Risk groups and risk factors associated with leptospirosis in Sindhudurg, Maharashtra state, India

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Abstract
In leptospirosis, risk groups are certain groups of humans in a population that are more likely to be exposed as a result of their occupational/recreational activities. Also there are many factors such as rain water contact, animal contact and recreational activities responsible for the risk of acquiring the disease. Leptospirosis outbreaks have been reported from different regions of Maharashtra State which is mainly associated with flooding. Retrospective analysis of the data was carried out to determine the risk groups and risk factors associated with leptospirosis cases reported at Sindhudurg District, Maharashtra State, India. Molecular and Serological diagnosis of 37 referred serum samples of leptospirosis suspected cases were carried out at the Nodal laboratory for Re-Emerging diseases at Haffkine Institute, Mumbai. Out of 37 samples, 24 (64.86%) tested positive for leptospirosis either by IgM ELISA or by Real-Time PCR. The data analysis revealed leptospirosis positivity of 35.13% among farmers, which is a substantial number followed by 16.21% in students. This analysis also demonstrated that water contact and animal contact are the primary risk factors responsible for leptospirosis in the study area. These observations highlight the importance of risk groups since they are more vulnerable for acquiring leptospirosis. Hence, it is necessary to create awareness among the risk groups as a measure to avoid leptospirosis.

Keywords: Leptospirosis, Risk groups, Risk factors, Diagnosis, Sindhudurg District

1. Introduction
Leptospirosis is a zoonotic disease of worldwide distribution. It affects both humans and animals and it is emerging as an important public health problem in India (Faine et al., 1999 [7]; Samasivaet al., 2003 [21]; Dalalet al., 1960 [5]; Joseph et al., 1966 [10]; Ratnamet al., 1983 [20]; Patil et al., 2014 [17]; Muthusethupathi et al., 1995 [16]; Sehgalet al., 1995 [22]; Sethiet al., 2010 [23]). It has been recognized as an important occupational hazard of agriculture manual laborers, sewage workers, animal handlers, forestry workers and other outdoor workers who work in wet conditions, and butchers. The transmission cycle involves interaction between one or more animal hosts harboring leptospires, an environment favorable for its survival, and human beings (Faine et al., 1999 [7]; WHO/ILS 2003 [30]; Vijayachariet al., 2008 [100]; Bharati et al., 2003; Levettet al., 2001) [13].

In leptospirosis, risk groups are certain groups of humans in a population that are more likely to be exposed as a result of their occupational/recreational activities. There are many factors such as rainwater contact and animal contact responsible for the risk of acquiring leptospirosis (Anagnaniet al., 2003 [2]; Swapnaet al., 2006 [27]; Lau et al., 2010 [12]; Hartskkelet al., 2011[9]; Terry et al., 2000 [26]; Radlet al., 2011 [19]; Sejyaret al., 2003 [24]; Shaw et al., 1992) [26]. Since year 1998, leptospirosis has been regularly reported from different parts of Maharashtra State (Bharadwaj et al., 2002 [5]; Ambekaret al., 2004 [3]; Karandeet al., 2002 [11]; Maskeyet al., 2006 [14]; Mathuret al., 2009 [15]; GOM, 2015) [8]. High risk areas include the coastal districts of Maharashtra. Majority of the reported leptospirosis outbreaks occur in association with flooding. There is significant underreporting due to unawareness of the disease, protein manifestations like fever, hepatorenal syndrome and pulmonary syndrome...
cases. Laboratory diagnostic facilities are not adequate to diagnose cases. The number of districts in Maharashtra reporting leptospirosis cases has expanded from 2 in 1998 to 10 districts in 2005. The coastal urban districts Mumbai and Thane districts report outbreaks during monsoon whereas Kolhapur and Sangli districts reported cases for the first time in 2005 post floods. Sindhudurg is one of the disaster prone districts in Maharashtra having 121 Kms coastal line. Floods are more common especially in Malvan, Kudal, Sawantwadi,Vengurla, Dogad and Kankavli due to overflowing rivers (GOM, 2015)8. The coastal districts of Sindhudurg also reports outbreaks in rice field farmers during the monsoon. A total of 2355 cases and 167 deaths were reported in 2005 mainly due to a large outbreak during the post-floods [DHS, 2015 [6]; WHO, 2006] 31]. The disease is presenting a cycle of epidemics to increasing areas of endemicity resulting in further outbreaks. Epidemics are associated with changes in human behavior, animal and sewage contamination of water and changes in animal reservoir density. Another cause for concern is the increasing outbreaks of leptospirosis in post-disaster situations like following the Orissa super-cyclone and floods in 1999 and Mumbai flash floods in 2005 [WHO., 2006] 31]. In 2006, cases of leptospirosis were reported from Sindhudurg district, Maharashtra, India. Since seropositivity might not indicate current leptospiral infection, molecular tools was also used for diagnosis. In the past, many sporadic cases and outbreak of leptospirosis has reported from Sindhudurg district of Maharashtra and is known to be endemic for the disease. Retrospective analysis of the leptospirosis cases were carried out to identify the risk group and risk factors responsible for leptospirosis in this region.

Materials and Methods

Study area

The State of Maharashtra is divided into seven divisions, which are themselves divided into several districts. Sindhudurg is one of the 5 districts of the Konkan division and is divided into 6 Talukas (Devagad, Kankavli, Kudal, Vengurla, Sawantwadi, and Malvan). Sindhudurg is the smallest district in the State, occupying only 1.65% of the total area of Maharashtra.

