Factors associated with leptospirosis in the district Valsad, Gujarat, India, 2008

By

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CERTIFICATION

This is to certify that this dissertation entitled ' **Factors associated with leptospirosis in the District Valsad, Gujarat, India, 2008** ' submitted by Dr. M. M. Lakhani in partial fulfillment of the requirements for the degree of Master of Applied Epidemiology is the original work done by him and has not been submitted earlier in part or whole for any other (Publication or degree) purpose.

Director,

National Institute of Epidemiology,

(ICMR), Chennai

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ABSTRACT

Background: Leptospirosis is endemic in Valsad since 1994. Average incidence is 3.5 per 1.00.000 population and case fatality 17.5 %. 1.6 million people are at risk. The disease incidence, case fatality increased in spite of preventive measures. We did the study with objectives of 1. To study factors associated with leptospirosis in Valsad. 2. Formulate recommendations.

Methods:

We did a prospective, matched case-control study in Valsad district, Gujarat during June to October, 2008. We defined a leptospirosis case as a disease with (1) >20 score in the WHO clinical assessment scale and (2) Titre > 1:80 in MAT or > 1:40 in ELISA IgM or four fold rising titre in paired serum sample by ELISA or PCR positive. We searched cases by active and passive surveillance. We recruited three controls per case at random from the same village matched for age group (+/- 5 year) and sex. The sample size was 44 cases and 132 controls. We collected information about general characteristics, potential exposures, and personal protective and hygienic practices using a pre-tested structured questionnaire. We did matched odds ratio and conditional logistic regression analysis of exposure factors.

Results: 74 suspected leptospirosis cases occurred in Valsad with incidence rate 4.46 per 1, 00,000 population and case fatality ratio 26 %. Highest and lowest incidence was 6 and 1 per 1, 00,000 in Valsad and Kaparada Taluka, respectively. The age group 45-59 years was affected most. No case was reported in 0-5 year age-group. Males were affected more but case fatality was equal. 10 factors were significantly associated with leptospirosis. Conditional multivariate logistic regression analysis indicated house compound wet(OR 16.28, 95% CI 3.12-84.91) and cuts/wounds on limbs(OR 8.92, 95% CI 2.37-33.53) as independent risk factors.

Conclusions: House compound wet and having cuts/wounds on limb are risk factors of leptospirosis in Valsad.

Factors associated with leptospirosis in the district of Valsad, Gujarat, India, 2008

Dr.M. M. Lakhani, MAE-FETP Scholar, VII Cohort, NIE (ICMR), Chennai, 27 Dec, 2008
Mentor: Dr. R. Ramakrishnan, Deputy Director, NIE (ICMR), Chennai, Tamilnadu, India.

1. Introduction:

Leptospirosis is an acute zoonotic disease caused by infection with spirochetes belonging to the genus leptospira[1, 2]. Clinically, it is characterized by fever, headache, myalgia, conjunctival suffusion, jaundice, breathlessness or oliguria. Main complications are renal, hepatic, pulmonary, haematological, brain and cardiac[1, 2]. Leptospirosis has high epidemic potential and the case fatality can be 05-40 %[1]. Rodents and animals are carrier of the disease. The urine of rodents, animals and dogs contains leptospira which contaminates the environment[3, 4, 5] Hence, leptospirosis is a potential occupational health hazards for those working in close proximity of such environment. The contaminated soil becomes source of infection for human beings. Heavy rain, wet and warm environment provides favorable environment for survival of leptospira[5]. Hence, Agricultural laborers, animal handlers, forest workers, sewage workers and outdoor workers who work in wet condition are at higher risk of leptospirosis. Cuts or wounds on limbs are known risk factors of the disease[5-8] Leptospirae can also be transmitted through mucosal surfaces, abrasions in skin, through the conjunctiva or by inhalation into lung of droplets containing leptospires[1]

The incidence of leptospirosis is very high in tropical and subtropical countries. It ranged from 18-128 per 100,000 in Barbados[9] and 40-60 per 100,000 in Seychelles[5]. Globally an average of 10,000 severe cases requiring hospitalization occurs annually all over the world[10]. Almost every country in South and Southeast Asia, South and Central America and several Island nations across the world are endemic to leptospirosis. Leptospirosis outbreaks have occurred in Seychelles (Indian Ocean) [5], Nicaragua[11], Argentina[12], Brazil[13]. Thailand[14], Colombia[15], Guana[16], Japan[17],Mexico[18], Bangladesh[19], Vietnam[20], Cubana[21] and India[22,23] . The incidence is increasing in developed countries.

In South East Asia Region, it is endemic disease as the economies of these countries are predominantly agricultural and more than 922 million people are engaged in agriculture. Rice is the major food grain cultivated in this region. Most of these countries have heavy rainfall resulting in water logging which predispose to occurrence of leptospirosis[24]. It affects reproductive group of people causing enormous economic loss[10].

In India epidemics have occurred in Andaman Islands[25], Gujarat[26], Maharastra[27], Kerala[28], Tamilnadu[29] and Orissa[30]. In Gujarat Valsad, Navsari and Surat are districts endemic for leptospirosis[26].1360 villages were affected in the 3 districts of south Gujarat region during 1994-2004. The case fatality was 7.6 % in 1994 with a peak of 22.5 % in 1996. Average case fatality was 11.4 % in this region[10,26].

In Valsad, leptospirosis cases occurred first time in 1994. 1.66 million people are at risk in Valsad district. Our field project of secondary data analysis indicated that during 2000-2006 the average incidence was 3.5 per 1,00,000

population and the case fatality 11.5 %. The highest incidence rate was 5 per 1,00,000 in the age group of 15-44 years. As compared to 2006, in 2007 the incidence rate increased from 2.5 to 6.38 per 1,00,000 population and the case fatality increased from 17.5 to 24 %[31]. Leptospirosis control programme was launched in 1996. Under the leptospirosis control programme of Gujarat health department[31], activities were done for (1) Information, education and communication (IEC) to increase awareness about leptospirosis disease (2) Daily house to house active surveillance by health workers during monsoon for early case detection and treatment of suspected cases (3) Weekly chemoprophylaxis with Cap. Doxycycline to the agricultural laborers in highly affected villages (4) Presumptive treatment of all cases of fever in high risk population with two capsule of doxycycline for seven days (5) Arrangement for prompt referral of the patients to higher treatment centre (6) Management of cuts, abrasions and treatment of the cases (7) Rodent control by pesticides during the month of May. Moreover, government of Gujarat had launched special campaign of cleanliness in Gujarat named as "Nirmal Gujarat"

The persistence of the disease and increase in the incidence and case fatality in spite of control measures emphasized the need for a study[32]. The information generated by this study will allow more effective prevention and control measures in the district as well as similar areas of Gujarat state with the disease. We did the study with following objectives of 1. To study factors associated with leptospirosis in Valsad district, Gujarat, 2008. 2. Formulate recommendations for prevention and control of leptospirosis.

2. Methods

Study population

The study population was permanent resident of Valsad district, Gujarat, India, 2008. The calculated midyear 2008 population of Valsad district was 16,60,000[33]. The characteristics of the area are plain fertile land, average rainfall of 200 cms per year and hot and humid climate. 73 % of the total population is rural. 70 % of rural population is engaged in agriculture work. Rice, sugarcane are main crops[34]. These factors are favorable for transmission of leptospirosis. Hence, the study population is relevant to answer the research question.

Study design

We conducted a prospective matched case control study during monsoon period of June to October, 2008.

Inclusion criteria: We included cases of leptospirosis confirmed by laboratory tests, as per case definition.

Exclusion criteria: We excluded all suspected cases that were not confirmed or who died before laboratory confirmation. We excluded malaria patient positive by blood smear examination. We also excluded cases belonging to other district.

Case definition

A leptospirosis case was defined as a disease with (1) >20 score in the WHO clinical assessment scale[1] and (2) PCR positive or Seroconversion for serology by Microscopic Agglutination Test (MAT) titre>80 or ELISA titer >40 in one serum sample or four fold rising antibody titre in paired sera taken 14 days apart[35,36]

Sampling procedure:

We searched for symptomatic cases by house to house active surveillance with help of multipurpose health workers of health department, in all the 470 villages of Valsad. We also searched cases by passive surveillance at primary health centre (PHC), community health centre(CHC) and all major hospitals of the Valsad district during June to October, 2008. The medical officer suspected case of leptospirosis, the block health officer verified and assigned a unique line listing number (LL No.) to the case-patient. We interviewed case-patients having > 20 score as per WHO scoring form[1]. 47 of the total 74 suspected cases were laboratory confirmed. We removed 3 cases randomly from the list and and recruited 44 cases for our study.

We selected persons as controls who were free from any signs/symptoms of leptospirosis for the last 30 days of onset of the disease in the case, We included controls, prospectively who were residing in the village at least for the last one year. To recruit controls, we selected a house in the neighborhood of the case-patient by using last number of any currency note taken randomly. A healthy person matching age (+/- 5 years) and sex was taken as first control. In the similar way, we recruited second and third control randomly. We recruited three age-sex matched controls for each case. Thus, we recruited 132 controls for the 44 cases. We followed the controls prospectively for period of 30 days to rule out occurrence of disease. No control had any sign/symptom of leptospirosis, during that period.

