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Distribution of serogroup specific antibodies against leptospirosis in livestock in Odisha

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ABSTRACT

The study was conducted to identify epidemiological sero-distribution of *Leptospira* serovars representing serogroups of antibodies in Odisha, Eastern part of India. Samples were collected from livestock during 2011–2014. A total of 537 non-purposive random serum samples from 12 districts in Odisha were tested at 1:100 dilutions in microscopic agglutination test (MAT) using live antigens of 18 reference *Leptospira* serovars. The overall prevalence of 36.69% (197/537) with 36.13% in cattle, 54.28% in buffaloes, 28.33% in goats and 44.44% in sheep was observed. Out of 197 reacted sera, 100 samples showed reactivity with more than one serovars representing 50.76% prevalence of multiple serovars. On analysis, the seroprevalence of leptospirosis in cattle (÷2 = 63.41, P <0.01), buffaloes (÷2 = 14.68, P <0.01), sheep (÷2 = 54.66, P <0.01) and goats (÷2 = 36.27, P <0.01) across different regions are significantly dependent. The predominant Leptospira serogroup specific antibodies against major serovars representing Hardjo (30.4%), Tarassovi (20.8%), Australis (19.19%), Bankinang (18.18%), Pomona (16.66%), Kaup (15.65%), Hebdomadis (11.11%), Pyrogenes (10.1%), Bataviae (9.59%), Icterohaemorrhagiae (9.09%), Shermani (7.57%), Djasiman/Javanica (6.56%), Hurstbridge (5.55%), Grippotyphosa (4.54%), Panama (4.04%), Canicola (3.03%) and Copenhageni (2.02%) against frequency distribution were noticed. The overall prevalence of antibodies detected against the serovars Hardjo, Tarassovi, Australis, Bankinang, Pomona, Kaup, Hebdomadis and Hurstbridge representing specific serogroups suggested that these serovars may be of use in the reference panels of Leptospira antigen in the MAT for this region in both human and animal state or district disease diagnostic laboratories.

Key words: Frequency distribution, Leptospirosis, Livestock, MAT, Odisha, Serovars

Leptospirosis is neglected and most widely spread re-emerging global zoonotic disease noticed in both developed and developing countries with more prevalence in tropical and sub-tropical rainfall regions. Natural calamities such as cyclone and floods increase the incidence of the disease (Sugunan *et al.* 2009) and large number of animals act as carriers or vectors. Environmental contamination by this bacteria present in the animal excreta is a source for human infection (Vijayachari *et al.* 2008). Sub-clinically infected animals (which are apparently healthy) with host adapted serovars serve as long term carriers and continuous shedders of the organism mainly through their urine. The animals pose a risk and source of infection to farm workers, occupational workers, livestock owners, etc. (Balamurugan *et al.* 2013a, 2016a). In India, the endemicity of the disease has been reported from Kerala, Tamil Nadu, Gujarat, Andaman and Nicobar Island, Karnataka and Maharashtra, Andhra Pradesh, Odisha, West Bengal, Uttar Pradesh, Delhi and Puducherry (Shivakumar 2008).

In general, most of the Leptospira cases remain undiagnosed due to the lack of awareness and difficulty in carrying out the laboratory based confirmatory tests, though it is estimated that 20% of undefined febrile illness are due to leptospirosis (WHO 2011). The domestic animals have been affected by *Leptospira* showing reproductive disorders such as abortion, infertility, stillbirths, birth of weak calves, reduced milk yield, productivity etc. This leads to huge economic loss in the countries' agricultural income (Vijayachari *et al.* 2008, Srivastava 2008). The local abundance of several species of pathogenic leptospires may be a useful indicator of transmission of *Leptospira* to humans and livestock. Knowledge of prevalent *Leptospira* serovar(s) in a particular geographical area either in incidental host or in carrier animals is essential to understand the epidemiology of the disease (Balamurugan *et al.*2013a,

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2016a). The comprehensive work on *Leptospira* serogroups specific antibodies in various livestock species, especially in certain part of India including Odisha state at various time period, cyclone, flood etc., is scanty or not much studied (a descriptive study of the sero-distribution and prevalence). Hence, the present study is first of its kind in Odisha which has dealt with using the samples from livestock species over a different time period.

