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SEROPOSITIVITY OF LEPTOSPIROSIS AMONG FISHERMEN IN AMARAPURA TOWNSHIP, MYANMAR

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ABSTRACT

Context: Leptospirosis is a worldwide zoonosis, caused by pathogenic *Leptospira* species. It is becoming significant public health problem, especially in resource-limited developing countries, as the aftermath of natural disasters. Leptospirosis is mostly present as mild acute non-specific febrile illness and some as severe leptospirosis, Weil's disease, pulmonary haemorrhagic syndrome or hepatic failure. **Objectives:** To determine the occurrence of leptospiral IgG antibodies in high-risk group in tropical urban region and identify the potential-associated factors such as demographic, socioeconomic and occupational-related factors among fishermen in Amarapura Township. **Methods:** A cross-sectional descriptive study was conducted among 151 fishermen in Amarapura Township to test for the presence of leptospiral IgG by using Enzyme-Linked Immunosorbent Assay during January to September 2018. **Results:** Among 151 participants, seropositivity in leptospiral IgG was recorded in 19 participants (12.6%) and most participants (16/19, 84.2%) were male. The highest seropositivity rate was shown in 31-40 years age group (15.4%). In the current study, there was no one who used boots or gloves to the working places or while working in the water. The leptospiral IgG was found in eight participants with injuries (8/42, 19.1%) and 14 peoples with animal contact (14/96, 14.6%). The results from this study showed no significant association between leptospiral IgG seropositivity and potential-associated factors. **Conclusion:** The results in the present study rendered the information on leprospiral seropositivity and related factors in fishermen and hopefully became informative to increase awareness of the disease among the fishermen and to contribute in preventing the transmission in endemic area.

KEYWORDS: Leptospirosis, Potential-associated Factors, Fishermen, Seropositivity.

INTRODUCTION

Leptospirosis, the disease caused by *Leptospira* species, is considered the most widespread zoonosis in the world especially in undeveloped areas. Higher incidence of leptospirosis occurs in the tropical and subtropical areas.[1] Leptospira were first detected as the agents of Weil's disease among coal miners in Japan.[2] Leptospirosis is transmitted to human by contact with infected animals or their urine.[3] High risk populations are those living in urban slum areas where environmental sanitation is poor and exposure with rat is more likely.[4]

Leptospirosis is considered not only as a zoonotic disease but also as an occupational one since it is an important occupational hazard of agriculture workers, miners, animal handlers, sewage workers, and other outdoor workers working in wet conditions.[5]

Transmission usually results from direct or indirect exposure to the urine or other tissues of the infected animals.[6] Abrasions, cuts, and damaged skin are particularly important as portals of entry. Walking barefoot and water sports in endemic areas are notoriously high-risk activities.[7] Environmental factors such as high rainfall, flooding, population explosion, urbanization, poor sanitation and hygiene favor the direct transmission.[8]

A study done in Pacific islands reviewed identified common behavioural risk factors and environmental drivers for leptospirosis infection across the Pacific region. The individual risk factors were farming, backyard livestock, lifestyle (fishing, hunting, walking bare-foot), contact with infected animals and contact with fresh water sources (washing laundry, recreational

use) while environmental drivers were mainly climate-related (flooding, heavy rainfall).[9]

Leptospirosis usually presents as a nonspecific, acute febrile illness with fever, headache, myalgia and may be confused with other diseases such as dengue fever and influenza. Severe outcomes may develop depending on three factors: epidemiological conditions, pathogen virulence and host susceptibility.[4] Leptospirosis can also cause severe systemic diseases (Weil's disease), hepatic and renal failure, pulmonary haemorrhage syndrome, extensive vasculitis and death. Case fatality rates for Weil's disease and pulmonary haemorrhage syndrome are more than 70% and 10% respectively.[10]

Leptospires isolation is time consuming and has low sensitivity. The reference serological test, microscopic agglutination test (MAT), requires technical expertise, can be done only in reference laboratories since they require live *Leptospira* strains, and is best interpreted with both acute and convalescent sera.[11] New diagnostic methods such as ELISA may facilitate early diagnosis and antibiotic treatment. For routine testing, most clinical microbiology laboratories use commercial Enzyme-Linked Immunosorbent Assay (ELISA).[12]