Sample size

We used Epi info 3.3.2 statcal, software to calculate sample size. The sample size was estimated for a power of 80%, an alpha error of 5% and a frequency of exposure of risk factor (agriculture population) among controls 70 % to detect an odds ratio of 3 with three controls per case. So, our sample size was 44 leptospirosis cases and 132 controls. We interviewed 10 % more cases and controls for non-response.

Data collection: We designed data collection instruments as semi-structured questionnaire. We translated the data collection instruments in the local language Gujarati and back translated in English. We pilot tested the data collection instruments in the field before actual study and made necessary changes. We trained medical officers and health staff during May, 2008. We collected the data with help of trained health staff using personal interview, observation of premises and review of medical and laboratory records. We collected information from cases and controls about general characteristics, demographic, socioeconomic characteristics, occupational exposure, cuts and abrasions, hygiene and health seeking behavior practice. Staff of agriculture and veterinary department helped in collecting information about animals and rodents population. The health supervisors supervised the health workers.

Laboratory specimen collection, transport and analysis

The multipurpose health worker/supervisor skilled in blood sample taking or laboratory technician collected first blood sample from the clinically suspected case of leptospirosis. We tested the specimens at microbiology department of government medical college, Surat, Gujarat. We collected

paired sera sample from the same patient after 14 days and tested in the similar way.

Data analysis:

Data entry: We used Epi info software version 3.3.2. Trained data entry operators entered data under our guidance. Two different persons had entered and re-entered the data and compared for the correctness.

Descriptive epidemiology:

We calculated incidence rate of leptospirosis by dividing total number of the suspected leptospirosis cases with population under study. We plotted the cases by week of onset to get epi-curve to know time trend. We calculated age-sex specific and blockwise attack rates. We plotted on a map geographical distribution of cases.

Analytical epidemiology:

We measured the frequency of exposures to specific factors among cases and controls. We calculated matched Odds ratio with 95 % confidence interval and p value to measure the strength of the association of the factors with leptospirosis. We did stratified analysis of risk factors to detect and minimize confounder and effect modifiers. We also did multivariate logistic regression analysis for the factors found significant in univariate analysis to find independent risk factors of leptospirosis.

Quality assurance

We developed protocol, submitted for peer review before the faculty and other MAE-FETP Scholars and revised. We sought clearance from ethical committee of National Institute of Epidemiology (NIE), Indian Council of Medical Research, Chennai. The principle investigator cross checked 10%

of the participant interviews. The block health officers and medical officers cross check 20% of the field works. We followed standard laboratory methods and procedures for collection, transportation and testing of specimens. The head of the department of microbiology, government medical college, Surat, Gujarat supervised for quality assurance.

Human subject protection

We could not find any leptospirosis case in vulnerable population of pregnant women or children. We followed the ethical guideline of Indian Council of Medical Research for human subject protection.

Risks

We interviewed patients without disturbing treatment schedule. We considered comfort and convenience of the participants. This laboratory investigation was a part of routine investigation for the treatment of the disease. We used a new syringe and needle to take blood samples and took all universal aseptic and antiseptic precautions. There was no adverse event due to the questionnaire or taking specimen. We managed the interviews with the help of the medical officers. We followed the ethical guidelines for testing of blood samples.

Benefits

The case-patients benefited for early diagnosis and treatment. We educated the controls for the disease. No compensation was given for participation in the study and this was informed to the participants before taking consent.

Confidentiality

To protect the confidentiality of study subject we used only an identifier code. We kept it under lock and key and destroyed after the project. We ensured privacy of the participants during an interview.

Informed consent

We explained the participants the objectives of the study in their local language Gujarati. We told them their rights and obtained written informed consent in presence of a witness.

Ethical committee clearance

We sought approval of study protocol the ethical committee of National Institute of Epidemiology (ICMR), Chennai, Tamilnadu, India.

3. Result:

Total 74 cases of suspected leptospirosis occurred in Valsad during June to October, 2008. The first case occurred on 7th June with peak in 2nd week of august and last case on 6th October, 2008. All over incidence rate was 4.46 per 1, 00,000 population. All the 5 blocks of Valsad district were affected. Highest and lowest incidence was 6 and 1 per 1, 00,000 in Valsad and Kaparada Taluka, respectively. All over case fatality of suspected cases of leptospirosis was 26 %. The age group 45-59 years was affected most with attack rate 16.87 per 1, 00,000 and cases fatality of 32 %. No case was reported in 0-5 year age group. Males were affected more than females with attack rate 6.37 against 2.38 per 1, 00,000 population. Sexwise case fatality was almost equal (25 % against 26 %). The cases were tested for leptocheck rapid test, ELISA, MAT and PCR. 47 (64%) of the 74 cases were

positive by at least one laboratory test. 32 (73%) cases were positive by ELISA and 27(61%) by MAT or PCR. The types of serovars found were *L. icterohaemorrhagiae, L. canicola, L.grippotyphosa, L.automonalis, L. pyrogen. L.hebdo L. australis,* and *L. Pomona*

We included 44 laboratory confirmed cases in our study. 24(55%) cases had liver and kidney involvement,14 (32%) cases had lung involvement (Table 2). All the 44 cases(100%) had fever followed by myalgia 33(75%), headache (66%), jaundice (48%) and conjunctival suffusion (32%). 31(70%) cases had contact possible contaminated water. 34(77%) cases had exposure to agriculture work.

The matched case control study indicated 10 factors as significantly associated with leptospirosis. In stratified analysis, association of house compound wet and leptospirosis was stronger amongst persons having rats at home (OR 9.94, 95% CI 3.60-27.46, P 0.0000) as compare to not having rats.

Conditional multivariate logistic regression analysis of all above significant factors identified two factors, house compound wet (OR 16.28, 95% CI 3.12-84.91, P 0.001) and cuts or wounds on limbs (OR 8.92, 95% CI 2.37-33.53, P .0012) as independent risk factors of leptospirosis.

4. Discussion:

Total 74 cases of suspected leptospirosis occurred in Valsad during June to October, 2008 with incidence rate 4.46 per 1,00,000 population which is comparatively less than other tropical countries[5,6,7](Figure 1). All the five blocks of Valsad district were affected (Figure 2). The case fatality of was 26 %. Incidence and case fatality was highest in of 45-59 year age group[10, 26].

Higher age group was affected in Valsad as compared to previous years[26]. Leptospirosis is known to affect male productive group of people . Males were affected almost three times more than females but case fatality was almost equal (Table 1). Liver and kidney involvement was more followed by lung involvement (Table 2). All the cases had fever. Other main signs/symptoms were myalgia, headache, jaundice and conjunctival suffusion (Table2). Agriculture workers were highest affected followed by other laborer.

We recruited 44 laboratory confirmed cases for analytical study. The analysis indicated factors that affected the risk of leptospirosis in Valsad district of Gujarat. Living in Kaccha house, illness in animals in house, rats at home, contact of contaminated water, agriculture work, outdoor occupation, swimming/bathing in river, house compound wet and having cuts or wounds on limbs were risk factors for leptospirosis. In further conditional logistic regression multivariate analysis, two of the factors were found significant. The first was related to condition of house premise and the second to hygienic and protective practices. A review of these results provides some understanding of the practices that expose the community to leptospirosis and provide useful direction to suggest behavior change interventions for prevention.

House compound wet and having cuts or wounds on limbs were strongly associated with leptospirosis in Valsad. The exposure to cuts or wounds on limbs was nine times higher and house compound wet was sixteen times higher in cases than controls. Other studies in different area indicated that walking on wet land was associated with leptospirosis[1,4,5,8]. Cuts or wounds

were reported as risk factors of leptospirosis in other studies done in Seychelles (Indian Ocean), Hawaii islands and Thailand[5,6,7,8]. Thus, our study is consistent with other studies.

Wet land provides environment to leptospirae organisms to grow effectively[1,5]. The wet compound of houses may be contaminated by urine of cattle or rats in rural area[1,3,4,5]. In our study, the stratified analysis indicated that the association between house compound wet and leptospirosis was stronger amongst persons having rat at home. In rural area of Valsad district, large proportion of houses are made of mud. The house compound remains wet due to rain and practices of people to discard waste water nearby houses. Cut or wounds on limbs are common in rural people and people have tendency to ignore them. This makes them vulnerable to leptospirosis. Cuts or abrasions in skin facilitates the entry of leptospires.[1], Hence, the biological correlation also supports the result of our study.

Our study suggests that there may be opportunities to prevent leptospirosis through addressing the sources of infection. These include house compound wet and having cuts or wounds on limbs. To reduce incidence a number of interventions need to take place. First, we need to advocate community for keeping house compound dry and clean, prevent unsanitary environment in the surrounding of houses, creating awareness by campaign of " Nirmal Gujarat" and doing minor engineering works in the house compound to prevent stagnation of water. Second, we need to promote hygienic and protective practices amongst the community for preventing cuts or wounds on limbs. Educate people regarding the risk of leptospirosis associated with having cuts or wounds on limbs and motivate them to wear footwear to

protect. We need to promote health seeking behavior of people to take early treatment for cuts or wound on the limbs. Educate them to minimize exposures to risk factors of leptospirosis while having cuts or wounds. Further studies could characterize the strength of association of leptospirosis with environment sanitation of housings and types, severity and duration of cuts and wounds. Finally, public health surveillance will provide an opportunity to evaluate the effectiveness of the proposed prevention, promotion and curative measures.

