

Seroepidemiology of Leptospirosis in Andhra Pradesh

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Abstract

Leptospirosis is worldwide zoonosis and considered as re emerging disease. Besides economic losses caused by *leptospira* to animal production, its zoonotic character makes it an important public health problem. Seroepidemiological study was conducted by MAT randomly according to season, region and district wise of Andhra Pradesh. A total of 2320 serum samples randomly from apparently healthy animals of cattle, buffaloes, sheep, goats, pigs and dogs and 258 serum samples from clinically suspected cases of both animals and humans were subjected for MAT. Overall seropositivity of 20 percent in apparently healthy animals, cattle, sheep, goat and dogs was found to be 19.1% 18.67%, 14.63%, 36.84% and 15.15% respectively. Similarly seropositivity in clinically suspected cases (33.37%) of cattle (28.94%) sheep (21.69%) pigs (50.0%) dogs (31.43%) and humans (61.76%) respectively. High seroprevalence in coastal regions (23.09%) followed by Rayalseema (17.49%) and Telangana (16.30%) was observed. High seropositivity was recorded during north east monsoon (28.29%) followed by south west monsoon (21.45%) and lowest in summer (7.26%).

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Introduction

Leptospirosis is a disease of animals and humans which has a worldwide distribution [17]. It is also emerging as important public health problem [14]. Leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira*, belongs to the family *Leptospiraceae* and order *spirochaetales* and is manifested by different syndromes including haemolytic crisis, chronic interstitial nephritis, decreased milk production, mastitis, death of young adults due to haemolytic anaemia and mastitis.

Leptospirosis is also an important worldwide cause of abortions, still births and reproductive failure in cattle and pigs in particular and there is an increasing prevalence of leptospirosis in humans resulting from contact with infected livestock. In humans the disease ranges from sub-clinical infection to severe syndromes of multi organ infection with high mortality. The seroprevalence of leptospirosis was reported from Tamilnadu [8, 4]; Kerala [1]; Karnataka [15]; Andaman [13]; Uttar Pradesh [2]; North eastern states [7]; Haryana [5]; Orissa [16]; Uttaranchal [10] and from West Bengal [9]. The disease has been reported earlier from Andhra Pradesh [11, 4]. The endemicity of the disease in Tamilnadu, Kerala & Karnataka, the adjoining states of Andhra Pradesh and inadequate information on leptospirosis in animals in Andhra Pradesh made us to study the seroepidemiology of leptospirosis among various species of animals and humans in different districts of Andhra Pradesh. Hence seroepidemiological study was conducted species wise and data was analyzed and presented during the investigation.

Materials and methods

A total of 2,320 serum samples were collected randomly from apparently healthy animals of different species includes Bovine (1499), sheep (316), goats (205), Pigs (133), dogs (99) were collected from different districts of Andhra Pradesh during the period 2006-2010. Similarly 258 serum samples were also collected from clinically suspected cases of Leptospirosis showing pyrexia and abortions in cattle (38), sheep showing fever, haemoglobinuria and abortions (83) Pigs having pyrexia and abortions (35), Dogs showing pyrexia, anorexia, hepatic dysfunction, jaundice (34) and from Humans with fever, jaundice, renal failure (68) and processed during the period of study. All the serum samples were inactivated at 56°C for 30 minutes and stored at – 20°C till used for analysis.

Microscopic agglutination test (MAT)

Serum samples were tested by using Microscopic agglutination test (MAT) according to the method followed at regional Medical research centre (RMRC), Port Blair, Andaman and Nicobar Islands. The serum samples were screened using a panel of 9 live leptospiral reference serovars namely *L. autumnalis*, *L. canicola*, *L. grippityposa*, *L. icterohaemorrhagae*, *L. hardjo*, *L. hebdomedis*, *L. javanica*, *L. pomona* and *L. patoc*, received from RMRC, port Blair, Andaman and Nicobar islands and maintained in our laboratory.

Leptospira reference antisera

Reference antiserum was procured from Regional Medical research center, Andaman & Nicobar Islands, Port Blair are presented in the **table.1** was used for testing antigens.

Preparation of antigen for MAT

The reference serovars strains were grown in Ellinghausen, McCullough, Johnson and Harris (EMJH) liquid medium. The cultures were incubated at 30°C for 5-7 days and examined for the growth of the organisms. The live cultures with density of 2-3 x 10⁹organisms/ml were used as antigens. Reactivity of the cultures for the MAT was tested using the homologous antisera. The panel of antigens used in MAT was presented in **Table 2**.