Leptospirosis in all its forms is amenable to treatment with antibiotics. Penicillin should be given as early as possible, during the early leptospiraemic phase. The destructive effects of leptospirosis cannot be reversed by antibiotics, but penicillin may have beneficial effects in severe leptospirosis, reducing mortality and duration of illness, when given intravenously, even at a late stage.[13]

It is not possible to prevent leptospirosis in all situations, because of its widespread nature all over the world. The best that can be done is to limit the effects of leptospirosis on humans and animals.[14] Personal protective equipment such as gloves, boots in high-risk occupations are important to prevent exposure of mucous membranes and skin, but can be difficult to implement in hot and humid conditions.[7] The cornerstone of prevention of leptospirosis is early detection and treatment of leptospires-infected animals, and immunization of uninfected animals.[4]

Pyae Hpone Maw studied the presence of leptospirosis in agricultural workers and sewage workers from North Okkalapa Township, Yangon Region and Pathein Township, Ayeyarwaddy Region during January 2016 to December 2016. A total of 136 samples were collected and tested with Leptospiral IgG ELISA kits. Nine (6.6%) were leptospiral IgG positive and 127 (93.4%) were negative.[15]

In 2017, 173 drainage sanitary workers of Mandalay City Development Committee were tested for the presence of leptospiral antibodies by using rapid test kits (SD Leptospiral IgM/IgG devices). Among them, leptospiral IgG was detected in 1.7% (3/173) and IgM was positive in 2.3% (4/173). Almost all of them were healthy workers and there was no signs and symptoms of leptospirosis at the study time.[16]

In Myanmar, there is still few researches on leptospirosis. The magnitude of leptospiral exposure in fishermen is unknown. The poor sanitation and the contact with stagnant water may favor transmission among the fishermen living around Taungthaman Lake which is the main working place for most fishermen in Amarapura Township. There is no current study on seropositivity of leptospirosis among fishermen in Myanmar.

The purpose of this study was to aid in increased awareness of the disease among the fishermen and health care personnel so that the disease can be recognized, treated as soon as possible and this study might contribute some information in preventing the transmission in endemic area especially during outbreak.

MATERIALS AND METHODS

Sampling procedure

A cross-sectional descriptive study was carried out in 151 fishermen living in Amarapura Township, Mandalay during January to September 2018. Samples were collected by using two stages simple random sampling method. Five wards were selected among 51 wards in Amarapura Township by simple random sampling method in first stage. All fishermen were listed to get a sampling frame for each ward in second stage and the participants were selected through simple random sampling from each ward.

After receiving written informed consent, pro forma was filled out for each subject. Under aseptic condition, three milliliter of blood was collected using sterile needles and syringes by venepuncture. The samples were placed in a sealed container during transport.

Serum preparation and storage

The samples were centrifuged at room temperature and the serum was separated into 2 sterile labeled 1.5 ml microvials using pipettes. Each vial contained 200 µl of serum (one for testing and another for back-up). The serum was stored at -20 °C deep freezer until tested at Public Health Laboratory, Mandalay.

Procedure for detection of leptospiral antibodies

The detection of the leptospiral IgG antibodies was performed in Public Health Laboratory, Mandalay. The samples were tested for presence of leptospiral antibodies by using ELISA IgG test kit (NovaTec, Germany). These tests were performed according to the manufacturer instructions. The sensitivity of the IgG ELISA test kit is >98% and specificity is 97.4%. For the equivocal results, blood was taken from the participants in 2-4 week time and repeated the ELISA test again.

Data Processing and Data Analysis

Data were collected by using pro forma. Then, all data were checked carefully for accuracy. Data entry was done by Microsoft Office Excel 2010. Data compilation and analysis was done by using e statistical software, STATA version 13. The results were shown in frequency distribution table, bar diagram, or descriptive statistics. Association between leptospiral seropositivity and its possible related factors was tested by using Chi square test. If assumptions for Chi square test did not meet, Fisher's exact test was used. Statistical significance was considered if p value was ≤ 0.05.