Limitation:

The regular health staff of health care system collected the data which might have caused selection and information bias. We minimized the probable bias by following random selection of controls using last digit of a currency note. The specificity of ELISA and MAT tests is 90 %. This could have lead to misclassification of cases. To minimize it we used case definition combining clinical and laboratory criteria. The patients were aware of their diagnosis at the time of interview which could result in bias leading to overestimation of strength of association. To overcome the bias we combined interview with observation of the environment for risk factors. The study was done in the restricted area of Valsad district. Epidemiology of leptospirosis may differ in other places. Hence, the risk factors may not be similar elsewhere. Findings can not be generalized to larger population, but can be generalize to similar areas having similar environment, socioeconomic and cultural habits. We did not test serum of controls to rule out leptospirosis infection, as our object was to study clinical cases and not prevalence of infection. To rule out misclassification of controls, we followed them for 30 days of incubation

period. We did not find any sign/symptoms of leptospirosis in controls during follow up period. We studied during monsoon period of June to October, 2008. Hence, the study might have failed to identify risk factors resulting from other seasonal activities. As such, cases were reported only in monsoon season in Valsad since 1994[26]. So, it is less likely to have other seasonal risk factors. We did not include water sports as risk factor in our study as they are not found in study area.

Conclusion:

House compound wet and having cuts or wounds on limb are risk factors of leptospirosis in Valsad district, Gujarat, India.

Recommendations:

We recommend creating awareness in the community regarding risk of leptospirosis associated with house compound wet and having cuts and wounds on limbs. Educate people for keeping house compound dry to protect them. Improve housing to ensure that house compound does not remain wet. The village Panchayat can take measures to prevent stagnation of water nearby houses during rainy season. Promote hygienic and protective practices amongst people for cleanliness of limbs and wearing footwear to prevent cuts and wounds on limbs. Promote health seeking behavior in the community for treatment of cuts and wounds. Educate people to minimize exposures to risk factors of leptospirosis while having cuts and wounds on limbs.

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***Figure 1* Cases of suspected leptospirosis by weeks of onset, Valsad, Gujarat, India, 2008**

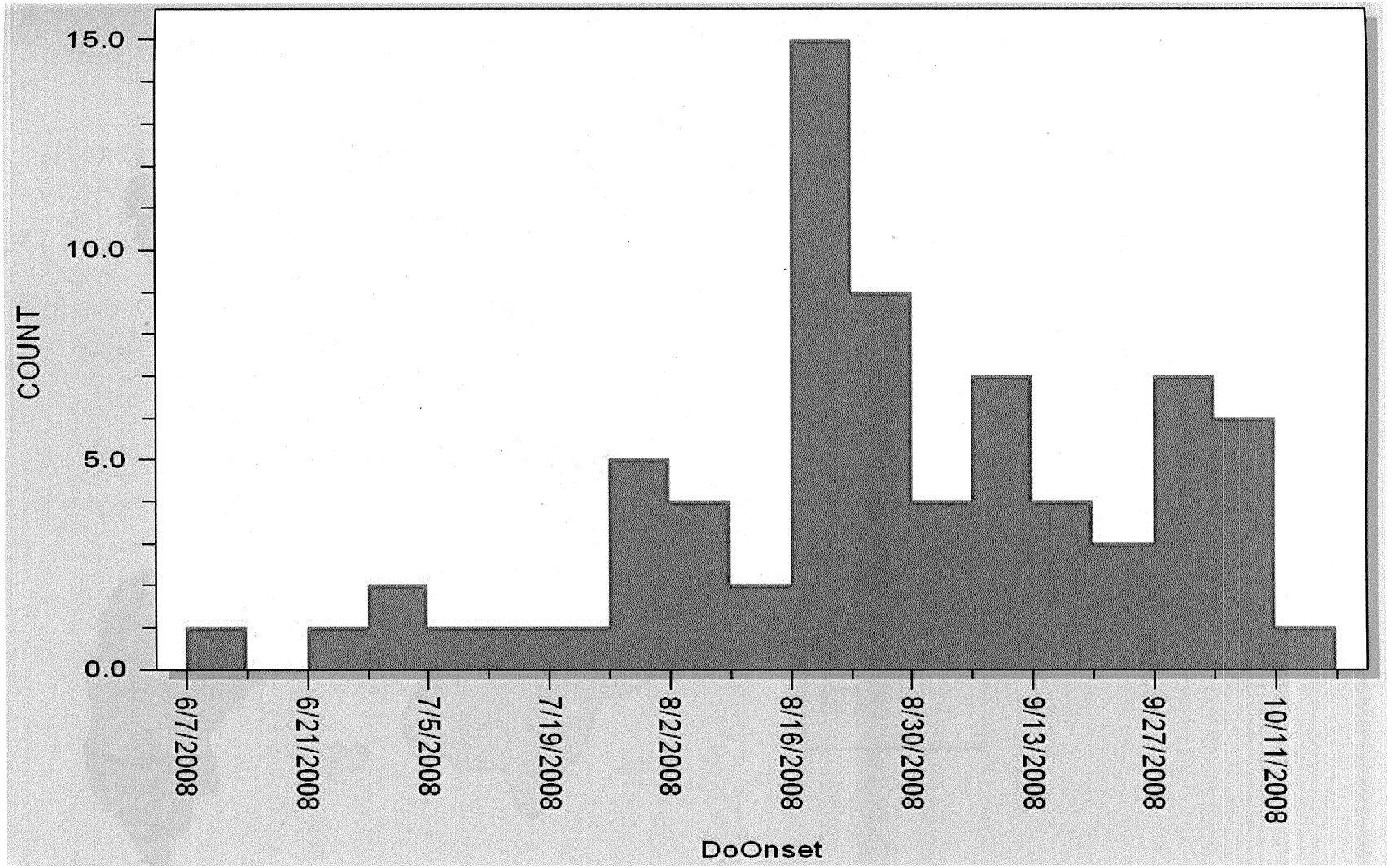

Figure 2 Incidence of suspected cases of leptospirosis by blocks, Valsad, Gujarat, India, 2008

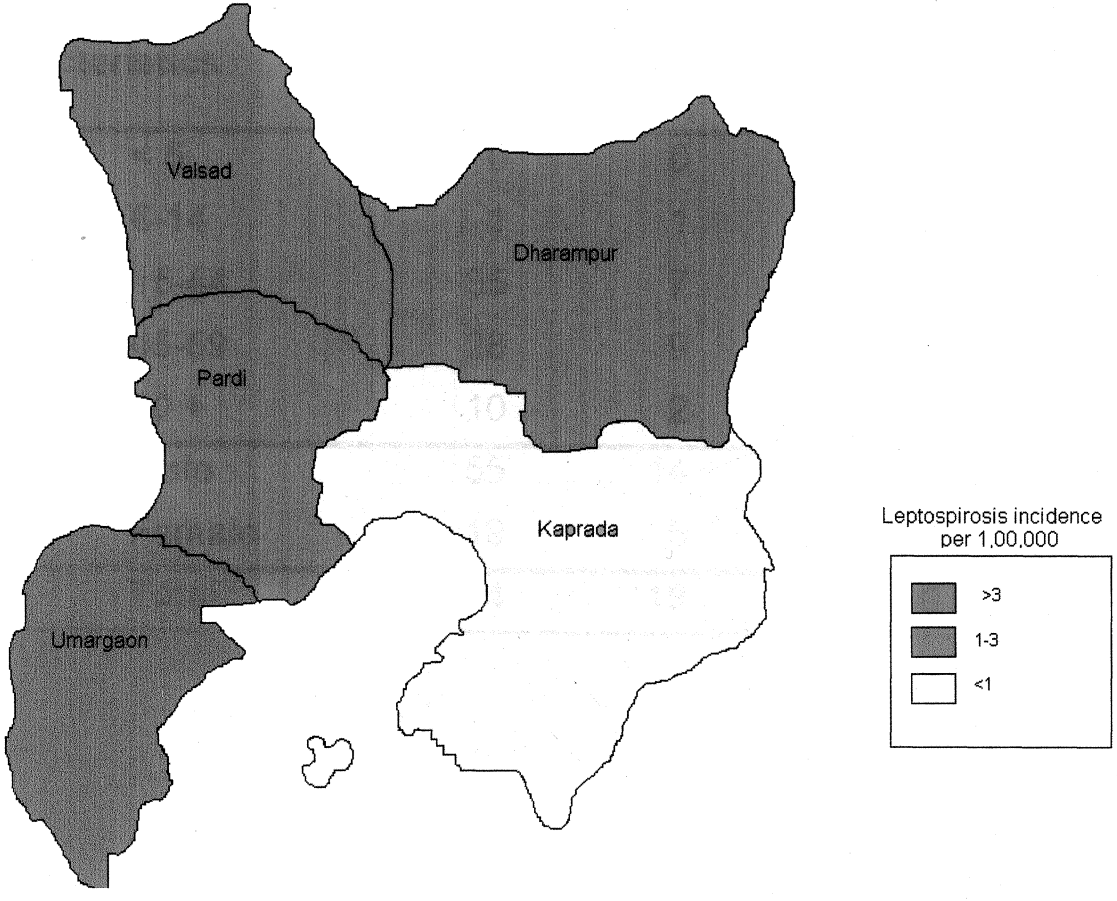

Table 1: Incidence of suspected cases of leptospirosis by age and sex, Valsad, Gujarat, India, 2008

