Original Article

Leptospirosis: An Emerging Infectious Disease in Nepal

Bhattachan B[1], Bhattachan A[2,] Sherchan JB[3], Dhoubhadel BG[4], Sherchand JB[1]
[1]Department of Microbiology and Public Health Research Laboratory, Tribhuvan University Institute of Medicine, Kathmandu, Nepal
[2]International Vaccine Institute, Seoul, South Korea&World Health Organization (WHO) Country office as name of Acute Encephalitis Syndrome (AES), Nepal,
[3] Department of Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel
[4]School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan
Corresponding authors: Mr. Balkrishna Bhattachan or Dr. Anuj Bhattachan
E-mail: balkrishna_bhattachan@hotmail.com/ or abhattachan@ivi.int

Abstract

Introduction: The aim of this study was to determine prevalence of *Leptospira* spp in Japanese Encephalitis negative cases, as well as its demographic and geographical picture in Nepal.

Methods: The Nepal government along with World Health Organization (WHO) country office Nepal had approved this surveillance project, which conducted from 2007 to 2008 throughout 47 districts in Nepal. JE was confirmed from Cerebrospinal fluid (CSF) samples using ELIZA Method whereas Leptospiraspp was identified by using Latex Agglutination Test kit method in Nepal Public Health Laboratory (NPHL), Kathmandu, Nepal.

Results: Among 2690 Acute Encephalitis Surveillance, the lumber puncture (LP) was done in 2145 patients. Among those tested, JE positive was confirmed in 771 patients. LP was not done in 381 cases. Among 993 JE negative cases, positivity rate of *Leptospira* spp was reported 41.8% (416/993). There was more male preponderance 63.5% (264/416) However, it was not statistically significant (p=0.713). In terms of age distribution, those above 15 years of age were 64.5% (268/416) while those below 15 years of age were 35.5% (148/416), (p=0.000). In eco- region, the rate of infection was highest in Terai region at 53.2% (222/416) followed by Mountain region at 45.2% (188/416) and Himalayan at 1.4% (6/416), (p=0.005). In terms of seasonal distribution, the rate of infection in autumn was at 44.2% (188/416) followed by summer at 40.1% (167/416), spring at 9.6% (40/416) and winter at 6.1% (25/416), (p=0.000).

Conclusions: Nepal government cannot neglect the increasing possibility of outbreaks of *Leptospira* spp in different parts of the country, so this study recommends for surveillance of this infection to prevent future outbreaks in Nepal.

Key words: Leptospirosis, *Leptospira*, JE,Nepal

Introduction

Leptospirosis is caused by spirochetes of the genus *Leptospira*. It is zoonotic disease widespread across the globe.[1,2] the clinical manifestations ranges from acute febrile illnesses to hemorrhagic manifestation associates with jaundice; renal nephritis,hemoptysis, meningeal irritation and sometime with cardiac arrhythmias.[3] It is considered among the differential diagnosis of febrile illnesses in Nepal.[4] The clinical features associated with leptospirosis are similar to a variety of other infectious diseases that are often accounted in the same geographic regions like scrub typhus, dengue, and malaria.[5] The World Health Organization (WHO) reported that approximately 10-100 cases per 100,000 people are annually infected with leptospirosis in tropical regions[6]and recognized this disease entity as re-emerging zoonotic disease[7] in developing countries, particularly in the Caribbean, Latin America, the Indian subcontinent, Southeast Asia, and Oceania.[1,8] where 1.7

million cases of severe leptospirosis are reported each year, with cases mortality rate about 10.0%.[8]

Leptospirosis is reported from countries of countries in South-East Asia Region. Themagnitude of the leptospirosis problem differs from country to country and depends on awareness and attitude of public healthcare decision makers in the country. Most of the human cases were reported from India, Indonesia,Thailand and Sri Lanka during the rainy season. Particularly, major outbreaks were reported in Jakarta (2003), Mumbai (2005) and Sri Lanka (2008). There are also anecdotal reports of human and animal cases in Bangladesh, Myanmar, Nepal and Timor-Leste[7]. In India, this has been a major problem with multiples epidemics in recent years associated with monsoons and poor sanitary situations.[24] In Sri Lanka, the disease is hyperendemic with the annual incidence reported more than 140 per million population as per the data available[1]. Some studies in Bangladesh have demonstrated frequent Leptospirosis infection among the rural population of Bangladesh[12].