MATERIALS AND METHODS

A total of 345 large ruminants (310 cattle, 35 buffaloes) and 192 small ruminants (120 goats, 72 sheep) serum samples were randomly collected from 12 different districts of Odisha by veterinary officers during different surveys conducted in the different regions of Odisha from 2011 to 2014. Serum samples, which are collected for other disease monitoring purposes were used and represented as non-purposive sampling. These non-purposive serum samples included were from 4 different periods in 2011 (n = 142 after cyclone and flood, winter), October-December 2013 (n = 295 winter with retreating monsoon, flood and cyclone) and April 2014 (n = 100, summer). The surveyed places are depicted in GIS Map based on their geo-coordinates (Fig. 1).

Ellinghausen McCullough Johnson and Harris (EMJH) liquid medium was prepared for the propagation of reference *Leptospira* serovar of live antigens cultures as per earlier described protocols (Balamurugan *et al.* 2013b) and all the serum samples were tested in MAT using 18 reference leptospira cultures antigens as described earlier (Balamurugan *et al.* 2016a) in addition other serovars Panama, Djasiman, Copenhageni, Bataviae were used.

Fig. 1. Sample surveyed places depicted in GIS Map based on their geo-coordinates using QGIS software version 2.12.3 Lyon.

Apparent prevalence with 95% confidence interval (CI) and data analysis were carried out using Microsoft Excel 2013 and Statistical Package for Social Sciences (SPSS) version 22. Chi-square test was used for test of independence {The H_0 is independent of seropositivity of species, sex groups and study regions (Vs- H_1 dependent)} as per standard statistical method and they were statistically tested for their significance at 5% ($P<0.05$) and 1% ($P<0.01$) level.

RESULTS AND DISCUSSION

The gold standard serological test to record the seroprevalence is MAT, which is accepted worldwide to detect the antibodies in both humans and animals (OIE 2013). Out of total 537 serum samples tested, 197 samples at 1:100 dilution reacted in MAT representing 36.69% of seroprevalence of leptospirosis in livestock (Table 1). Out of 197 reactors, 75 samples showed positive reactivity at 1:200 dilution, representing 13.96%. Among different ruminants, the seroprevalence of 36.13% in cattle (112/310), 54.28% in buffaloes (19/35), 28.33% in goats (34/120) and 44.44% in sheep (32/72) were observed. Among the district, the Kalahandi showed the highest prevalence (74.0%) followed by Angul (72.0%), Ganjam (68.2%) Subarnapur (60.0%), Jajpur (54.5%), Puri (53.0%), Bolangir (32.0%), Dhenakanal Sadar (30.0%), Khurda (18.4%), Cuttack (13.8%), Jagatsinghpur (12.7%) and Kendrapara (9.8%).

On analysis of coastal districts (Ganjam, Puri, Jagatsinghpur and Kendrapara), the districts adjoining the coast (Jajpur, Khudra and Cuttack) and the non-coastal districts (Kalahandi, Angul, Subarnapur, Bolangir and Denkanal), the flood prone (the non-coastal region) showed more prevalence than the coastal or regions proximal to costal region. The most prevalent serovars among the reactive samples were Hardjo (30.45%, 60/197) and Tarassovi (20.81%, 41/197), representing Sejroe and Tarassovi serogroup. However, 100 samples from 197 reactors showed reaction with multiple serovars representing 50.76% prevalence. The number of samples showed reaction (48 cattle, 14 buffaloes, 19 goats and 19 sheep) with more than one *Leptospira* antigens representing prevalence of 18, 14, and 19% in cattle, buffaloes and sheep and goats, respectively.

Antibodies against different serovars have been reported from Andaman (24.2%) and Tamil Nadu, (Natarajaseenivasan *et al.* 2011), Karnataka (4.6%), Andhra Pradesh (10.5%), Maharashtra (7.3%) and Uttar Pradesh (4–8%), Odisha (42.5%) (Balamurugan *et al.* 2013a), Kongon Region of Maharashtra (41%) (Balamurugan *et al.* 2016b), Gujarat (12.8%) (Patel *et al.*2014) during different surveys. In the earlier study, the seroprevalence of leptospirosis in various Indian states was reported as 5.4% in buffaloes, 7.5% in cattle, 12.5% in sheep, 14.6% in horses and 15.9% in dogs (Srivastava 2008) and 12.7% in organized dairy cattle farm of India (Balamurugan *et al.* 2016c).