Demographic characteristics		Cases	Death	2008 population	Attack rate per 100,000	Case fatality ratio(%)
Age group (Years)	< 5	0	0	215800	0	0
	6-14	1	1	398400	0.26	100
	15-44	35	7	763600	4.58	20
	45-59	28	9	166000	16.87	32
	60 +	10	2	116200	8.6	20
Sex	Male	55	14	863200	6.37	25
	Female	19	5	796800	2.38	26
	Total	**74**	**19**	**16,60,000**	**4.46**	**26**

Table 2: Characteristics of leptospirosis cases, Valsad, Gujarat, India, 2008 (n=44)

Characteristics		#	(%)
Severity	Without complication	14	32
	With complication	30	68
Organ involvement	Liver	24	55
	Kidney	24	55
	Haematological	23	52
	Lung	14	32
	Cardiac	0	0
	Brain	0	0
Clinical description	Fever	44	100
	Myalgia	33	75
	Headache	29	66
	Jaundice	21	48
	Oliguria	16	36
	Conjunctival suffusion	14	32
	Bleeding	3	7
	Breathlessness	3	7
	Cough	3	7
	Meningism	0	0
Other characteristics	Contact contaminated water	31	70
Laboratory results	MAT Positive	27	61
	ELISA Positive: Single high titre	32	73
	ELISA Paired sera rising titre	23	52
	PCR Positive	27	61

Table 3: Frequency of baseline characteristics among leptospirosis cases and controls, Valsad, Gujarat, India, 2008. (cases=44, controls=132)

1. Baseline characteristics		Cases	%	Controls	%	Chi Sq.	P
Age	>20 years	44	100	132	100	0	1
Sex	Male sex	33	75	99	75	0	1
Living area	Rural	41	93	123	93	0.12	1
Caste	ST/SC/OBC	44	100	119	90	3.35	0.04
Education	Illiterate	16	36	36	27	1.31	0.25
Income	Monthly Income<1200	21	48	42	32	3.63	0.056

Table 4: Univariate matched odds ratios, confidence intervals of potential exposures in leptospirosis cases and controls, Valsad, Gujarat, India, 2008

Exposure within 30 days of onset / interview		Cases(44), Control(132) Concordant for exposure status		Discordant for exposure status)		Matched OR	95% CI
		Exposed	Unexposed	Exposed	Unexposed	M-H	
1. Baseline characteristics							
Caste	ST/SC	99	6	24	3	8	1.28-49.86
Education	Illiterate	17	65	31	19	1.63	0.73-3.61
Income	Monthly Income<1200	28	55	35	14	2.5	1.07-5.8
2. Living environment							
Status of house	House in low-lying land	0	128	3	1	3	0.19-47.96
	House compound wet	52	6	65	9	7.22	2.76-18.92
Type of house	Kachcha house	58	26	35	13	2.69	1.1-6.58
Animals in house	Any animal	49	38	26	19	1.37	0.59-3.16
	Cattle	33	51	33	15	2.2	0.94-5.17
	Goats	6	101	12	13	0.92	0.30-2.81
	Dogs	3	118	6	5	1.2	0.29-6.31
Illness in animals	Any illness noted	0	108	21	3	7	1.59-30.80
Exposure to rodents	At home	96	5	27	4	6.75	1.40-32.66
	At work place	52	41	26	13	2	0.78-5.16
Water source for drinking	Stream	0	132	0	0	0	0
	Well	12	97	9	14	0.64	0.19-2.17
	Hand pump	65	29	22	16	1.37	0.54-3.48
	Tap	19	96	8	9	0.89	0.23-3.46

Table 4 Continues...	Characteristics	Exposed	Unexposed	Exposed	Unexposed	Match OR	95 % CI
	Stream	2	128	1	1	1	0.02-50.40
Water source for washing	Well	14	90	16	12	1.33	0.47-3.76
	Hand pump	64	32	20	16	1.25	0.49-3.2
	Tap	12	100	6	14	0.42	0.10-1.78
	Cow dunk/urine in yard	27	53	33	19	1.74	0.80-3.78
Peri-domestic environment	Animals in the yard	35	52	22	23	0.96	0.42-2.19
	Pigs around house	3	123	6	0	undefined	
Contact	Contact contam. water	37	26	56	13	4.31	1.84-10.10
	With animals	13	81	26	12	2.17	0.85-5.52
	Milking cattle	6	99	12	15	0.8	0.27-2.38
Activities Working	On a farm	48	19	52	11	4.91	1.97-12.26
	Occupation outdoor	104	8	16	4	4	0.78-20.50
	Wash/bath after work	122	3	4	3	0.133	0.16-11.14
	Swim in stream/river	3	96	30	3	10.00	2.67-37.42
	Walk barefooted	119	3	4	6	0.67	0.12-3.78
Hygiene and protective practices	Poarch cleaned	51	32	24	25	0.96	0.43-2.12
	Cuts/wounds on limbs	9	47	69	7	9.86	4.09-23.74
	Medicine taken	8	82	19	23	0.83	0.35-1.97
	Antibiotic taken	2	105	7	18	0.39	0.11-1.39

Table 5 Conditional logistic regression of significant exposures of leptospirosis, Valsad, Gujarat, India, 2008.

Exposures in 30 days	Odds Ratio	95 % C.I.	P-Value
House compound wet	16.278	3.12-84.91	0.00
Rats at home	9.8628	0.90-108.36	0.06
Cuts-wounds on limbs	8.923	2.37-33.53	0.00
Swim/bath in river/stream	3.9504	0.60-26.17	0.15
Agriculture work done	3.2561	0.55-19.13	0.19
Contact contaminated Water	1.9921	0.50-8.00	0.33
Monthly income<1200	1.4313	0.34-5.95	0.62
Handle animals	1.3761	0.30-6.41	0.68
Animal ill in house	0.9991	0.06-17.32	1.00
Cow/ox in house	0.1754	0.026-1.16	0.07

ANNEXURE: 1

Consent form for study on factors associated with leptospirosis in the district Valsad of Gujarat, India, 2008

Hello,

I am and am working with the health department, district panchayat, Valsad to look into factors that may put you at risk for leptospirosis or protect you from them. We are doing this research as a response to more than hundred cases of leptospirosis in Valsad between June and October in the year 2007. The National Institute of Epidemiology, Chennai is also working with us.

To find out factors associated with leptospirosis disease, we need to ask questions about the risk factors for the disease to persons who had leptospirosis and to healthy persons. Thus, between June 08 and October 2008, we will be asking the same questions to some of the persons with leptospirosis, as well as to some healthy members of the area. We would like to confidentially ask these few questions to you once. Answering these questions should take about 30 minutes of your time.

For cases only: For this study, we will go to your village and ask the same questions we asked you to three of your neighbors. However, we will not mention any medical information about you and we will not mention that we have come and see them because there was a case of disease in their area. We would like to make a few blood tests and take 5 ml or one tea spoonfuls of blood on two occasions, at intervals of fourteen days. We will use a new, single use syringes and needle and follow standard hygiene rules. You may experience only a slight pain during collection of the blood

sample. These tests are also part of the regular medical care that you need. They will be sent for testing at the microbiology department, Government medical college, Surat, Gujarat. The blood will be tested for leptospirosis only and nothing else. Any leftover blood will be discarded.

Taking part in this survey is voluntary. No compensation will be paid to you for taking part in this study. You can choose not to take part. You can choose not to answer a specific question. You can also stop answering these questions at any time without having to provide a reason. This will not affect your rights to health care in the primary health centre, community health centre/referral hospital, Civil Hospital, Valsad, or any other rights. There is no specific benefit for you if you take part in the survey. However, taking part in the survey may be of benefit to the community, as it may help us to understand the problem, its causes and potential solutions. When the results will have been analyzed, a report will be shared with all the participants and the local health officials concerned with public health, so that the right measures can be taken to prevent and control leptospirosis in Valsad as well as Gujarat.

The information we will collect in this survey will remain between you and the health department. We may ask questions about various specific things you do. This does not mean that we think that these things you do would put you at risk for leptospirosis. It does not mean either that we think that these things you do would protect you from leptospirosis either. We will not write your name on this form. We will only use a code instead. Only the doctor will know the key to this code. It will be kept under lock and key. It will be destroyed after the project.

If you wish to find out more about this survey before taking part, you can ask me all the questions you want. You can contact Dr.M.M.Lakhani, MAE-FETP Scholar (VIIth Cohort) and principal investigator of this survey attached to the National Institute of Epidemiology, Chennai, at the health branch, Valsad or chief district health officer attached to the health branch, Valsad, who will be happy to give you more details. If you are OK to take part, we will go ahead now.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw from the study at any time without in any way it affecting my further medical care.