In Nepal, there is no program for surveillance of leptospirosis. Nonetheless, there are scientific papers[9] that reported detection of anti-leptospiral antibodies in military personnel. In the study, the prevalence of confirmed leptospirosis was 9.0% among hepatitis cases and 8.0% among febrile cases participating in an efficacy study of a hepatitis E virus vaccine in Nepal. Similarly, Rai et al (2000)[10] detected 32.0% in sero-prevalence Leptospiraspp infection by using one-point MCA method.

Nepal is a country landlocked between China and India. Geographically, it is divided into three regions: Himalayan, Mountain, and Terai region. The total population is nearly 30 million. Leptospirosis is under - diagnosed and under - reported disease in Nepal. The reason is due to lack of awareness among clinicians, occult manifestations, diagnostic difficulties and inadequate diagnostic facilities in many areas of the country. The major objective of this study is to determine the national prevalence rate of Leptospira infection, demographic study, seasonal variation and geographical distribution in Nepal.

Method

Study site

In Nepal, there exists an integrated surveillance of Vaccine Preventable Diseases (VPDs) including Japanese encephalitis (JE) conducted by Nepal government through the involvement of Epidemiology and Disease Control Division (EDCD), Child Health Division (CHD), National Public Health Laboratory (NPHL) with support from World health Organization (WHO) country office Nepal in the name of Acute Encephalitis Syndrome (AES) surveillance. This study of *Leptospira* spp was conducted among JE negative cases in 47 districts in Nepal.

Sampling process

In 2007 to 2008, AES and JE surveillance was done using a structured reporting form, information on age, gender, region and seasonal variation patients from reference hospital by trained volunteers. Experts were isolated Cerebrospinal Fluid (CSF)and blood from patients by Lumber puncture and using sterile syringe respectively. Cerebrospinal Fluid (CSF) and Blood samples collected for laboratory testing.Then both samples were labeled and stored at 4° to 20°C in cold-box till to reach Diagnostic Laboratory of Nepal Public Health Laboratory (NPHL), Teku, Kathmandu. Sample transporting process took no more than 4 hours by Plane or road.

Diagnosis

JE was confirmed by the presence of JE IgM from1 ml CSF by using IgMCapture ELISA (MAC ELISA) kit method, which were IgM negative used for Leptospirosis. 5-10 ml human blood samples were collected in small test tube.Blood samples were immediate centrifuged to obtain sera at diagnostic Laboratory, and stored −20°C until 2nd and 3rd week for *leptospira* testing or stored at 4°C to 8°C. Because of antibodies (IgM and IgG) begin to appear at the end of 1st week and attains very high titre (1:10,000) on 3nd and 7th week[11] and were tested at the National Public Health Laboratory (NPHL) of the Department of Health Services in Kathmandu.Leptospirosis tested in serum by using Latex agglutination test.

Latex agglutination test

The entire tests were done by LeptotekDri Do, BioMerieux, Netherland. This is a Latex Agglutination Assay, which is used to detect *Leptospira* specific antibodies IgM and IgG in human sera.[11,12] This kit has sensitivity of 91% and specificity of 91%.[2,4]Human sera were kept in card. *Leptospira*-specific antibodies are detected when it agglutinate blue latex particle that have been coated with antigen from the Lely 607 strain. A dry spot of these latex particles, affixed to a white card was mixed with 10 µl serum, string with a sterile

spatula. After gently swirling the suspension for 30 seconds, each test spot was recorded positive, negative and intermediate (weak positive) according to the degree of agglutination observed.