RESULTS

Among 151 fishermen, leptospiral IgG seropositivity was recorded in 19 participants (12.6%). Most of participants were male (110/151, 72.8%). The most common age group of this study population was 18-30 years (38.4%). Mean age was 36.23 ± 12.10 with the minimum age of 18 years and maximum age of 60 years. Most respondents had primary education (56.3%). In this study, there were 126 respondents (83.4%) having income of 100,000-200,000 kyats per month. The socio-demographic characteristics of study population were described in Table 1.

Table 1: Socio-demographic characteristics of the study participants (n=151).

Socio-demographic characteristics	Frequency	Percentage
Gender		
Male	110	72.8
Female	41	27.2
Age (year)		
18-30	58	38.4
31-40	39	25.8
41-50	35	23.2
51-60	19	12.6
Education		
Read & write	28	18.5
Primary school	85	56.3
Middle school	35	23.2
High school	3	2.0
Monthly per capita income in family (Kyats)		
100,000-200,000	126	83.4
> 200000	25	16.6
Wards		
Pan-bae	58	38.4
Taung-gyi	41	27.2
Sin-zwe-poot	27	17.9
Shwe-gwan-htoke	16	10.6
Shwe-kae	9	5.9

Leptospiral IgG seropositivity rate was highest in 31-40 years age group (6/39, 15.4%), followed by 18-30 years age group (8/58, 13.8%), 41-50 years age group (4/35, 11.4%) and 51-60 years age group (1/19, 5.3%). In this study, 16 male participants showed seropositivity (16/110, 14.5%).

Among five different wards randomly selected from Amarapura Township, fishermen living in Pan-bae ward were found at highest seropositivity rate of 22.4% (13/58). The seropositivity rate of fishermen living in wards was shown in Figure 1.

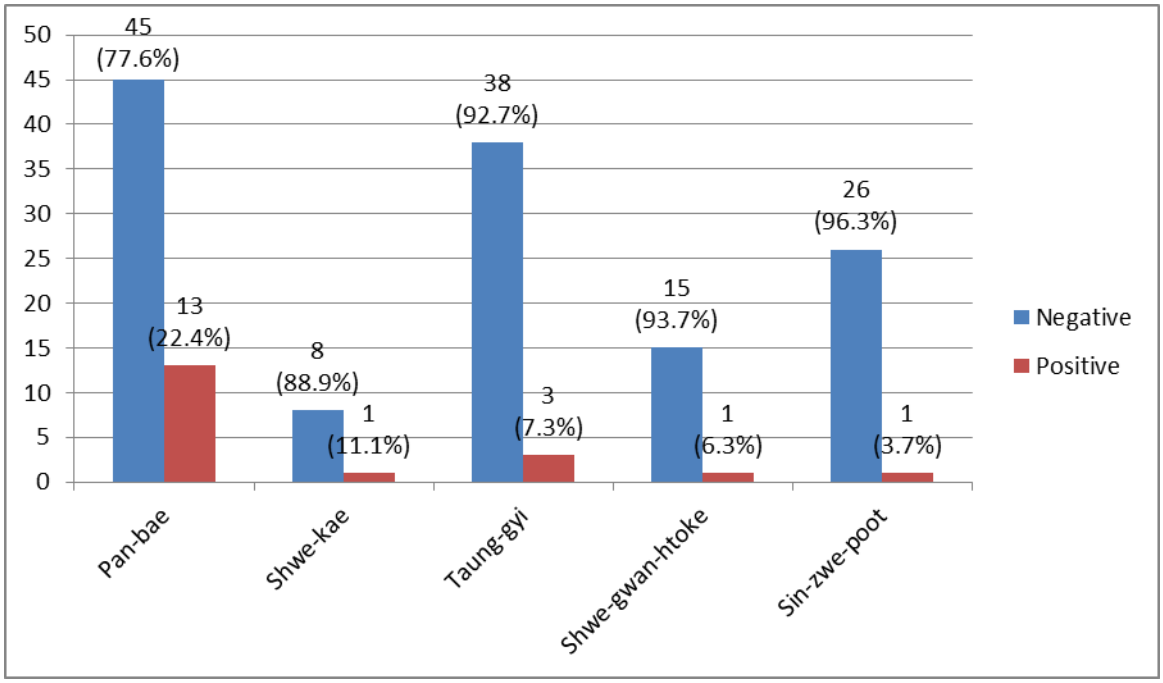

Figure 1: Distribution of leptospiral IgG seropositivity according to different wards in Amarapura Township.