Name of the study participant or guardian (if minor)	Signature/thumb impression of the study participant or guardian
Name of the witness	Signature of the witness
Name of the interviewer	Signature of the interviewer

Annexure 2

Identifier collection sheet

Personal identifier segment

Status of participant: Case/Control Date of the interview:

Name of participant: _____

Primary health center's name: _____

Name of sub centre: _____

Name of the village:_____

Age in complete years: ____ years

Gender: 1. Male 2. Female

Religion: 1. Hindu 2. Muslim 3. Christian 4. Others

Caste: 1. General 2. ST 3. SC 4. Other backward caste (OBC)

Participant's identifier code: | | | | | | |

First box is for district Valsad (V), second for the block, third for rural (R) or urban (U); four to six are for numbers of the volunteer. The Block codes are given below:

	Valsad	Pardi	Umergaon	Dharampur	Kaparada
Codes	Va	Pa	Um	Dh	K

Annexure 3

Data collection instrument items for inclusion in the questionnaire / structured observation guide, Date of interview:

Leptospirosis study, Valsad, Gujarat, India, 2008.

Participant's Identifier Code | | | | | | |

• What is your age?	_____	Years
• What is the respondent's sex?	1. Male	2. Female
• What is your religion	1. Hinduism	2. Muslim
	3. Christianity	4. Other
• What is your caste?	1. General	2. Scheduled tribe
	3. Schedule caste	4. backward caste
• Type of residence	1. Urban	2. Rural
• Level of house	1. Average	2. low lying
• Type of house	1. Kaccha (made of mud wood)	2. Pakka (made of brick and cement)
• What is your occupation?	1. Animal herding	2. Farming
	3. Other labour	4. Trade
	5. Home maker	6. Teaching
	7. Office work	8. Student
	9. Unemployed	9. Other
• Could you tell me what is your monthly average family income ?	_____	Rupees
• Up to what level have taken education?	0. No education	1.<=7 std.
	2. 8-12 std	4.>=graduate
• Do you have animals in the house?	1. Yes	2. No
• If yes, which animals are there ?	1. Cow/ox	2.Buffelo
	3. Goat	4. Dog
	5. Cat	
• How many animals do you have?	1.<5	2.>=5
• Was any animal ill within 30 days prior to the onset of symptoms/ before?	1.Yes	2. No

• How frequently did you see rodents in / around your house within 30 days prior to the onset of symptoms/ before?	0. Never	1. Occasionally (1-3 times a week)
	2. Often (>3 times a week)	3. Daily
• How frequently did you see rodents in your areas of activity within 30 days prior to the onset of symptoms/ 30 days before?	0. Never	1.Occasionally (1-3 times a week)
	2. Often (>3 times a week)	3. Daily
• Do you rear animals in the yard ?	1. Yes	2. No
• If yes, how many:	_____	Animals
• Is there cow dunk-urine in the yard ?	1.Yes	2. No
• How often do you find pigs around your house?	0. Never	1. Occasionally (1-3 times a week)
	2. Often (> 3 times a week)	3. Daily
• Did you have contact of dirty water within 30 days prior to the onset of symptoms/30days before?	1. Yes	2. No
• Did you handle animal in 30 days prior to the onset of symptoms/ before?	1. Yes	2. No
• Did you do agriculture work within 30 days prior to the onset of symptoms/ before?	1. Yes	2. No
• Were you involved in milking of cattle within 30 days of onset of symptoms/30days before?	1. Yes	2. No
• Did your house compound remain wet within 30 days prior to the onset of symptoms/30days before?	1. Yes	2. No
• How frequently did you pass across wet land within 30 days prior to the onset of symptoms/30days before?	0. Never	1. Occasionally (1-3 times a week)
	2. Often (>3 times a week)	3. Daily

• From where do you get water for drinking?	1. Stream	2.Well
	3. Hand pump	4. Tap
• From where do you get water for drinking?	1. Stream	2.Well
	3. Hand pump	4. Tap
• How frequently did you suffer from cuts/wounds within 30 days prior to the onset of symptoms/30days before?	0. Never	1. Occasionally (1-3 times a week)
	2. Often (>3 times a week)	3. Daily
• If suffered, did you take treatment for cuts/wounds within 30 days prior to the onset of symptoms/30days before?	1. Yes	2. No
• How many times did you wash yourself/bathe after the daily activities within 30 days prior to the onset of symptoms/30days before?	0. Never	1. Occasionally (1-3 times a week)
	2. Often (>3 times a week)	3. Daily
• How many times did you swim in a stream within 30 days prior to the onset of symptoms/30days before?	0. Never	1. Occasionally (1-3 times a week)
	2. Often (>3 times a week)	3. Daily
• How frequently did you use tools to clean premise within 30 days prior to the onset of symptoms/30days before?	0. Never	1. Occasionally (1-3 times a week)
	2. Often (>3 times a week)	3. Daily
• Did you take any medicine within 30 days prior to the onset of the symptoms/ in 30 days?	1. Yes	2. No
• If yes, mention the details (Verify the prescription/bills)		

Signature of the interviewer

SECTION: 2

LITERATURE REVIEW

Section II: Review of literature: Leptospirosis

1. Introduction

Leptospirosis is an acute bacterial infection caused by spirochetes belonging to family leptospiraceae[1.2.3.4.5]. It is a zoonosis of worldwide distribution, endemic mainly in countries with humid subtropical or tropical climates and has epidemic potential[6]. Heavy rain, hot and humid environment favors the survival of the leptospires organisms[1,2,3,10] In the developed world, most cases are associated with recreational exposure to contaminated water[2,7,9]. The incidence is increasing in developing countries. It is an occupational hazard in the south east Asia region (SEARO) for the people working in the agriculture, animal herding, forest and sewer cleaning, abattoir , mine works[8] . Thailand, where leptospirosis is under surveillance, has recorded increase in incidence[17] . The incidence of leptospirosis is very high in tropical and subtropical countries. It ranged from 18-128 per 100,000 in Barbados[18] .In a study the incidence of leptospirosis in Seychelles was found 40-60 per 100,000[10.] On an average 10,000 severe cases requiring hospitalization occur annually all over the world[19] Outbreaks have been reported in other countries of Nicaragua[20], Argentina[21]and Brazil[22]. Thailand[23], Colombia[24], Guana[25], Japan[26],Mexico[27], Bangladesh[28], Vietnam[29], Cubana[30] and India[31,32]

Leptospirosis is emerging disease in India[32] . In India epidemics have occurred in Andaman Islands[34], Gujarat[35], Maharastra[36], Kerala[37], Tamilnadu[38] and Orissa[39]. Some of these were as a result of natural calamities such as post cyclone outbreak in Orissa[39] and post flood in Mumbai[36].

In Gujarat Valsad, Navsari and Surat are districts endemic for leptospirosis[23,35].1360 villages were affected in the 3 districts of south Gujarat region during 1994-2004. The case fatality was 7.6 % in 1994 with a peak of 22.5 % in 1996. Average case fatality was 11.4 % in this region[23]. In Valsad, leptospirosis cases occurred first time in 1994. 1.66 million people are at risk in Valsad district. Our field project of secondary data analysis indicated that during 2000-2006 the average incidence was 3.5 per 1,00,000 population and the case fatality 11.5 %.

Historical aspects

Stimson demonstrated the first one of leptospira (Abdussalam M *et al*, 1965) which was subsequently confirmed by WHO research fellows doing work on leptospirosis during 1962-65. Faine S. has mentioned about similar occupational hazard of farmers in china

Public health importance

Leptospirosis is worldwide public health problem (World Health Organization, 2003). Leptospirosis affects productive group of people which cause great socioeconomic impact. It has high case fatality ratio of 5-40% (Singh SS *et al*, 1999).Leptospirosis accounts for about 12.7% of cases of acute febrile illness attending Hospitals in India (Sehgal SC *et al*, 2003). In Thailand, the annual incidence of leptospirosis increased from 0.3/100,000 to 3.3/100,000 population during the period 1982 – 1995 to 1997 – 98[17]. Sehgal SC *et al* (1995) reported leptospires as the aetiological agents in Andaman Haemorrhagic Fever (AHF) in North

Andaman in 1993. Zaki SR & Sheih WJ (1996) also reported that leptospires was the aetiology of the acute febrile illness with pulmonary haemorrhage in Nicaragua in 1995. Ko AI *et al* (1999) reported leptospirosis outbreaks in urban Salvador in Brazil through a hospital based surveillance system where the case fatality rate was 15%. In a study following the super-cyclone in Orissa in 1999, 14% of the study subjects had febrile illness due to leptospiral infection (Sehgal SC *et al* (WHO, 2001). A study by Karende S *et al*(2002) in Mumbai during heavy rainfall and floods in July – August 2001, 30 (32%) the 93 children having suspected leptospirosis were confirmed for leptospirosis based on rapid diagnostic tests. A seroprevalence in North Andaman was of 54% among apparently healthy population (Murhekar MV *et al*, 1998). The study also indicated seroprevalence more than 72% in older adults. High seroprevalence was observed among some of the primitive tribes of Andaman and Nicobar Districts (Sehgal SC *et al*, 1999a).