Method for conducting MAT

The protocol followed at Regional Medical research center (RMRC), WHO collaborating center for diagnosis, reference and training on leptospirosis, Port Blair, Andaman and Nicobar islands was used for conducting MAT. In brief the MAT was carried out in the U shaped microtitre plate consisting of 96 wells. 50ul of PBS added to all wells except 2nd col-

Table 1. Leptospiral reference antisera used in the present study.

S.No	Serogroup	Serovar	Strain
1	Autumnalis	Bankinang	Bankinang 1
2	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
3	Canicola	Canicola	HV IV
4	Sejroe	Hardjo	Hardjo prajitro
5	Hebdomedis	Hebdomedis	Hebdomedis
6	Grippotyphosa	Grippotyphosa	Moskova
7	Javanica	Javanica	Poi
8	Patoc	Patoc	Patoc
9	Pomona	Pomona	Pomona

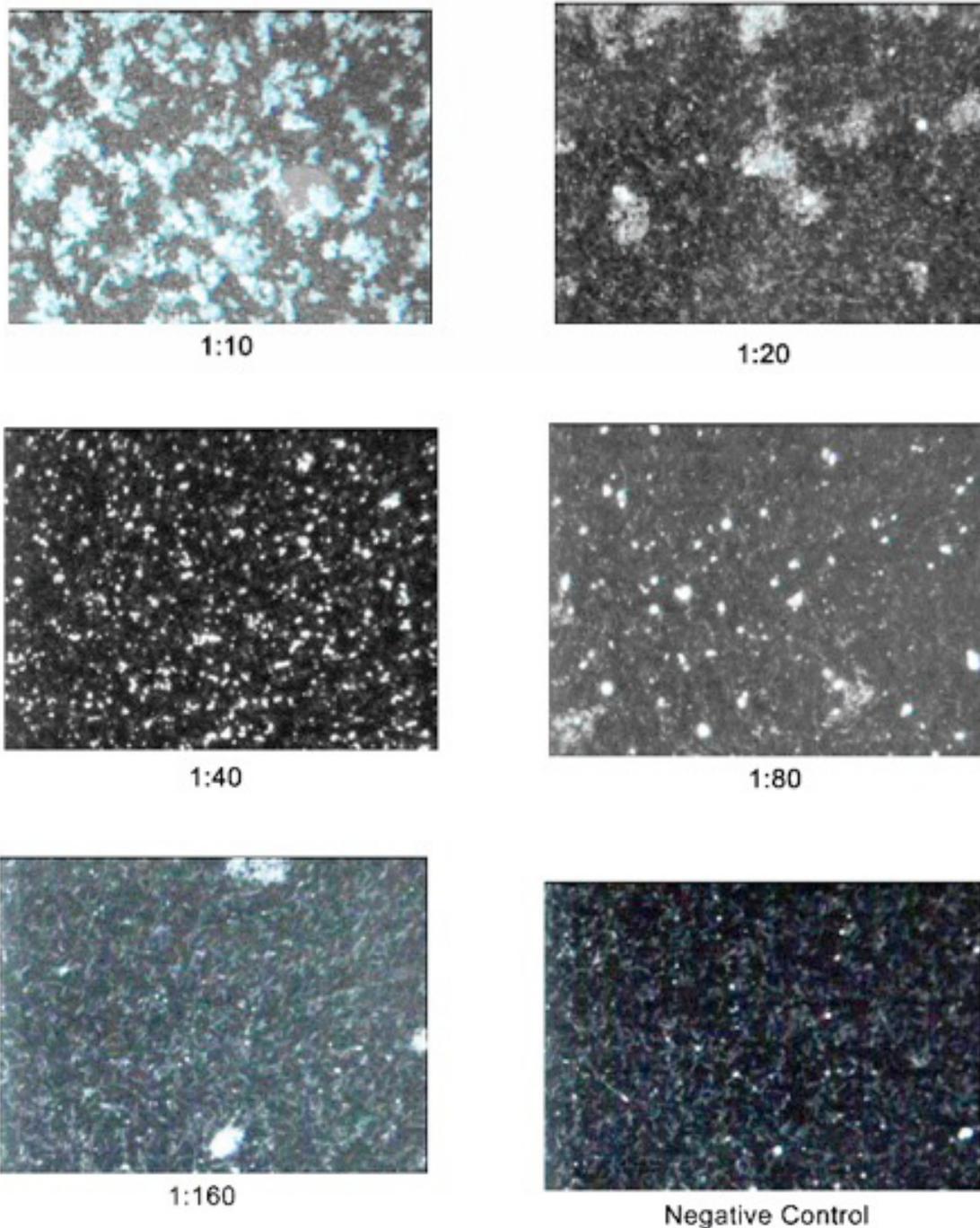
Table 2. Reference strains of leptospires used in the present study.