Statistical analysis

Win-Pepi Software programme was used for data analysis. P-value calculated from Pearson's Chi square test where $\chi < 0.05$ was considered statistically significant.

Ethics Approval:

Ethical approval was obtained and the study was recommended by WHO and Government of Nepal, Ministry of Health and Population.

Results

Figure 1: Flow chart

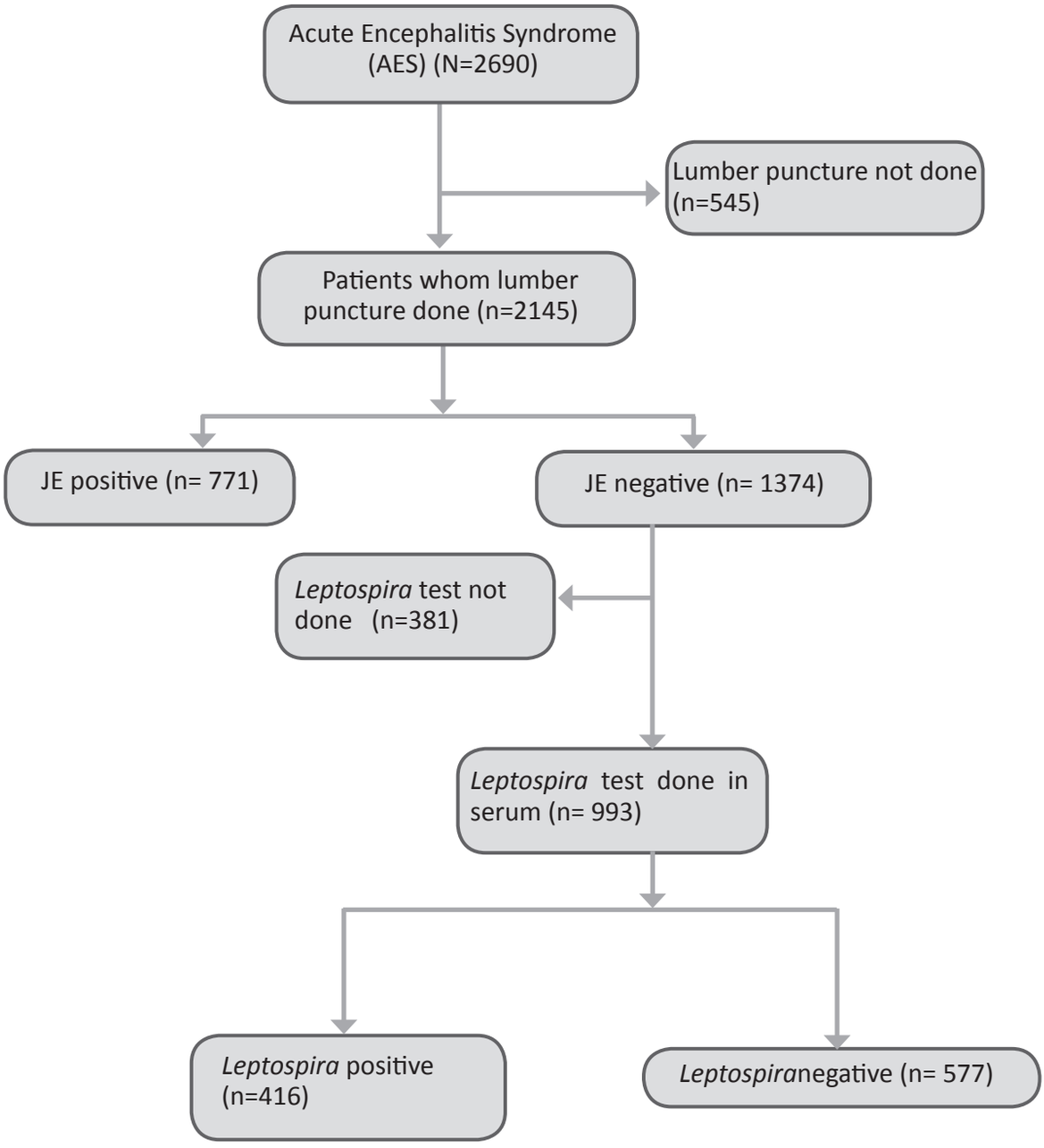
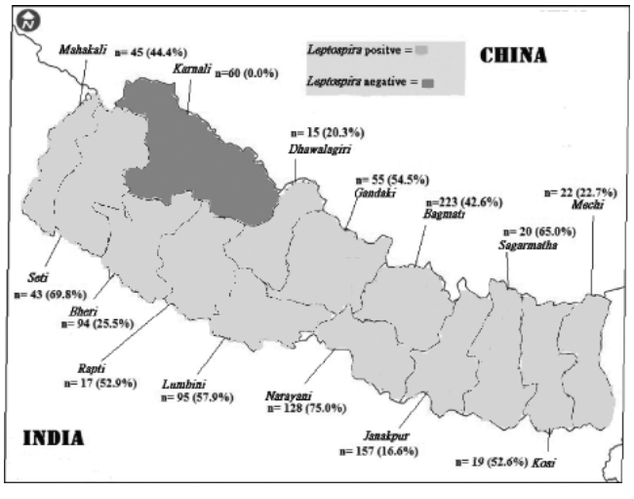