Ratnam S *et al*(1983) studied the seroprevalence among the people of a village near Chennai City following an outbreak of leptospirosis in cattle. 47% of the participants showed antibodies against leptospires. In a study conducted Vado-solis I *et al*, 2002 in Yucatan State of Mexico 57 (14.25%) of the 400 participants were found positive for leptospires. A study in Seychelles (Bovet P *et al*, 1999) showed 9% point prevalence of assymptomatic leptospiral infection as proved by positive PCR. (Sehgal SC *et al*, 2000a)reported 27% seropositivity in Andaman Island.

Epidemiology of leptospirosis:

Agent factors:

Pathogenic leptospires are the agent of leptospirosis which belong to the genus *Leptospira. They are* long corkscrew-shaped bacteria, too thin to be visible under the ordinary microscope. The organism leptospira is tightly coiled, thin spirochete measuring 5-7 micron. The end is bent into a hook[11] . They are motile by a flagellar action[2]. They are Gram negative. There are 25 groups and 200 serovars of leptospira[5] . WHO reports more than 240 pathogenic serovars[3]. Genus leptospira consist of two spieces L. interogan and L.Biflexa. The former is pathogenic strain. Other leptospires recognized include *L. icterohaemorrhagiae, L. canicola, L.grippotyphosa, L. Valsada, L. australis, L. bataviae, L. tarassovi* and *L. Pomona.* Rodents and cattle are carrier that sheds leptospira in urine for years. Leptospira can survive for long periods in water and wet soil. Some serovars of leptospires are known to survive in soil for up to 74 days and can grow, multiply and retain their infective potential[2,3].

Hosts: Leptospirosis affects most of vertebrate animals. Rodents, cattle, dogs, cats and wild animals are main hosts. From animals the disease is transmitted to human[8] .

Factors in human

Age: Leptospirosis affects the productive age groups of people due to their exposures to outdoor occupations.

Sex: Males are affected more due to more exposure to outdoor occupation.

Caste: Lower socioeconomic people are affected more.

Occupation: Agriculture workers, animal handlers, abattoirs, sewer workers, fisherman and outdoor laborer working in close proximity of contaminated environment by leptospira are affected most.

Housings: Mud thatched house is found to be a risk factor.

Environmental factors: Hot, humid or wet environment favor growth of leptospirosis[10]. Heavy rain and water lodgment is good media for leptospires. Hence the disease has high incidence in such areas[33] .

Salinity of soil: Alkaline type soil favors growth of leptospires[3] PH of 7.00 to 7.4 is favorable for growth.

Source of infection: Contaminated environment by leptospires, water bodies, soil are source of infection of leptospirosis.

Modes of transmission: The transmission of the disease can be direct or indirect. Direct transmission in animals occurs by haematogenous, by suckling milk from infected mother, by sexual transmission or Tran placental. Ellis WA *et al* 1986 reported that by this mode, leptospires enter new host from body fluid or urine of infected animal or body fluid. Direct transmission is common in butchers, veterinarians, cattle, pig handlers and rodent control workers. Bolin CA and Koellner P indicated in 1988 the possibility of human-to-human transmission through breast-feeding. Indirect transmission of leptospirosis occurs through contaminated environment by urine of infected animals. Cuts and abrasions in skin facilitate entry of leptospira(Faine S).[2] Leptospira can also enter the host through intact skin[11]. The leptospira are also transmitted through skin lesions and mucus membranes of mouth, pharynx, and esophagus by ingestion of food and drink contaminated by the urine of the reservoir

animal[12] . Leptospirosis has been recognized as a potential hazard of water sports and other recreational activities. Ingestion of contaminated water was reported to cause leptospirosis by Corwin A *et al in* 1990. He who investigated an outbreak of leptospirosis at Okinawa, Japan and reported that swallowing water while swimming was significantly associated with leptospirosis. The incubation usually lasts about 10 days (2 to 30 days)[3,4].

Clinical features:

The clinically leptospirosis is characterized by mainly two forms. The icteric form represents with jaundice. Other signs/symptoms includes fever, headache, conjunctival suffusion[1,2,3,13] .

The complications include myocarditis, acute meningitis, renal failure, pulmonary filtration, haemorrhage, hepatic involvement, iridocyclitis. Vinetz JM, reported pulmonary haemorrhage as the complication of leptospirosis from china[7]. Acute Respiratory Failure was studied by Silvia RRV & Brauner JS in 2002. Myocarditis was reported by Ramachandran S & Perera MVF in 1977. Uveitis is also a complication of leptospirosis[12].

In south Gujarat area the complications included involvement of kidney, liver, lung, haematological, cardiac and brain[13]. Renal involvements are very common in leptpospirosis[42]

Clinical diagnosis:

As per WHO guideline for leptospirosis Faine, scoring of more than 20 in scoring scale indicates suspected case of leptospirosis. In south Gujarat a suspected case of leptospirosis was considered when a case of fever had

any two of the symptoms namely1. Myalgia2. Conjunctival suffusion and 3. Agriculture worker or a person with history of contact with animals[35].

Laboratory diagnosis:

Diagnostic tests for leptospirosis are Rapid test, ELISA, MAT, PCR, immunofluroscent Tests, Dark field illumination microscopic test, Isolation of leptospires and cultures. Leptospirae can be detected from Blood, urine, CSF. ELISA serological test can detect leptospirosis as early as 3 days in acute stage. A titre of >40 in single test has diagnostic value. Four fold rise in the titre is considered as diagnostic[2] . The sensitivity and specificity of the test is 93%. Microscopic agglutination test (MAT) is done using live battery of leptospira. Cut point for minimum titre for diagnosis is 1:80.

Polymerase chain reaction array test can detect antigen of leptospirosis as early as 3 days. Leptospires can be detected in urine after 2nd week of infection. Other investigations include renal, hepatic function and haematological blood tests. High level of Serum transaminase in leptospirosis cases is useful in differentiating it from hepatitis[13]. Serum urea, creatinine are increased in renal involvement. Serum urea more than 40/dl of blood is significant. The platelet count below 60,000 per 100ml blood can lead to haemorrhagic manifestations[13]

Differential diagnosis:

In tropical and temperate countries the important diseases should be differentiated from leptospirosis. These include malaria, dengue, viral fever, yellow fever, enteric fever, scrub typhus and hanta virus fever.

Treatment:

Due to rapid complications leading to high case fatality early detection and treatment can save life. The Leptospirosis Information Center on website recommends the treatment as follows: Severe infections should be managed with IV benzyl penicillin and will require hospital admission. Adult dose is 5MU to 8MU per day for five days although in some studies the doses have been routinely very much higher - up to 20MU. There is no evidence that doses over 8MU have an additional benefit, but doses below 5MU may be inadequate. Other authors also recommend penicillin as drug of choice. Benzyle penicillin 1.5 mega units 6 hourly IV for 7 days is effective treatment[11,43]. In patients with penicillin allergy, a program of erythromycin can be used at 250mg QID for five days. In mild to moderate cases oral medication using amoxycillin, erythromycin, doxycycline or ampicillin can be used, subject to contraindications and age limits. Amoxycillin/ampicilin is useful in case of pregnant women. Co-trimoxazole is preferred in children. Cap. Doxycycline 100 mg BD daily for 7 days is effective when given within 3 days of onset of leptospirosis[12] Typical dosage for doxycycline is 100mg BID for ten days. 3G cephalosporins (cefotaxime, etc.) are known to be somewhat effective but the primary drug of choice is always penicillin[44] Leptospires are usually resistant to vancomycin, chloramphenicol, rifampicin and metronidazole. Multiple antibiotic therapy is not required. Prophylaxis with cap. Doxycycline 200 mg weekly for 6 weeks can be given to agriculture workers during high transmission season[4] .

Treatment for complications

Peritoneal or haemodialysis for renal failure, platelet replacement for thrombocytopenia, blood transfusion in haematological complication and ventilators in pulmonary involvements are useful measures.

Supportive care

Fluid and electrolyte balance need to be maintained. ECG monitoring is also important as cardiac arrhythmias are common. Psychological manifestations are common, and patients may require sedation if they become aggressive or psychotic. These symptoms are temporary and would not normally require specific treatment, however longer-term depression, fatigue and other symptoms. Follow up of cases treated is useful for knowing late sequelae

Risk factors of leptospirosis

Leptospira, the causative organs are maintained in nature by animal hosts such as cattle, pig, dog, cat, mongoose, wild animals and laboratory animals. The rodents such as rats, squirrel are also carriers of leptospirosis. The animals shed Leptospires through urine. They survive in the environment for long period of time. The environment contaminated with leptospires is also source of infection. Hence, contact with animals, animal tissue, animal urine, wet environment, occupational and recreational exposure to contaminated water bodies are risk factors for leptospirosis.