The seropositivity rate was highest in participants with primary school education (15/85, 17.7%). Three participants with middle school education (3/35, 8.6%) and only one participant who could only read and write (1/28, 3.6%) showed positive results.

Leptospiral IgG antibodies were found in six fishermen getting incomes of >200,000 kyats (6/25, 24%) and in 13 fishermen getting income between 100,000-200,000 kyats (13/126, 10.3%).

The occupational-related factors like wearing gloves or boots in the working places, presence of injuries or abrasions in last 3 months and the contact with animals at home or work were studied in this current study. Among 151 participants, there was no one who wore boots or gloves to the working places. Among 151 fishermen, there were 42 participants (27.8%) with injuries or abrasions in last 3 month and leptospiral IgG was positive in eight peoples with injuries (8/42, 19.1%) (Table 2).

Ninety six out of 151 participants (63.6%) had come into contact with animals and among them, 14 participants (14.6%) showed seropositive results. Most of people who had contact with animals in this study had more than one type of animals at their homes or work. There was no significant association between potential related factors and prevalence of leptospirosis (Table 2).

Table 2: Occupational related factors in relation to leptospiral seropositivity (Presence of injuries or abrasions and animal contact).

Occupational related factors		Numbers of fishermen				*p* value
		Total (n=151)		Seropositive (n=19)		
		No.	%	No.	%	
Presence of injuries or abrasions in last 3 months	Yes	42	27.8%	8	19.1%	0.137
	No	109	72.2%	11	10.1%	
Contact with animals	Yes	96	63.6%	14	14.6%	0.327
	No	55	36.4%	5	9.1%	

DISCUSSIONS

In this study, only 19 participants (12.6%) showed seropositive results. Pyae Hpone Maw studied the presence of leptospirosis among 136 agricultural workers and sewage workers from North Okkalapa Township, Yangon region and Pathein Township, Ayeyarwaddy region and only nine (6.6%) were seropositive.[15] The findings in this study were not correspondence with study in India where high prevalences were found in hospital sanitary workers (56.2%) and fishermen and fisher folk (52.8%) with overall seropositive rate 37%. It may be due to difference in study population and different demographic area.[17]

The higher leptospiral IgG seropositivity was found in people around 31-40 years because these age groups were more involved in outdoor activities like fishing, animal rearing and agricultural works. Many studies

reported higher prevalence of leptospirosis in age group between 20-40 years and the findings in this study were consistence with these studies.[17,18] The finding was not coincided with the study conducted in Yangon. The highest seroprevalence rate was found in old age group of 51-60 years (14.3%),[15] The difference between these two studies might be due to different study population.

The seropositivity rate of leptospirosis was higher in male than female with the ratio of 5.3:1. The predominance of leptospirosis among male participants is in agreement with other serosurveys. Males are more affected than female because males are mostly engaging in outdoor works like animal rearing and fishing.

Fishermen living in Pan-bae ward were found at highest seropositivity rate of 22.4%. This might be due to the poor sanitation around the Pan-bae ward. In this ward, animal rearing was more common than other wards and the housing was more overcrowded. The working area also had waste accumulation and hampered water outflow. During the rainy season every year, flooding often occurs in this area. The poor-quality water supply also contributes to this higher seropositivity. The combined effect of poor sanitation and flooding risk is the major risk factor which favours the increased transmission of the leptospirosis. A similar consistency was found in the studies conducted in other countries. In Rio de Janeiro, higher rates were observed for census tracts inside the flood-risk area and in the vicinities of waste accumulation sites.[19] However, Alvarado-Esquivel et al. stated that people dwelling in rural area had higher leptospiral IgG seropositive rate (37.5%) than those dwelling in urban area (9.9%) with overall seropositivity of 17.7% in Mexico.[18] The difference between the studies may be due to the rural works like rearing animals and agriculture were also risk factors for leptospirosis.