S.No	Serogroup	Serovar	Strain
1	L.autumnalis	Rachmati	Rachmat
2	L. icterohaemorrhageae	Icterohaemorrhagiae	RGA
3	L.canicola	Canicola	HV IV
4	L.hardjo	Hardjo	Hardjo prajitro
5	L.hebdomedis	Hebdomedis	Hebdomedis
6	L.grippotyphosa	Grippotyphosa	Moskova
7	L.javanica	Poi	Poi
8	L.patoc	Patoc	Patoc I
9.	L.pomona	L.pomona	L.pomona

umn in which 90ul was taken. Then 10ul of serum samples were added to all the wells of 2nd column of plate mixed thoroughly and transferred 50ul to 3rd column in the same row and so on. Dilute serially and discard 50ul from the last well, which gives the dilution 1:10, 1:20, 1:40, 1:80, 1:160 and so on. Then added 50ul of fresh leptospiral culture with a density of 2-3 x 10⁹ organisms/ml to all the wells of the plate. Mixed thoroughly in shaker and incubated at 37⁰C for 2 hours. Later serum antigen mixtures from each dilution were examined fewer than 20x dark field microscope. The reciprocal of the highest

dilution of the serum where 50 % agglutination of leptospires occurs was considered as MAT titer. Test samples were compared with known positive and negative controls. The cutoff titer to differentiate positive and negative serum samples was determined using known positive and negatives serum samples collected from Andhra Pradesh. MAT titer of 1:80 was found optimum to differentiate positive and negative samples. Hence, MAT titer of 1:80 is considered as cut off point to differentiate positive and negative samples used in the study (**Fig. 1**).

Fig. 1. Results of microscopic agglutination test.



Results

A total of 2,320 serum samples collected randomly from apparently healthy animals of different species viz. cattle, buffaloes, sheep, goats, pigs, dogs, 485 (20.9 %) (**Table 3**) samples were positive for

leptospiral antibodies. Similarly of the 258 serum samples from clinically suspected cases of bovine, sheep, pigs, dogs and humans, 99 samples (38.37 %) were found positive for antibodies to leptospira on MAT (**Table 4**).

Table 3. Percentage of seroprevalence of *Leptospiral* antibodies of apparently healthy animals in different species in Andhra Pradesh.

S.No	Species	Total Sera samples tested	Total positives	% positive	Autumnalis	Canicola	Grippityphosa	Hebdomedis	Hardjo	Ictero	Javanica
1	Bovines	1499	285	19.01	55 (19.29)	26 (9.12)	75 (26.31)	12 (4.21)	37 (12.98)	14 (4.91)	36 (12.63)
2	Sheep	316	59	18.67	3 (5.08)	-	12 (20.33)	6 (10.16)	26 (44.06)	-	12 (20.33)
3	Goats	205	31	15.12	2 (6.45)	-	9 (29.63)	-	12 (38.70)	-	8 (25.80)
4	Pigs	133	49	36.84	11 (22.44)	10 (20.40)	13 (26.53)	-	15 (30.61)	-	-
5	Dogs	99	15	15.15	2 (13.33)	6 (40)	-	-	1 (6.66)	-	6 (40)

Table 4. Percentage of seroprevalence of leptospiral antibodies in clinically suspected cases.