Animal reservoir

Leptospirosis in animals as a veterinary problem and a possible source of infection to human beings was identified in late 1930s and 1940s[2]

Waitkins SA reported in 1987 that the transmission cycle of leptospirosis involves the carrier hosts, the environment and human beings. Most of rodent and mammal can be carrier and excretes leptospires[2]

"Leptospiral carrier state and seroprevalence among animal population—a cross-sectional sample survey in Andaman and Nicobar Islands" A total of 494 sera samples from different domestic animals and 85 samples from rats (Rattus rattus) were tested by microscopic agglutination test using nine serogroups prevalent in these islands. Antibodies to leptospires were detected in 164 samples giving an overall seroprevalence of (33.11%). The seroprevalence was highest among cows (40.32%). Of 85 rat (Rattus rattus) samples tested for antileptospiral antibodies six (7.1%) were positive. The two isolates from rats were found to be pathogenic, belonging to serogroup Grippotyphosa[16]. Leptospiral infection among cattle was first recorded in Russia[2]. Cattle all over the world may be infected with serovars Hardjobovis,Pomona, and Grippotyphosa. Infection with Icterohaemorrhagiae, Bratislava, Hebomedis, Autumnalis, Australis, Sejroe, Canicola and Bataviae also occurs[1]. Leptospirosis in cattle could be totally unapparent or may result in acute febrile illness or severe complications. W.A. Ellis and colleagues studied leptospirosis in cattle in Northern Ireland. In a study on aborted bovine foetuses Ellis WA *et al,* 1978 identified 6.9% leptospiral antibodies in the aborted foetuses, whereas none of the 196 non-aborted foetuses had antibodies. The study provided clue for trans-placental transmission of leptospirosis in cattle.

Certain serotypes are associated with some animal species. Several studies on cattle leptospirosis were conducted in other countries such as Spain and Australia also. Alonso-Andicoberry C *et al* (2001) studied 762 diary cattle in Spain. He used microagglutination test (MAT) with 11 leptospiral serovars as antigens. 8% of the cattle showed antibodies.

A study in Andaman and Nicobar Districts showed seropositivity in 40% of the cows and 26% of the bulls[16]. Ratnam S *et al* screened 40 cows in a village near Chennai. Antibodies against leptospires were found in 68% of the cows. A survey by Sehgal SC et al, in villages affected by an outbreak of leptospirosis in Thane in India in 2001showed a similar seroprevalence among cattle.

Other animals that are carrier of leptospirosis are pigs, sheep, dog, cat. Canicola and Icterohaemorrhagiae are the common serovars that infect dogs[2]. Scanziani E *et al* conducted a serosurvey in 2002 among 245 kennelled dogs in Italy, 72 were found to be seropositive. Venkataraman KS & Nedunchelliyan S (1992) reported an outbreak of leptospirosis in human beings and dogs in Madras City. Following the outbreak, a serosurvey was conducted among humans and dogs. Seroprevalence was 50.5% among humans and 21.3% in dogs. Leptospira belonging to serovar Icterohaemorrhagiae was isolated from a human patient and Canicola from a dog.

Wild animals and laboratory animals are also affected by leptospirosis (Ruiz-Pina HA *et al* (2002). Seroprevalence rate of 38.2% was found in sea lions in California by Colagross-Schouten AM *et al* (2002).

Smythe LD *et al* (2002) studied Australian flying foxes (bats) in Queensland and found 28% seropositivity. Bunnell JE *et al* (2000) conducted a study among wild mammals in Peruvian Amazon basin. Seroprevalence was 39% in the opossums and 35% in bats (35%) The study indicated that wild mammals could be more important reservoirs of leptospires.

Rodents

The role of rodents in the transmission of leptospirosis was understood after the discovery of leptospires as the cause of Weil's disease. Rodents are the first recognized carriers of leptospires. They are source of infection to human beings. Icterohaemorrhagiae serovar is associated with rodents and other serovars have also been isolated. Various studies done in different countries provided evidence for it. A study by Matthias MA & Levett PN (2002) estimated seroprevalence rates of 28.2% and 40.7% in mouse and mongoose respectively in Barbados. Leptospires were isolated from mouse. A test to detect anti-leptospiral antibodies in mammals was developed by modifying the lepto-dipstick test as reported by Gussenhoven GC *et al*, 1997. Eighteen of the 60 (30%) wild rodents were positive in the test in a study by Kollars TM Jr *et al* in 2002. Alder H *et al* (2002) studied rodents and shrews in Zurich, Switzerland for knowing leptospiral carrier rate. They sreened 190 Kidney specimens from small animals by polymerase chain reaction (PCR) and 12.6% were positive. In another study in Turkey conducted in *Rattus norvegicus rodents showed* 27.1% positivity in the kidney samples and 16.9% of brain samples (Sunbul M *et al*, 2001). Saravanan R *et al* (2000) studied 28 rats and 58

bandicoots at Avadi, Chennai, India Anti-leptospiral antibodies were seen in 14.3% of the rats and 16.1% of the bandicoots. Natarajaseenivasan K *et al* (2003) studied field rodents near the rice mills of Salem, Tamil Nadu, India following leptospirosis in rice mill worker. A study in Andaman Island seroprevalence rate among rats was found 52.1%[16] . We reviewed the following studies for risk factors.

Table1. Studies on risk factors of leptospirosis reviewed.

Authors	Study title	Place	Year
Bovet P *et al*	Risk factors associated with clinical leptospirosis: a population-based case control study in the Seychelles (Indian Ocean) Seychelles District	Seychelles (Indian Ocean)	1999
Tangkanakul W *et al*	Risk factors associated with leptospirosis in North-eastern Thailand, 1998	North eastern Thailand	2000
Sugunan AP	Risk factors of leptospirosis in Andaman Island- A matched case control study	Andaman Island, India	2002

Risk factors associated with leptospirosis in North-eastern Thailand, 1998.

A case-control study was conducted in Nakhornratchasrima Province in the Northeastern region of Thailand in 1998 by Waraluk Tangkanukul, Piyanit et al with the objective to identify risk factors leptospirosis on admission which could be useful for early diagnosis and treatment in Thailand. The study was conducted between August and December, 1998. For this study, a suspect case was defined as a resident of Nakornratchasrima Province greater than 15 years old who presented to the community or provincial hospitals between August 22 and

December 31, 1998 with fever, headache, and myalgias. A confirmed case was defined as a suspect case with a positive test result for anti-leptospiral positive IgM antibody using the Panbio enzyme-linked immunosorbent assay (ELISA) (Panbio, Inc, Brisbane, Australia).

Two persons from the neighborhood of the cases who did not have any illness during the previous 30 days and found negative in IgM ELISA test were selected, after matching to the corresponding case for age (+/-5 years) and sex, as controls. Total 59 cases and 118 controls were included in the study. A standardized questionnaire was used to collect information from the study participants on activities associated with water and animals and environmental conditions of house and workplace. An environmental survey was also conducted at the house and workplace.

During the study period 62 cases of the 80 suspects identified from Nakhornratchasrima Province were confirmed by IgM ELISA. Three patients died that were excluded from the study. Among the remaining 59 cases, 51 cases had two controls matched for age-sex. Remaining 8 cases had one control each. Univariate matched analysis suggested that activities in wet fields for more than 6 hours per day (ploughing, pulling out sprouts, replanting sprouts and fertilizing), walking through water, having cuts on feet in the 2 weeks before were significantly associated with leptospirosis. Keeping dog was protective. Sowing, fishing and not wearing boots were not significantly associated. The authors did multivariate conditional logistic regression analysis. The adjusted odds ratio indicated that walking through water in two weeks prior to illness was found associated. Pulling out sprouts, plowed and fertilizing in wet fields

for more than 6 h/day were significant independent variables associated with leptospirosis.

IgM ELISA used to diagnose or exclude asymptomatic controls has sensitivity and specificity of about 93%. Hence, false positive and negative tests would result in some differential misclassification leading to altered estimation of the strength of association. 77.5% positivity in IgM ELISA test inspite of a liberal case definition used is matter of consideration. The test can detect IgM antibodies upto about three months of infection. Infection in controls prior to three months can not be detected. This would result in misclassification. The study might have failed to identify risk factors of other seasonal activities as the study was done during August to December. The exclusion of died cases severe cases would result in an under-estimation of the strength of association.

The identified risk factors are modifiable. Behavior change communication in agriculture workers by awareness campaign and adoption of newer techniques of cultivation are important measures to address the disease.

Risk factors associated with clinical leptospirosis: a population-based case-control study in the Seychelles (Indian Ocean).

This study was conducted in Seychelles Islands by Pascal Bovet, Claude Yersin et al from 1 April 1995 to 31st March,1996. The objectives were to determine the incidence of acute leptospirosis, the prevalence of subclinical infection in the healthy population and to identify risk factors of acute leptospirosis in an endemic tropical setting. In previous study high incidence of leptospirosis (40 – 60/100,000) was found in Seychelles.

The study was conducted in two big hospitals of the country having facility of treatment. All the physicians of the country were informed to refer patients of suspected leptospirosis to these hospitals. Suspects who had seroconversion (to a minimum titre of 1:400) or four-fold rise in titre in microagglutination test (MAT) or had a positive result in PCR assay were considered as cases. Eligible controls were selected from a list of 1067 subjects aged 25-64 randomly selected from census data and who had participated in other study in past. For each case-patient, age, sex and occupation matched persons were selected from the list. Those controls screened by MAT and PCR and found negative were considered as definite control for the case. Controls were matched for time period by selecting and investigating them within 15 days of identification of case, Information about demographic, social, educational, occupational, environmental and behavioral variables was obtained using a questionnaire in interviews of cases and controls. In the study 75 of the 125 identified suspects met the laboratory criteria for a definite diagnosis. Among 125 controls identified, 60 were positive in either MAT or PCR. Hence they were excluded. Of these 75 cases and 65 controls, 38 were original matched pairs.