Table 1:Demographic and regional data with JE and *Leptospira*

Name of Category	Feature	JE positive n=771	JE negative n=1374	P	*Leptospira* positive n=416	*Leptospira* negative n= 577	P
		Number(%)	Number(%)		Number(%)	Number(%)	
Age	< 15 years	449(58.3)	719(50.3)	0.008	148(35.5)	199(44.9)	0.723
	>15 years	322(41.7)	655(47.7)		268(64.4)	378(55.1)	
Sex	Male	417(54.1)	683(49.7)	0.052	264(63.5)	286(49.6)	0.000
	Female	354(45.9)	691(50.3)		152(36.5)	291(50.4)	
Eco-region	Himalayan	129(16.7)	203(15.1)	0.342	6(1.4)	30(5.2)	0.005
	Mountain	343(44.5)	594(43.0)		188(45.2)	235(40.7)	
	Terai	299(38.8)	577(41.9)		222(53.4)	312(54.1)	
Season	Autumn	138(17.8)	446(32.5)	0.000	184(44.2)	210(36.4)	0.000
	Winter	151(19.5)	252(18.3)		25(6.1)	68(11.8)	
	Spring	130(16.8)	389(28.3)		40(9.6)	100(17.3)	
	Summer	352(45.9)	287(20.9)		167(40.1)	199(34.5)	

This data is only surveillances. In *Leptospira*, aged of >15 years patients was found higher than < 15 years. Similarly, infection rate of male was found higher than in female. In similar way,infection rate in male was found higher than in female in JE. Eco-region and Seasonal variation data can see in table 1.

Fig 2: Surveillances of Leptospirosis in Nepal



Note: n indicates the total samples of zone.

Among 14 zones, 13 zones were suspected from *Leptospira* positive except Karnali Zone. *Leptospira* positive rate in Narayani Zone has highest whereas lowest in Janakpur Zone. Detail information was depicted in fig 2.

