagiae, Grippotyphosa, Javanica, Djasiman, Panama, Bataviae
Major serogroup-specific antibodies in dairy farms	Sejroe, Pyrogenes, Canicola, Javanica, Hebdomadis, Icterohaemorrhagiae, Pomona, Shermani, Grippotyphosa, etc.

aforesaid determined seven major serovars. These serovars may be considered as highly infective serovars in different regions of surveyed farms. These results on seroprevalence from this study correspond with earlier reports. It is a well-known fact that Hardjo serovar is the common one and dairy cattle have a role as a natural host of serovars Hardjo, Pomona and Grippotyphosa (Leonard et al. 2004). Moreover, different serogroup-specific antibodies have been reported from different parts of India by various researchers viz. from Karnataka (4.6%), Andaman (24.2%), Tamil Nadu (51.4%) (Natarajaseenivasan et al. 2011) and Andhra Pradesh (10.5%) (Srivastava 2008), Maharashtra (7.3%) and Uttar Pradesh (4 to 8%) (Srivastava and Kumar 2003), Odisha (37.74%) (Balamurugan et al. 2017), Konkan Region of Maharashtra (41%) (Balamurugan et al. 2016c), Gujarat (12.8%) (Patel et al. 2014) and organized cattle farm (12%) (Balamurugan et al. 2016b) during different surveys.

Table 8 Details of the samples reacted with the *Leptospira* intermediate and other species serovars

Region/location/state	Serovars (no. of reacted samples)
Maharashtra	Hus (2), She (2), Pan (5)
Gujarat	Hus (3), She (2), Kau (2)
Tamil Nadu	Hus (1), Kau (3), Pan (2)
Haryana	Hus (3), Pan (4)
Telangana	Hus (4), She (2), Kau (2), Pan (8)
Karnataka	She (3), Kau (2), Pan (4)
Chhattisgarh	Hus (1), Pan (1)
Andhra Pradesh	She (1), Kau (1), Pan (2)
Sikkim	She (28), Kau (14), Pan (13)
Uttarakhand	She (1), Kau (3), Pan (1), Hus (5)

Kau Kaup, *Hus* Hursbridge, *She* Shermani, *Pan* Panama

The reacted serovars (Kaup and Hurstbridge) representing *Leptospira* intermediate species may be of significance, as the prevalence of this species in India has been reported (Balamurugan et al. 2013, 2017). However, most of the reacted samples also showed multiple reaction with either of the aforesaid major serovars, and only a few samples showed reaction with serovars representing *Leptospira* intermediate species. It is to be noted that reacted samples to Kaup, Hustbridge serovar representing the Tarassovi serogroup, did not react with the serovar Tarassovi (Perepelicin strain) belonging to species *L. borgpetersenii*. Further, on inclusion of the *L. santarosai*, *L. noguchii* and *L. kirschneri* serovars, it is possible to detect additional reacted positive samples, which helped in knowing or identifying the prevalence of emerging or re-emerging serovars in various geographical regions as

Table 9 State-wise coverage of the positive reacted samples with selected panel of the serovars

State	Total no. of positive reacted samples	Reacted samples with major serovars[a]	Reacted samples with other unique additional serovars inclusion in the test panel							
			Gri	Cop	Tar	Aus	Pan	Dja	Hus	Kau
Maharashtra	28	7	2	11	4	1	1	1	1	
Gujarat	15	9	4	1	1	–	–	–	–	–
Punjab	3	3	–	–	–	–	–	–	–	–
Tamil Nadu	39	35	2	–	–	1	–	–	–	1
Haryana	6	5	–	1	–	–	–	–	–	–
Telangana	21	17	–	–	–	–	3	–	–	1
Jharkhand	5	4	–	–	–	–	–	1	–	–
Karnataka	26	20	–	2	–	–	3	1	–	–
Chhattisgarh	12	11	–	–	–	–	–	1	–	–
Andhra Pradesh	19	17	1	–	1	–	–	–	–	–
Sikkim	62	56	1	–	–	3	1	1	–	–
Himachal Pradesh	5	5	–	–	–	–	–	–	–	–
Uttarakhand	22	21	–	–	–	–	–	–	1	–

[a] If the panel contains major reacted serovars Hardjo, Pyrogenes, Canicola, Javanica, Hebdomadis, Pomona and Icterohaemorrhagiae

Aus Australis, *Tar* Tarassovi, *Kau* Kaup, *Gri* Grippotyphosa, *Hus* Hurstbridge, *Pan* Panama, *Dja* Djasiman, *Cop* Copenhageni

reported earlier (Balamurugan et al. 2016a, 2017). The local abundance of several species of pathogenic *Leptospires* may be a useful indicator to assess the potential for transmission to humans and livestock, and it may help to take appropriate control measures to reduce the impact of leptospirosis (Chadsuthi et al. 2017).