The district occupies an area of 5207 km² and has a population of 868,825 of which 9.47% were urban (as of 2001). As of 2011 it is the least populous district of Maharashatra (out of 39). Sindhudurg has a semi-tropical climate and remains warm and humid in most of the year. It has three clear seasons: Rainy (June-October), winter (November-mid February) and summer (mid February-May). Temperatures vary between Max. 32°C and monsoon winds bring heavy rains (average rainfall 3240.10 mm) (Wikipedia, 2015) 32].

The total population of the district is 862,000 people, 92% of who live in the rural part (710 villages). 31% of them are considered to live below the poverty line. The rural population in the district experience difficulties such as under employment, unemployment, a lack of access to income generating resources, fragmented land holding and problems such as common ownership and land inheritance disputes. In this context, farming has become an occupation for subsistence for a majority of farmers. Agriculture is the main occupation of the people of the district. However, cashew, mango, coconut and other forest fruits plantations and fishing related activities the predominant traditional economic activities (UNIDO, 2003) 20].

Ethics Clearance

The study was approved by the Institutional Ethics Committee of Haffkine Institute for Training, Research and Testing, Mumbai, India.

Clinical specimen processing and storage: During November 2006, a total of 37 blood samples from the patients suspected of leptospirosis (as per WHO guidelines for diagnosis of Leptospirosis) from Sindhudurg district in Maharashtra were referred to the Nodal laboratory for Re-Emerging Diseases at Haffkine Institute (WHO/ILS., 2003) 33]. The blood samples were allowed to clot. Tubes were then centrifuged and the separated sera were distributed into aliquots in sterile serum vials, and stored at −80°C until further processing.

Serology: All 37 serum samples of the suspected cases of leptospirosis were screened using Anti-leptospira IgM ELISA kit (M/s IVD Research Inc., USA) for detection of IgM antibodies against leptospira. The test was performed according to the manufacturer’s instructions.

Molecular Diagnosis: Real time PCR was carried out on all 37 serum samples to determine the presence of leptospira. DNA was first extracted using a modified Boom’s method (DNA sorb B; M/s Sacace Biotechnologies S.r.l., Italy) and was used for the amplification of leptospiral DNA using a Real Time PCR system (SmartCycler; M/s Cepheid, USA). Fluorogenic data for the amplified DNA was collected during the reaction through the FAM channel. Appropriate positive and negative controls were used for each run (Paul et al., 2005) 31].

Results

Molecular and Serological Diagnosis

Of the 37 samples tested, 5 (13.51%) were positive by Real Time PCR and 24 (64.86%) were positive by IgM ELISA. More males tested positive and the highest positivity was seen in the age group of 14-25 years.

Clinico-epidemiological Analyses

Risk groups associated with leptospirosis

In this retrospective analysis of leptospirosis, an attempt was made to determine the risk groups and risk factors for leptospirosis. It was observed that out of 24 ELISA positive samples, the highest positivity for leptospirosis was observed amongst farmers (35.13%), followed by students (16.21%) making farmers the highest risk group for acquiring leptospirosis (Figure 1).
Risk Factors associated with leptospirosis

An attempt was also made to determine the risk factors responsible for the leptospirosis. From this retrospective analysis, it was observed that water contact by boating was the most common risk factor (35.13% positive), followed by animal contact with dogs (24.32% positive) for acquiring leptospirosis (Figure 2).

Correlation of Risk Groups and Risk Factors associated with leptospirosis

A correlation analysis of Risk Groups and Risk factors revealed that farmers had the maximum animal contact (highest with cattle; 16.22%) as well as water contact (highest by boating; 29.73%) (Figure 3).
Leptospirosis is now identified as one of the globally emerging infectious diseases, having an increased incidence in tropical locations and those exposed by virtue of their occupation. The specific risk group and factors for acquiring leptospirosis infection can be unique to each community. An understanding of the transmission cycle of the disease in the community and the modifiable risk behaviors is essential for planning strategies for prevention and control (Faine et al., 1999; Anagnani et al., 2003; Swapna et al., 2006; Lau et al., 2010; Hartskeerl et al., 2011). Hence, in the present study an attempt was made to determine the risk groups and risk factors for leptospirosis in affected area of Sindhudurg, Maharashtra. In this study, farmers were found to be affected the most followed by student. Farmers were found to be mainly affected because of their occupation which has higher chances of getting infection through different activities of livelihood. The study highlights the importance of risk groups since they are more vulnerable for acquiring Leptospirosis. Hence, it is necessary to create awareness among the risk group as precautionary measure to avoid leptospirosis. An attempt was also made to determine the risk factors responsible for getting infection. From this study, it was observed that water contact through boating was the most common risk factor for acquiring the disease. This could be mainly due to getting water contact during commuting from one village to other via boats and fishing activities. Rodent urine contamination of stagnant water in wet land is another predisposing factor for leptospirosis as it is most common with farmers in India. A spur in incidence of leptospirosis in human is an annual feature in some developing countries during monsoon. Rural population are at high risk of getting leptospirosis infection accidentally through direct or indirect contact with the infected animal and considered to be a “dead end host” (Sambasiva et al., 2003; Sehgal et al., 2010). The study also demonstrated that contact with dogs and livestock is important risk factor for getting infected with leptospiroa. An association between risk groups and risk factors identified support the view the prevention of leptospirosis needs be multifactorial; particularly in tropical areas where leptospires are ubiquitous and where outdoor activities bring along several risk factors for leptospirosis infection. Therefore health education is of paramount importance and should stress the need to apply all available measures relevant to risk activities, such as wearing closed footwear. Identification of risk groups and potential risk factors can help in understanding the transmission dynamics of the leptospirosis and formulate public health interventions.

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Declaration of Conflict of Interest
The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

References
31. WHO, Representative to India, New Delhi and Regional Medical Research Centre (ICMR). Report of the Brainstorming Meeting on Leptospirosis Prevention and Control, 2006