S. No	Clinical signs	Total no. of samples tested	Total positivity on MAT		Details of positivity of serovars							
			No	Percentage	L. autum	L. canicola	L. grippityphosa	L. hebdomedis	L. hardjo	L. ictero	L. javanica	L. pomona
1	Cattle Pyrexia, Abortions	38	17	44.73	3 (17.64)	1 (5.88)	5 (29.41)	-	2 (11.76)	-	-	6 (35.29)
2	Sheep Fever, haemoglobinurea, abortions	83	29	34.93	3 (10.34)	1 (3.44)	4 (13.79)	2 (6.89)	9 (31.03)	-	1 (3.44)	11 (37.93)
3	Pigs Pyrexia Abortions	35	18	51.42	1 (5.55)	2 (11.11)	3 (16.66)	-	5 (27.77)	-	-	7 (38.88)
4	Dogs Pyrexia, anorexia, hepatic dysfunction, jaundice	34	18	52.94	4 (22.22)	6 (33.33)	-	-	5 (27.77)	2 (11.11)	-	1 (5.55)
5	Humans Fever, jaundice, renal failure	68	45	66.17	7 (15.55)	5 (11.11)	5 (11.11)	6 (13.33)	12 (26.66)	4 (8.88)	3 (6.66)	3 (6.66)
	Total	258	127	49.22								

Cattle

Out of 1,499 serum samples collected from apparently healthy cattle and buffaloes, 285 (19.1%) serum samples were found positive for leptospiral antibodies on MAT. On MAT seroprevalence of *L. grippityposa* (26.31 %) was found to be highest followed by *L. autumnalis* (19.29 %), *L. hardjo* (12.98 %), *L. javanica* (12.63 %), *L. icterohaemorrhagiae* (4.91 %) and *L. hebdomedis* (4.21 %) respectively.

Of the 38 serum samples collected from cattle showing pyrexia and abortions subjected for MAT, the seropositivity was reported to be 44.73 %. The predominant serovar was *L. Pomona* (35.25 %), followed by *L. autumnalis* (17.64 %) and *L. hardjo* (11.76 %).

Sheep

The seroprevalence of leptospira in apparently healthy sheep in the present study was 18.67 %. Serovar analysis of the results revealed the predominance of the serovar *hardjo* (44.06 %) and *L. autumnalis* (5.08 %) in sheep of Andhra Pradesh.

Seroprevalence of leptospirosis in sheep (83) showing the clinical signs of fever, haemoglobinuria, found to be 21.00 %. The high prevalence of *L. pomona* (37.93 %) followed by *L. hardjo* (31.03 %), *L. autumnalis* (10.34 %), *L. hebdomedis* (6.80 %) and *L. javanica* and *L. canicola* (3.44 %). In addition to the serovars reported from apparently healthy sheep, *L. hebdomedis* and *L. icterohaemorrhagiae* were found circulating in sheep showing clinical signs.

Goats

Among 205 serum samples, the sero positivity was found to be 15.12 %. Serovar *hardjo* (38.7%)

followed by *L. grippityposa* (29.63%), *L. javanica* (25.8%) and *L. autumnalis* (6.45%) were reported.

Pigs

The seropositivity in healthy pigs (133) was found to be 36.84 %. The predominance of serovar *L. hardjo* was 30.61 % followed by *L. grippityposa* (26.53 %), *L. autumnalis* (22.44%) and *L. canicola* (20.4%). Seroprevalence of leptospira in clinically suspected cases (35) was 51.12 % (18). The predominance of serovar *L. pomona* (38.88 %) was noticed followed by *L. hardjo* (27.77 %), *L. grippityposa* (16.66 %), *L. canicola* (11.11 %) and *L. autumnalis* (5.55%).

Dogs

Over all seropositivity of 15.15 % (99) was recorded in apparently healthy dogs compared to the clinically suspected cases of 52.94 % (34). The serovar *L. canicola* and *L. javanica* (40%) dominated by *L. autumnalis* (13.33%) and *L. hardjo* 6.66 % in apparently healthy dogs, whereas, the dominance of *L. canicola* (33.33 %) followed by *L. hardjo* (27.77 %), *L. autumnalis* (22.22 %) and *L. pomona* (5.55%) were observed in clinically suspected cases. In addition the serovar *L. icterohaemorrhagiae* (11.11%) was also recorded in clinically suspected dogs.

Humans

Out of 68 serum samples screened from clinically suspected cases, 66.17 % seropositivity was reported. The predominance of serovar *L. hardjo* (26.66 %) followed by *L. autumnalis* (15.55 %), *L. hebdomedis* (13.33 %), *L. canicola* and *L. grippityposa* (11.11%), *L. javanica* and *L. pomona* (6.66%) and *L. icterohaemorrhagiae* (8.88 %) were noticed.

