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Seroprevalence of Leptospirosis among Army Personnel in Northeastern Malaysia

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Abstract

Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira*. The disease affects various high-risk groups including army personnel, who are at risk because of field activities that bring them into close contact with zoonotic reservoirs. This cross-sectional study determined the seroprevalence of leptospirosis among 616 asymptomatic army personnel in Northeastern Malaysia. Sociodemographic data were obtained using validated questionnaires. Serological screening for leptospirosis was performed using an ELISA method and confirmed by microscopic agglutination test (MAT). Samples indeterminate and positive according to ELISA (n = 117) were sent for MAT testing. A MAT titer ≥ 100 was considered positive evidence of exposure to leptospirosis. The overall seroprevalence of leptospirosis among the subjects was 16.2% (95% confidence interval: 13.32, 19.15). The most common reaction obtained with the sera tested by MAT was against a strain of *Leptospira* spp. isolated from Terengganu (38.3%) followed by *L. biflexa* serovar Patoc (35.2%). In conclusion, the seroprevalence of leptospirosis among army personnel in Northeastern Malaysia is high.

Keywords

Seroprevalence, Leptospirosis, Army Personnel, Northeastern Malaysia

1. Introduction

Leptospirosis is a zoonotic disease that can be transmitted from animals to humans [1] [2]. Humans are usually the incidental hosts [3] [4]. The prevalence of

leptospirosis varies among countries. In the Philippines, there are 147 cases per 100,000 people [5]. Furthermore, the seroprevalence of leptospirosis in Laos is reported to be 23.9% [6].

Leptospirosis is an occupational disease reported to affect soldiers in the UK and the former West Germany [7]. Army jobs are high-risk occupations for leptospirosis [8] [9] [10]; this is because army personnel perform operation and military exercise activities that bring them into contact with zoonotic reservoirs [7] [11] [12]. However, few studies worldwide indicate a risk of infections at training sites [10] [13].

Most studies on leptospirosis involving army personnel to date have focused on outbreak episodes rather than merely exposed personnel; thus, the literature on asymptomatic infection is sparse. Furthermore, few outbreaks involving military personnel have been reported worldwide [8] [9].

Enzyme-linked immunosorbent assays (ELISAs) are based on chemical reactions rather than live bacterial specimens. There is an ELISA that only reacts to one type of antibody (*i.e.*, IgM, IgG, or IgA) that can help determine if the titer is of current or historical origin [14]. In the present study, the Pan Bio Leptospira IgM ELISA test (PanBio, Queensland, Australia) was used for the qualitative detection of IgM antibodies to *Leptospira* in sera. This ELISA can detect infections caused by several *L. interrogans* serovars including Hardjo, Copenhageni, Pomona, Madanesis, Australis, Kremastos, Nikolaevo, Celledoni, Canicola, Grippotyphosa, Swajzak, Djasima, and Tarrasovi [15].

The microscopic agglutination test (MAT) is the gold standard for confirming leptospirosis because of its high sensitivity and specificity compared to other currently available tests [16] [17]. This test is most appropriate for seroprevalence studies and is generally performed in reference laboratories. However, it is operator-dependent as well as difficult to perform, interpret, and maintain; therefore, the use of the MAT is limited, especially in developing countries where leptospirosis is endemic [18]. Therefore, the use of different tests in other studies depended on the availability of the test during the study period.

2. Methods

This cross-sectional study involved 616 army personnel in four camps in Northeastern Malaysia. Socio-demographic data were obtained using a validated questionnaire. Informed consent was obtained from all subjects.

Venous blood (5 mL) was taken from each subject for the qualitative detection of IgM antibodies against *Leptospira* by ELISA (Panbio, Queensland, Australia). A score of < 9 units indicates a negative result or no detectable IgM antibodies, 9 - 11 units indicates an indeterminate result, and >11 units indicates a positive result and thus the presence of *Leptospira*-specific IgM antibodies. The samples were analyzed by trained laboratory personnel in the Microbiology Laboratory of Universiti Sains Malaysia.

The indeterminate and positive samples were subsequently sent to the Insti-

tute for Medical Research (IMR) for the MAT. The samples were tested against the following 17 serovars: *L. biflexa* serovar Patoc, *L. interrogans* serovar Autumnalis, *L. interrogans* serovar Bataviae, *L. interrogans* serovar Canicola, *L. interrogans* serovar Celledoni, *L. interrogans* serovar Hardjobovis, *L. interrogans* serovar Icterohaemorrhagiae, *L. borgpetersenii* serovar Javanica, *L. interrogans* serovar Pomona, *L. interrogans* serovar Pyrogenes, *L. interrogans* serovar Hardjoprajitno, *Leptospira* spp. isolated from Melaka, *Leptospira* spp. isolated from Terengganu, *Leptospira* spp. isolated from Sarawak, *L. interrogans* serovar Copenhageni, *L. interrogans* serovar Australis, and *L. interrogans* serovar Lai. A MAT titer ≥ 100 was considered evidence of exposure to leptospirosis [18] [19]. The seroprevalence of leptospirosis was calculated and presented as proportions and 95% confidence intervals (CIs).