Table 1. Samples and statistical analysis of results of samples screened for leptospirosis by MAT.

Analyses		Cattle				Buffalo				Goat				Sheep			
		T	R	P	*CI*	T	R	P	*CI*	T	R	P	*CI*	T	R	P	*CI*
District-wise analysis	Kalahandi (NC)	13	8	61.5	35-82	13	12	92.3	66-98	12	12	100.0	75-100	12	5	50.0	19-68
	Angul (NC)	26	15	57.7	39-74	-	-	-	-	9	7	77.8	45-93	15	14	93.3	70-98
	Ganjam (C)	22	15	68.2	47-83	-	-	-	-	-	-	-	-	-	-	-	-
	Subarnapur (NC)	5	3	60.0	23-88	-	-	-	-	-	-	-	-	-	-	-	-
	Jajpur (PC)	11	6	45.5	28-78	-	-	-	-	-	-	-	-	-	-	-	-
	Puri (C)	66	35	53.0	41-64	-	-	-	-	-	-	-	-	-	-	-	-
	Bolangir (NC)	20	3	15.0	5-36	5	-	-	0-43	16	5	31.3	14-55	9	8	88.9	56-98
	Dhenakanal Sadar (NC)	26	11	42.3	25-61	-	-	-		12	1	8.3	1-35	12	3	25.0	8-53
	Khurda (PC)	34	8	26.5	14-43	17	7	41.2	21-64	23	1	4.3	1-23	24	2	4.2	2-25
	Cuttack (PC	24	3	12.5	4-31	-	-	-		5	1	20.0	3-62	-	-	-	-
	Jagatsinghpur (C)	20	-	-	-	-	-	-		35	7	20.0	10-35	-	-	-	-
	Kendrapara (C)	43	5	11.6	5-24	-	-	-		8	-	-	-	-	-	-	-
	Total	310	112	36.1	31-42	35	19	54.3	38-69	120	34	28.3	21-37	72	32	44.5	33-55
	χ^2 Value	63.41***				14.68***				54.66***				36.27***			
Sex-wise analysis	Male	80	27	33.8	24-44	11	8	72.7	43-90	13	3	23.1	8-50	7	4	57.1	25-84
	Female	230	86	37.4	31-43	24	11	45.8	27-64	107	31	29.0	21-38	65	28	43.1	31-55
	Total	310	112	36.3	31-42	35	19	54.3	38-69	120	34	28.3	21-37	72	32	44.4	33-55
	χ^2 Value	0.001^{NS}				0.06^{NS}				0.001^{NS}				0.007^{NS}			

T, Number of serum samples tested; R, positive reactor in microscopic agglutination test (MAT); P, per cent seroprevalence (%); CI, confidence interval at 95% level; ***compared with sample regions versus sero-positivity (P<0.01) level; NS, not significant; C, coastal district; PC, proximity to coast; NC, non-coastal district.

The overall results (Table 1) revealed *Leptospira* seropositivity in animals are region dependent (associated) and sex independent (not associated), implying leptospirosis occurs in similar geographical environment and affect animals independent of sex. Further, it may be due to same agro ecological, environmental and animal rearing practices prevailing in the sample regions. However, sex-wise prevalence in cattle and goats showed females having high percentage of leptospirosis whereas in buffaloes and sheep, males having high leptospirosis.