In overall, with the selected major reacted serovars in the panel, it is possible to provide the diagnostic screening more than 80% in MAT with coverage ranges from 90 to 100% in some states. However, in the present study, as the samples were limited to few farms in some states, it has to be further tested and verified with large samples size. In other way, initially, dairy cattle farm samples are to be screened by using these selected major serovars panel in MAT followed by screening of negative samples with remaining panels of *Leptospira* serovars antigens, which will avoid tedious effort and time and funds associated with handling of more number of reference *Leptospira* serovars in the panel for providing specific diagnosis. Further, study supports that dairy cattle maintaining important several serovars in endemic states of India warrants mitigating measures to reduce the disease burden by an intensive surveillance programme, preventive vaccination and control strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Balamurugan, V., Gangadhar, N.L., Mohandoss, N., Thirumalesh, S.R.A., Dhar, M., Shome, R., Krishnamoorthy, P., Prabhudas, K. and Rahman, H., 2013. Characterization of *Leptospira* isolates from animals and humans: phylogenetic analysis identifies the prevalence of intermediate species in India. Springer Plus, 2, 362. doi:https://doi.org/10.1186/2193-1801-2-362

Balamurugan, V., Alamuri, A., Veena, S., Bharatkumar, K., Patil, S.S. and Govindaraj, G., 2016a. Investigation on the prevalence of leptosira serovars Hardjo in organized cattle dairy farms of India. Indian Journal of Animal Science, 86 (10), 1145–1147

Balamurugan, V., Thirumalesh, S.R.A., Sridevi, R., Govindaraj, G., Nagalingam, M., Hemadri, D., Gajendragad, M.R. and Rahman, H., 2016b. Microscopic agglutination test analysis identifies prevalence of intermediate species serovars in ruminants in endemic states of India. The Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences, 86(2), 469–475

Balamurugan, V., Thirumalesh, S.R.A., Veena, S., Alamuri, A., Nagalingam, M., Sridevi, R., Govindaraj, G., Hemadri, D., Gajendragad, M.R. and Rahman, H., 2016c. Investigation on the distribution of *Leptospira* serovars and its prevalence in bovine in

Konkan region, Maharashtra, India. Advances in Animal and Veterinary Sciences, 4(2s), 19–26

Balamurugan, V., Veena, S., Thirumalesh, S.R.A., Alamuri, A., Sridevi, R., Sengupta, P.P., Govindaraj, G., Nagalingam, M., Hemadri, D., Gajendragad, M.R. and Rahman, H., 2017. Distribution of serogroup specific antibodies against leptospirosis in livestock in Odisha. Indian Journal of Animal Sciences, 87 (5), 546–551

Chadsuthi, S., Bicout, D.J., Wiratsudakul, A., Suwancharoen, D., Petkanchanapong, W., Modchang, C., Triampo, W., Ratanakorn, P. and Chalvet-Monfray, K., 2017. Investigation on predominant *Leptospira* serovars and its distribution in humans and livestock in Thailand, 2010-2015. PLoS Neglected Tropical Diseases, 11(2), e0005228

Leonard, N., Mee, J.F., Snijders, S. and Mackie, D., 2004. Prevalence of antibodies to *Leptospira* interrogans serovar hardjo in bulk tank milk from unvaccinated Irish dairy herds. Irish Veterinary Journal, 57, 226–231

Levett, P.N., 2001. Leptospirosis. Clinical Microbiology Reviews, 14, 296–326

Natarajaseenivasan, K., Vedhagiri, K., Sivabalan, V., Prabagaran, S.G., Sukumar, S., Artiushin, S.C. and Timoney, J.F., 2011. Seroprevalence of *Leptospira* borgpetersenii serovar javanica infection among dairy cattle, rats and humans in the cauvery river valley of southern India. Southeast Asian Journal of Tropical Medicine and Public Health, 42(3), 679–686

Office International des Épizooties (OIE-World Organisation for Animal Health)., 2013. Manual of diagnostic tests and vaccines for terrestrial animals, OIE. Paris. Chapter 2.1.9. Pp. 251–264

Patel, J.M., Vihol, P.D., Prasad, 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

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R., 1994. Clinical veterinary microbiology. Wolfe Publication Ltd. Spain. Pp. 296–303

Snedecor, G.W. and Cochran, W.G., 1989. Statistical Methods, Iowa State University Press, 8th Edition. 1989

Srivastava, S.K., 2008. Current status of leptospirosis in India in animals and humans. Indian Journal of Veterinary Pathology, 32 (2), 179–186

Srivastava, S.K. and Kumar, A.A., 2003. Seroprevalence of leptospirosis in animals and human beings in various regions in the country. Indian Journal of Comparative Microbiology Immunology and Infectious Diseases, 24, 155–159

Vijayachari, P., Sugunan, A.P. and Shriram, A.N., 2008. Leptospirosis: an emerging global public health problem. Journal of Biosciences, 33(4), 557–569.

World Health Organisation., 2011. Report of the Second Meeting of the Leptospirosis Burden Epidemiology Reference Group. ISBN 978 92 4 150152 1. Printed by the WHO Document Production Services, Geneva, Switzerland. Pp. 7–14