Discussion

In Nepal, JE is a disease of public health importance in terms of its high mortality and disability rate in the community especially in Terai districts. Nepal government responded to the public health threat by integrating JE in the surveillance network working closely with district health system, thereby improving the sensitivity of the surveillance system.In general, total 2690 AES samples of hospitalized patients were collected from different districts of Nepal. Lumber puncture was done in 79.7% (2145/2690) patients where as LP did not in 545 cases due to lack of patient's informed consent and serious illness case. In addition to, JE positive found 35.9% (771/2145) in LP whereas JE negative found in 1374. Similarly, in 1374 JE negative, Leptospira test (LT) was done in 993 patients whereas LT did not done in 381 patients due to patient's informed consent, serious cases, and clinical complication. In addition, Leptospira positive found 41.8% (416/993) where Leptospira negative found in 58.1% (577/993) patients. In general, positivity rate of *Leptospira* spp was found 41.8%. This finding is similar with Victoriano et al, 2009[13] and dissimilar with (Rai et al 2000: Sulzar et al 1978).[9, 14]

In Age, *Leptospira* infection rate in above 15 years was higher than below 15 years patients. This finding is similar with Rai et al 2000[9] where dissimilar with (Murhekar et al1988 :Amarasekera et al 2013),[17, 23] with no significant difference (p=0.723).This might be adult are suffered from other medical illness like Jaundice, fever, febrile illness rather than less than 15 years and sample size affects its rate.

In gender, the *Leptospira* infection rate in male was higher than in female.This finding is similar with (Everad et al 1985: Merien et al 1996)[15, 16]and dissimilar with Rai et al 2000[9], with significant difference (p=0.000). It might be due to male have to work outside or expose in environment. So,there is high chance of Leptospira contaminated host animal and pathogen vectors in outer environment.

In terms of ecological distribution, the infection rate was highest in Terai region while the rate is lower in Mountain and Himalayan with significant difference (p=0.005). Most of Terai people loves host animal in home, so, loving behavior of people in animal and throughout contaminated host animal like mice, cat, and dog might be transmitted *Leptospira* infection in people. It is also reported that leptospires are able to survive for longer periods in higher temperatures and humid environments.[2]Therefore, it could be partly because environmental temperature is high compound with low socio-economic condition in Terai region. Moreover, glacial retreat in the Himalayas is expected to increase the volume of water flow into major river systems, further contributing to the flooding risk.[18, 19]

In season, leptospirosis was reported high in the autumn and summer season whereas low in winter and intermediate in spring season with significant difference (p=0.000). This finding is similar to (Rai et al 2010 :Murherker et al 1988 : Myint et al 2010 : Tangkanakul et al 2005).[10, 17, 9,21]In summer season, heavy rainfall and flood are major risk factors to transmit bacterial *Leptospira* infection from contaminated animal host like mice. In rainfall, leptospira infected mice discharge urine in small pond formed on road then contaminated pond water transmits into people due to their activities. Outbreak of leptospirosis is linked with rainfall, have raised the public profile of zoonotic disease.[22]

In autumn, flooding and other natural disasters can also increase the risk of infectious diseases such as leptospirosis by disrupting public health services and infrastructure, damaging water and sanitation networks, displacing populations,damaging homes and increasing environmental exposureto pathogens.[20]

Thousands of people can potentially become infected in a short time during epidemics, and put enormous stress on healthcare facilities. Implementation of public health measures for prevention, control and surveillance will also put added stresses on the health system. In addition, leptospirosis can threaten livestock, thus compounding economic losses.Leptospirosis reported in clinical practice since 80's, however without much significant notice as a public health threat in the community. Therefore, this disease is under-reported and under diagnosed.[25]

Due to the limitation of the laboratory capacity and existing case definition, we could not perform biochemical test (Bilirubin, AST, ALT value), other related clinical test and death cases or outcome. This data is only surveillance. Therefore, a further study with proper laboratory support is necessary.These findings highlight that leptospirosis reveal as an emerging infectious disease in Nepal.It might be re-emerge in current JE suspected area. It is therefore, further Leptospirosis surveillance and detail research should conduct in Nepal.

Conclusion

Leptospirosis is recognized emerging infectious disease in Nepal. Evidence suggests that there is always possibility of outbreaks related to *Leptospira*spp in rainy and summer season. Therefore, Government of Nepal should exercise on formulating policy related with the surveillance program in an effort to prevent and control leptospirosis in the community in Nepal.

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