The predominant *Leptospira* serogroup specific antibodies were determined by frequency of distribution of the serovars Hardjo (30.4%) followed by Tarassovi (20.8%), Australis (19.19%), Bankinang (18.18%), Pomona (16.66%), Kaup (15.65%), Hebdomadis (11.11%), Pyrogenes (10.1%), Bataviae (9.59%), Icterohaemorrhagiae (9.09%), Shermani (7.57%), Djasiman/Javanica (6.56%), Hurstbridge (5.55%), Grippotyphosa (4.54%), Panama (4.04%), Canicola (3.03%) and Copenhageni (2.02%) respectively, with their titre ranging from 1:100 to 1: 3200. Along with these, serovars such as Icterohaemorragiae, Pyrogenes and Bataviae have shown individual serovar reactivity for the tested samples. Further, on comparison between the large and small ruminants, prominent serovars observed were Hardjo, Australis, Tarassovi, Bankinang, Pomona, Kaup, and Hebdomadis (Table 2). The cross reactivity observed between the different serovars tested is shown in Table 3. The majority of the cross reactions were between the serovars Australis/Bankinang and Pyrogenes/Tarassovi/Kaup followed by Hardjo and Hebdomadis/Pomona. However, Australis and Bankinang are showing the highest cross reactivity. The most prevalent serovars were Hardjo, Tarassovi, Australis and Bankinang and distribution of pathogenic serovars reactive percentage with individual livestock in MAT are presented in Table 4. The seropositive MAT titers according to livestock tested in MAT varies from 1:100 to 1:3200. The overall seroprevalence during different climatic conditions and the serum samples collected for this survey is summarized in Table 5.

Generally, the most recent infection shows the higher titre as the fresh infection produces more specific antibodies or it can also show multiple sero reactivity while only one serovar may be present. Based on this, the samples showing

Table 2. Sero distribution of *Leptospira* serovars in large and small ruminants

	Aus	Ban	Can	Had	Heb	Ict	Pyr	Tar	Pom	She	Kau	Gri	Hus	Jav	Pan	Dja	Cop	Bat
Large ruminants	30	24	4	40	20	17	14	24	16	5	18	7	4	13	6	9	2	7
Small ruminants	12	12	2	20	2	1	6	17	17	10	11	1	7	1	2	3	2	11
Total	42	36	6	60	22	18	20	41	33	15	29	8	11	14	8	12	4	18

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.

Table 3. Cross-reactivity among different reference leptospira serovars

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

The majority of cross-reactive serovars are: 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.

Table 4. Distribution of pathogenic serovars reactive percentage with individual livestock in MAT

Species / Serovars	Cattle	Buffaloes	Sheep	Goats
Australis	8.33	16.98	-	15.00
Bankinang	17.84	15.90	5.45	11.25
Canicola	1.96	-	1.82	1.25
Hardjo	17.16	9.43	-	5.00
Hebdomadis	9.31	1.89	-	1.25
Icterohaemorrhagiae	5.39	11.32	-	1.25
Pyrogenes	2.94	13.21	-	7.50
Tarassovi	8.82	5.66	7.27	15.00
Pomona	6.37	5.66	14.55	10.00
Shermani	1.96	1.89	2.73	3.75
Kaup	8.82	1.89	5.45	10.00
Grippotyphosa	2.45	3.77	-	1.25
Hurstbridge	1.96	-	5.45	5.00
Javanica	6.32	-	-	-
Panama	1.96	3.77	-	2.50
Djasiman	3.43	3.77	1.82	5.00
Copenhageni	0.98	-	-	3.75
Bataviae	1.96	3.77	20.00	-

the positivity at higher dilutions (i.e., 1:200; 1:400 to 1:3200) can be concluded to be infected recently whereas the remaining samples of 1:100 (n = 122) may be from past infections as disease is endemic. It is obvious from the Table 5 that serovars Hardjo, Australis, Bankinang, Tarassovi, Pomona, Kaup, Hebdomadis and Icterohaemorragiae were quite prevalent during all the periods. After the cyclone (October-November 1999), 142 patients with febrile illness and haemorrhagic manifestations were evaluated from human serum samples suspected of leptospirosis in a village of Mayurbhanj district in north Odisha during June-July 2002. The people were exposed to contaminated water in a canal which was probably the source of infection (Shivakumar 2008). Balamurugan *et al.* (2013b), reported the seroprevalence of leptospirosis 42.5% (51/120) in bovine population (cattle, bulls and bullocks) in Odisha in one season. During 2008, a leptospirosis survey was done among bovine in West Bengal regions which has the similar agro-climatic conditions as Odisha (Mandal *et al.* 2008). This survey showed a district wise prevalence of 16.84% and Hardjo being the major serovar. Puri, Cuttack, Jajpur, Kalahandi, Angul and Dhenkanal though being urban areas had high prevalence (Table 1), which may be due to the poor sanitation, drainage system and improper management of the flood water which would form damps and clogs making perfect environments for the continued existence of *Leptospira*. Further, Kalahandi, Angul, Subarnapur and Bolangir areas being a non-coastal region showed higher seroprevalence, as it has forest areas (sub-tropical) with lots of bamboos and it is obvious that rodent and rat populations are higher in such areas, which could be the cause for the *Leptospira* contamination. Puri is a major tourist destination and also being a coastal region it has been hit by many cyclones which may be the cause of *Leptospira* contamination from animals to human.