The seroprevalence of leptospiral antibodies in different regions of Andhra Pradesh was studied dur-

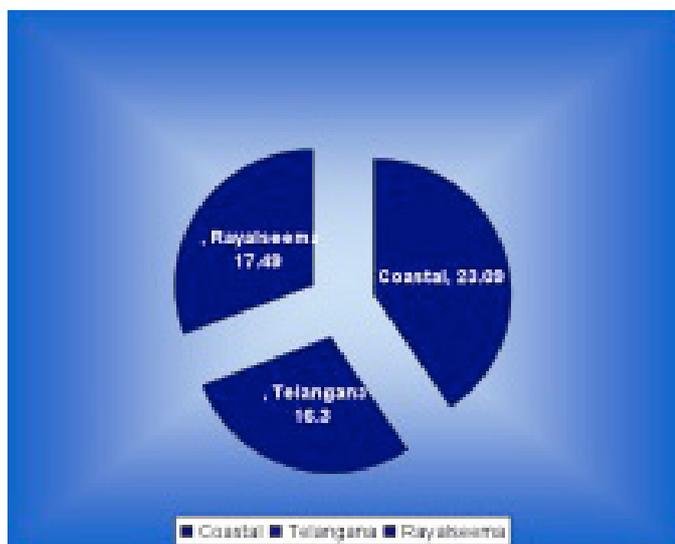


Fig. 2. Region wise percentage seroprevalence of leptospiral antibodies in Andhra Pradesh.

ing the period (**Fig. 2**). The coastal region had the highest seroprevalence of 23.09% (1433) followed by Rayalseema with a seropositivity of 21.51% (703) and 16.03 % (184) in Telangana region.

Similarly the prevalence of leptospiral antibodies in different seasons of Andhra Pradesh was also studied during the period (**Fig. 3**). The highest seroprevalence of 28.29% during the South West monsoon followed by 21.45% in North east monsoon period. The lowest seroprevalence of 7.26% during summer followed by 12.22% in winter was observed.

District wise analysis of the results of the seropositivity of *Leptospira* in Andhra Pradesh revealed high seropositivity rate in west Godavari (34.00 %) followed by East Godavari (28.72 %) and low seropositivity of 4.83 % in Anapapur District was observed during the period of study (**Fig. 4**).

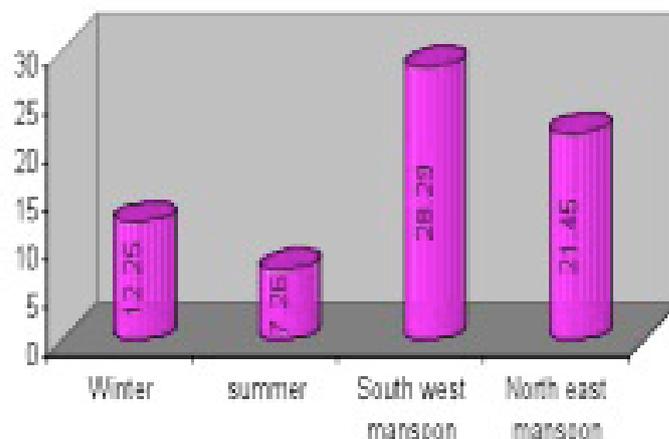


Fig. 3. Season wise percentage seroprevalence of leptospiral antibodies in Andhra Pradesh.