The highest rate of seropositive level (17.7%) was seen in participants with primary school education. In this study, the association between educational level and seropositivity of leptospirosis was not strongly significant. In Pyae Hpone Maw study, seropositivity was equally distributed among all the education level.[15] A similar consistency was concluded in the study in Selangor, Malaysia, seropositivity of leptospiral antibodies among respondents who received informal education was 29.4% while the rate of those received formal education was 24.5%.[20] Similarly, lower education level was found to be not associated (p=0.13) in a study in Northern Mexico.[18] A study conducted in Western Uganda also excluded the educational level as a risk factor.[21]

There was no strong association between income and seropositivity in this study (p=0.060). This finding was coincided with Pyae Hpone Maw study where no significant conclusion could be achieved regarding the association between leptospiral IgG seropositivity and income of volunteers.[15] A study in Mexico concluded that 17% of volunteers with low socioeconomic status were seropositive while the rate was 10.9% in those with medium socioeconomic status (p=0.24) showing socioeconomic status was not enough to be regarded as a risk factor of leptospiral IgG seropositivity.[22]

In this study, wearing boots and gloves while working or to the work and seropositivity might be associated but p values could not be calculated due to lack of people wearing boots and gloves which made analysis of working practices insufficient to ascribe the seropositivity of leptospirosis significantly. But in other studies, walking barefoot and standing in water while working, were already established risk factors. A study in Middle Andaman, India stated that seropositivity was associated with those walked barefoot (p=0.005).[23] In 2014, people who had direct contact with water or mud during work had seropositivity rate of 51 (72.9%) suggesting that association between walking barefoot and seropositivity was significant (p=0.001).[24]

Only eight participants with injuries show seropositive results (19.1%). There was no strong association between the presence of injury and seropositivity (p=0.137) as the current study was conducted in a confined area. However, the skin abrasion was the strongest risk factor in other foreign studies in Thailand, India and France which may be due to broken skin that probably facilitates the entry of *Leptospira* directly into the bloodstream and increases the number of bacteria that enter the host in a given exposure period. In Thailand, Kamath et al. stated that people with presence of cut or wound during work showed seropositivity rate of 72.9% with highly significant with p value (p=0.001).[24] The major occurrence of leptospirosis in peoples with injury or abrasion was in agreement with the study in Middle Andaman, India in which the presence of skin wounds and seropositivity of leptospirosis was statistically significant with p=0.014.[23]

The animal contact and the seropositivity was not positively associated in this study (p=0.327). This may be depended on the type of animal because the most common carrier hosts were pigs, cattles, dogs and rodents. In this study, these types of animals were not common in works or homes except dogs. Rearing animals was identified as one of the risk factors in leptospirosis in other studies. A study conducted in India, among 70 people, 41 peoples with seropositive results had dogs at their homes (58.5%). Rearing domestic animals at home was identified as a risk factor.[24]

CONCLUSIONS

The results from this current study rendered the representation about the occurrence of leptospirosis in Myanmar which appears to be lower than that in other countries. These results also portrayed the associated factors among the high risk groups like fishermen. Lots of peoples participated were unaware of the disease. The

magnitude of leptospiral exposure in fishermen was unknown before this current study.

The limitations of this study are small samples sizes and short duration of the study. Moreover, the study was conducted in a confined area. Hence the findings cannot be generalized for the larger areas. Also, the seropositive samples cannot be confirmed by the reference methods, Microscopic Agglutination Test because this test is not available in our country.

Community based studies with alternative studies designs studying for serovars, comparing data between high risk groups, wide study areas and comparing more than one serological test in parallel which may increase the sensitivity would give better data. However, this study may help further more studies to describe the trend in incidence of leptospirosis in Myanmar and may depict the general features of the disease and to aid in preventive measures of the diseases transmission.

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Ethical Considerations

The study received approval from board of studies of University of Medicine, Mandalay, Myanmar for permission to proceed (140(Micro)/UMM/2018).

Competing interests

The authors declare that they have no competing interest.

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