Rice fields also idyllic for the growth and spread of organism by a major population of reservoir or carrier rodent's species. These paddy fields are warm, wet with the pH close to neutral and slightly alkaline which is optimum for the survival of this pathogen. It also shows that alluvial plains soil is highly fertile, but vulnerable to flood and drought and soil sediments formed due to floods with more prevalence of leptospirosis than the coastal and Tarai regions (Mandal *et al.* 2008). This has a similar effect on the plains of Odisha and probably effects may be same Odisha also has many natural calamities (floods and cyclones) causing alluvial soiland flood disasters ever year. This re-emphasizes that natural calamities can increase the prevalence of leptospirosis as stated earlier (Sehgal *et al.*

Table 5. Prevalence of leptospira serovars in different periods in Odisha

Samples from different periods	Percent Positivity and overall seroprevalence (%)	Serogroup prevalence
November 2011 (after cyclone and flood) *n*= 142	38 (26.76)	Javanica, Hebdomadis, Australis, Autumnalis and Icterohaemorragiae
April-May 2013 *n*=120 (Balamurugan *et al*., 2013b)	51 (42.50)	Australis, Sejroe, Tarassovi, Kaup (Tarassovi), Pomona and Hurstbridge
October 2013 (flood and cyclone) *n*=203	41 (20.20)	Sejroe, Tarassovi, Kaup (Tarassovi), Hebdomadis, Icterohaemorragiae, Pyrogenes, Pomona and Bataviae
December 2013 (after flood and cyclone) *n*=92	66 (71.74)	Sejroe, Tarassovi, Australis, Autumnalis, Bataviae, Hebdomadis, Pomona and Kaup (Tarassovi)
April 2014 *n*=100	52 (52.00)	Australis, Autumnalis, Pomona, Kaup (Tarassovi), Sejroe, Shermani, Icterohaemorragiae and Tarassovi
Overall cumulative serovars prevalence by MAT in Odisha *n*=657	248 (37.74)	Australis, Sejroe, Autumnalis, Pomona, Kaup (Tarassovi), Hebdomadis, Tarassovi. Hurstbridge Icterohaemorragiae, Javanica, Pyrogenes, Bataviae

2002). This indicates the evidence of contact of leptospires in different species of livestock which would have probably spread through the shedding of the urine by infected/ carrier animals contaminating the soil and water dams and the transmission due to rain and flood, thus leading to the agricultural economic loss and disease infection to mankind.

The present findings provided base line information about the prevalence of *Leptospira* serogroup specific antibodies in Odisha. The overall prevalence of antibodies detected representing specific serogroups against the serovars Hardjo, Tarassovi, Australis, Bankinang, Pomona, Kaup, Hebdomadis and Hurstbridge. This suggested that these reference serovars may be of use in the panels of *Leptospira* antigen in MAT for this region in both human and animal state and district disease diagnostic laboratories. Further the seroprevalence of the pathogenic *Leptospira* in these areas has to be studied in more details using purposive sampling in relation with the reservoir/carrier host and human to find the exact distribution of pathogenic *Leptospira* serovars for providing early and accurate diagnosis of leptospirosis so that rapid and opt treatment to reduce the extent of problem associated with the disease. The awareness regarding the disease prevalence and spread needs more attention especially in endemic region with varying agro-climatic conditions.

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