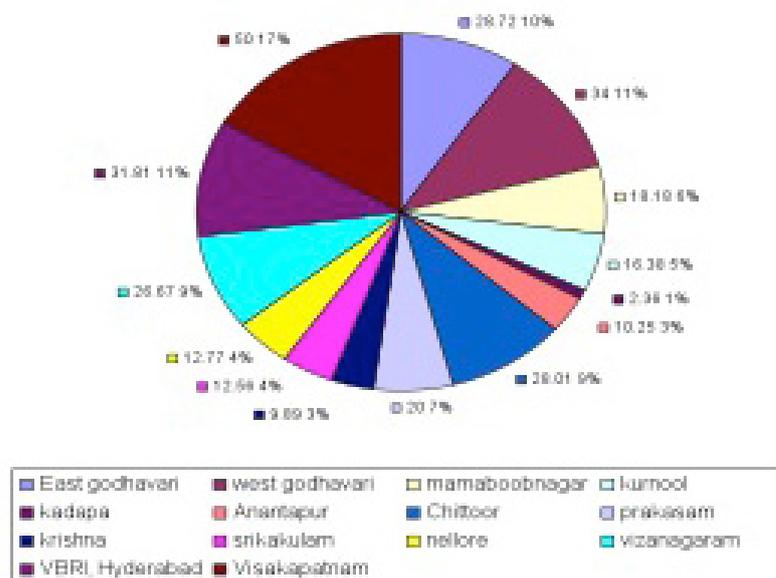
Discussion

A total of 2,320 serum samples representing different climatic zones, different species of animals and humans were collected during the period under study and subjected to MAT. Overall seropositivity of 20.09 % was observed in the state. The data was analyzed species wise, region wise and seasonal wise to understand the distribution of disease.

Seropositivity in case of clinically suspected cases of bovine, sheep, pigs, dogs and humans was reported to be higher compared to the apparently healthy animals due to active infection.

District wise analysis revealed the high prevalence in West Godavari district, due to natural vegetation with marshy lands and small ponds along with low humidity and temperature which is very much optimum for leptospiral growth, survival and perpetuation in the environment. Further, the habit of bathing of cattle in water bodies mostly contaminated with infected urine in the area helps in transmission of leptospires resulting in high seropositivity [11].

Fig. 4. District wise percentage seroprevalence of leptospiral antibodies in Andhra Pradesh.



The second highest prevalent district is East Godavari (28.72%), due to the presence of more number of rice field infested with rats that acts as carriers for leptospira [6]. Also the warm wet conditions with a pH close to neutral and slightly alkaline provides an optimum survivability of leptospira [19, 20]. After that comes Chittoor District (15%) followed by Prakasam and Nellore Districts (13.3%). Lowest seroprevalence was reported in Anantapur (4.83%) and Kadapa districts (5.63%). The low prevalence was attributed to low rainfall with high temperature prevailing in the districts. The maintenance of animals in households separately for milking purpose under clean hygienic conditions could also be one of the factors for low prevalence in the area. These conditions did not favor the transmission of the disease [3].

Significant difference in seropositivity was observed between the rainy seasons, in southwest and north east monsoons. The difference is due to favorable environmental conditions such as low temperatures and water logged areas. Overflow of sewages mixing and spreading of contaminated urine etc suitable

for survival and transmission of leptospira. Similarly, the favorable conditions at Andaman and Nicobar Islands (55 %age) reserved in high endemicity of the disease [14]. More number of cases was also reported from Tamilnadu during the rainy season [21].

Conclusion

The seroprevalence of leptospira in Andhra Pradesh was analyzed by Microscopic Agglutination Test (MAT). A total of 2,320 serum samples randomly from apparently healthy animals of cattle, buffaloes, sheep, goats, pigs, dogs and 258 serum samples from clinically suspected cases of both animals and humans from different districts of Andhra Pradesh were collected and subjected for MAT. The seropositivity of apparently healthy cattle, sheep, goats, pigs and dogs was found to be 19.0%, 18.67%, 14.63%, 36.84% and 15.15% respectively. Similarly seropositivity in clinically suspected cattle, sheep, pigs, dogs and humans was recorded as 28.94%, 21.69%, 31.43%, 50.00% and 61.76% respectively. The serum samples were screened against 9 refer-

ence serovars procured from ADMAS, Bangalore. Grippotyphosa followed by autumnalis and hardjo in cattle, hardjo followed by grippotyphosa in sheep, hardjo followed by grippotyphosa and javanica in goats, hardjo followed by grippotyphosa and autumnalis in pigs, canicola, javanica, autumnalis and hardjo in dogs due to environmental, pH of the soil, season, grass lands and rice fields of the region, small ponds with stagnated water and presence of rodents in particular areas are the main factors for the prevalence of respective serovars in different species of animals. In humans, hardjo, autumnalis, grippotyphosa and canicola were found to be commonly circulating serovars. The main predominant serovar in humans was observed during the study in Chittor district because of dairying and animal

husbandry activities. The percentage of seroprevalence of leptospirosis in clinically suspected cases (38.37%) was high compared to the apparently healthy ones (20.9%) indicating acute infections.

The prevalence of leptospirosis was noticed high during south west and North east monsoons. High prevalence related to the congenital environment for the survival of leptospire during the monsoons. The seroprevalence of leptospirosis in coastal regions was high (23.09 %), followed by Rayalaseema (20.90 %) and Telangana (16.30 %) regions.

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