The authors did univariate conditional and unconditional regression analysis. 10 and 15 variables were found statistically significant, respectively. Multivariate backward logistic regression indicated that the strongest independent correlates of acute leptospirosis were, by decreasing odds ratios, gardening, cats at home, skin wound, drinking home brews, wet soil around home, refuse not being collected by public

service, home built of corrugated iron and inversely indoor occupation. Combination of gardening, skin wound and wet soil around home accounted for 37 % variance in predicting acute leptospirosis.

The paired MAT and PCR are highly sensitive and specific standard tests. The tests were used to confirm cases and exclude leptospirosis in controls. Hence the chances of misclassification are minimal. The incidence of leptospirosis calculated (101/100,000) was higher than the previously reported annual incidence in Seychelles. This indicates better sensitivity than that in the routine surveillance system.

The study period was one year covering all seasons. Hence seasonal exposure factors are taken care of. Due to large sampling frame of controls identifying of matched controls for all cases was possible to get better outcome. Moreover, it was not proper to do an unmatched analysis on partially matched pairs. There is no clarity regarding multivariate logistic regression model whether it was conditional or not.

The modifiable risk factors identified in the study are drinking home brews, skin wounds, house refuse and gardening. A control programme can address these issues. People can be made aware of the risk factors and encouraged to protect them by wearing footwear and protective clothing during gardening or walking on wet lands. Treatment of skin wounds can reduce the risk of leptospirosis.

Risk factors of leptospirosis in Andaman Islands- A matched case control study.

A.P. Sugunan conducted this matched case control study as a part of MAE-FETP study. The objective of the study was to identify potentially modifiable independent risk factors associated with acute leptospirosis in Andaman Islands. The study was conducted in Rangat and adjoining villages in Middle Andaman, Andaman & Nicobar Islands during October–November, 2002. A suspected case of leptospirosis in Rangat was defined as any patient who reported to CHC, Rangat on or after 1 October 2002 with complaints of fever associated with any of the following symptom/signs (a) Severe muscle tenderness (b) Any bleeding tendencies including sub-conjunctival haemorrhage (c) Jaundice (d) Cough, haemoptysis and breathlessness (e) Oliguria (f) Signs of meningeal irritation. A confirmed case was defined as a suspect who showed serological evidence of current leptospiral infection

A set of eligible controls for each case was selected from the neighborhood of the cases matching for age (=< 5 years) and sex. The selected persons were screened for serological evidence of asymptomatic or past leptospiral infection. Two seronegative persons were selected for each case as controls. A blood sample was collected from the suspected cases during the acute phase and another sample 10 – 14 days later. One blood sample was collected from all the eligible controls.

Information about potential risk factors was obtained by interviewing the cases and controls using a structured questionnaire and observation. The information included type of house, house surroundings, proximity to water bodies, ownership of house and agricultural land, contact with animals, participation in agricultural activities, fishing, contact

with garbage and sewage, cleaning animals, direct contact with animal urine, and recreational activities such as swimming.

information about such exposure was considered during a four week period prior to onset of symptoms in the case of cases and during the corresponding period for their matched controls.

Total 114 suspected cases of leptospirosis were admitted in Rangat CHC. 79 paired blood samples were tested 38 showed seroconversion and 17 had four fold rise in paired titre. Diagnosis was confirmed in 58 cases. The case fatality was 9.4%.

Thatched roof, use of stream water for drinking and keeping cattle and pigs in house were significantly associated with leptospirosis. Among occupational and behavioral factors, walking barefoot, having wounds, harvesting, cleaning sewage, clearing garbage were significantly associated working standing in water. The strength of association was highest for keeping cattle in house (OR: 5.63) followed by harvesting (OR: 5.43) and using well water for drinking (OR: 5.0). Having goats, cats and dogs in house, rat infestation of houses, fresh water fishing, swimming in streams and having direct contact with animal urine were not found to be significantly associated with acute leptospirosis.

The author did multiple logistic regression with backward elimination process. Five variables remained in the model. Presence of cattle in the house had the strongest association with leptospirosis (OR: 5.1) followed by use of streams as a source of drinking water. Cleared garbage, walks barefoot, worked standing in water were other significant factors.

The author followed standard procedures sampling and laboratory methods. The analysis is acceptable one. The author had excluded six fatal cases due to non availability of results. It could have resulted in underestimation of strength of association. The author did not estimate various gradations of exposures.

Prevention and control

Prevention of leptospirosis essentially is by identifying the source and interrupting the transmission[1]. The large number of serovars and of infection sources and the wide difference in transmission conditions make leptospirosis difficult to prevent. Preventive measures should be based on knowledge of those groups at higher risk of infection and of local epidemiological factors; they include: Identifying and controlling the source of infection (e.g. open sewers, contaminated wells). Control of feral reservoirs is often not feasible but control measures can be highly effective in small, defined area[3,4,6]. Selective rodent control may be important. For interrupting transmission, thereby preventing infection or disease in the human host, the following steps are important:

1. Wearing protective clothes and equipment;
2. Disinfecting contaminated surfaces such as stable and abattoir floors;
3. Marking areas with increased risk exposure (warning signs).
4. Preventing infection or disease in human hosts:
5. antibiotic prophylaxis of exposed persons in areas of high exposures may be effective, e.g. soldiers (doxycycline 200mg in one weekly dose);
6. Raising awareness of the disease and its modes of transmission.

Epidemics and m*anagement:*

Conditions leading to an increase of contaminated surface water or soil, such as rain, floods and disasters increase the risk of leptospirosis and may lead to epidemics. During periods of drought both humans and animal reservoirs may be attracted to spare water places, hence increasing the risk of infection. Social and recreational activities that expose persons to a contaminated environment increases also the risk.

Early diagnose and prompt treatment is the key of control. Rapid diagnostic screening tests are useful for early diagnosis. Identification of the serovar and the source is important for appropriate measures. Attack rate and seropositivity amongst populations over a time period are useful indicators of control. Infection source and transmission conditions should be identified. Animal serology may give information on serogroup status. Isolation followed by typing gives definite information on serovar.

In different epidemiological setting, different animal species could be the primary source of infection. In the case-control study conducted in Seychelles[10] cats were found to be associated. Sarkar U *et al*, 2002 reported that rats were found to be associated with leptospirosis in Brazil. Animals were associated with leptospiral seropositivity in Andaman Islands[16] Murhekar MV *et al*, 1998 reported cattle associated with leptospirosis. Significant carrier state in animals could be potential source of infection to humans, although seropositivity is not a reliable indicator of carrier state (Ellis WA *et al*, 1981). Excretion of leptospires in the urine by bacteriological or molecular tools can be conclusive evidence.

Measures targeting human beings include vaccination and chemoprophylaxis. Vaccines have been developed for use in man (Torten M *et al*, 1973), however the existence of a large number of serovars of leptospires makes it difficult to develop a universally effective vaccine. Chemoprophylaxis with doxycycline was tried in soldiers from non-endemic areas visiting endemic areas and was almost 100% effective (Takafuji ET *et al*, 1984). However, Sehgal SC *et al*, 2000 found it only 54% protective in a study conducted in endemic area during an outbreak. Chemoprophylaxis can only be used in outbreak situations or in travelers. Cap. Doxycycline 100 mg BD for 7 days can be given within 3 days of onset of symptoms[12].

Developing policy, protocol and programme for prevention and control measures is important for success of measures. Adequate resource of manpower, materials and fund should be ensured at local level. Interdepartmental co-ordination of health, veterinary and agriculture experts is essential for the control and management. Environment sanitation campaign like "Nirmal Gujarat campaign" can help a lot. For reducing reservoir of infection antirodent activities in high risk area can be considered judiciously. Minor engineering works in housing can reduce exposures to risk factor in rainy season. Prevention and treatment of cut-wounds, house compound wet are modifiable risk factors and need to be addressed. Creating awareness by health education by specific IEC is basic tool for prevention. Capacity building by training of health, medical, veterinary, agriculture staff is useful for case detection, referral and treatment. Early case detection by active surveillance, prompt referral and

treatment can be life saving. Public private partnership, community participation and political commitment are essential requirement of leptospirosis prevention and control. Resource allocation in time should be ensured. The authority need to identify research needs of local area and can make provision and motivation for it.

Summary

Leptospires are widespread pathogens and have a large number of animal hosts. Human infection results from accidental interaction of people with carrier animals or environment contaminated with leptospires. Although the basic principles of prevention such as reduction of source, environmental sanitation, more hygienic work-related and personal practices etc, are same everywhere, there is no universal control method applicable to all epidemiological settings. A good understanding of the ecological, epidemiological and cultural characteristics of a community that faces the problem of leptospirosis is the essential prerequisite for evolving an effective and acceptable control measure

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