3. Results

The majority of subjects were Malay males. Their mean (SD) age was 29.28 (7.15) years. The sociodemographic characteristics of the participants are shown in Table 1.

When a MAT titer ≥ 100 was considered positive, the overall seroprevalence of leptospirosis was 16.2% (95% CI: 13.32, 19.15) (Table 2). Table 3 shows the distribution of serovars determined according to positive MAT results among the 100 seropositive cases.

Table 1. Sociodemographic characteristics (n = 616).

Variables	Frequency (%)	Mean (SD)
Age		29.28 (7.15)
Sex		
Male	608 (98.7)	
Female	8 (1.3)	
Race		
Malay	525 (85.2)	
Others	91 (14.7)	

Table 2. Seroprevalence of leptospirosis (n = 616).

	Frequency	(%)	95% CI
Positive	100	(16.2)	(13.32, 19.15)
Negative	516	(83.8)	(80.85, 86.68)

Table 3. Serovar distribution for positive MAT (titer ≥ 100) among 100 army personnel.

Serovars tested	Frequency	%
**Leptospira* spp. isolated from Terengganu	74	38.3
L. biflexa serovar Patoc	68	35.2
L. interrogans serovar Celledoni	11	5.7

Continued

L. interrogans serovar Hardjobovis	5	2.6
L. interrogans serovar Australis	4	2.1
L. interrogans serovar Canicola	4	2.1
**Leptospira* spp. isolated from Sarawak	4	2.1
L. interrogans serovar Autumnalis	3	1.6
L. interrogans serovar Copenhageni	3	1.6
L. interrogans serovar Lai	3	1.6
**Leptospira* spp. isolated from Melaka	3	1.6
L. interrogans serovar Pyogenes	3	1.6
L. interrogans serovar Bataviae	2	1.0
L. interrogans serovar Hardjoprajitno	2	1.0
L. borgpetersenii serovar Javanica	2	1.0
L. interrogans serovar Icterohaemorrhagiae	1	0.5
L. interrogans serovar Pomona	1	0.5

*Local strain.

4. Discussion

The seroprevalence of leptospirosis varies among professions; it was also influenced by other factors such as region, weather, and climate [5] [20] [21] [22]. The seroprevalence of leptospirosis among army personnel in the present study was high (16.2%) based on a MAT titer ≥ 100. This finding was consistent with those of other local studies [20] [23]. Tan reports the seroprevalence of leptospirosis among afebrile Malaysian army personnel ranged from 13.0% - 17.2% using a sensitized erythrocyte lysis (SEL) test with a positive titer cut-off of ≥ 80. Meanwhile, Supramaniam reports a slightly wider range of the seroprevalence (12% - 22%) in a similar population.

However, the seroprevalence of leptospirosis among army personnel in the present study was higher than in a previous study in Nepal, which reports the prevalence of cases confirmed by MAT to be 8% [24]. Another seroprevalence study among military recruits following an outbreak after a training exercise in a jungle reports the seroprevalence of leptospirosis was 28% [9], this value may be higher than in the present study owing to the fact that their study population was symptomatic with fever and other symptoms suggestive of leptospirosis. Most outbreak studies involving army personnel to date have focused on symptomatic patients rather than merely exposed individuals; thus, the literature on asymptomatic infection is sparse.

In comparison to other occupational risk groups, the seroprevalence among army personnel in this study was lower than reported among civilian service workers. Using similar methods and MAT titer cut-off, Sulong *et al.* found the overall seroprevalence of leptospirosis among civilian service workers in Malay-

sia to be 24.7% [25]. Studies in Madras, India and Singapore report very similar seroprevalence rates of 24.8% and 20.8%, respectively [26] [27]. However, the seroprevalence among army personnel in the present study was higher than low-occupational-risk group (e.g., school teachers, housewives, and indoor workers), which was reported to range from 1.5% - 9.4% [20].

The seroprevalence of leptospirosis among army personnel in the present study was higher than symptomatic patients in the general population. In a hospital-based cross-sectional study performed in Malaysia, the seroprevalence of leptospirosis among the general population was 8.4% [28]. A study in India reports a very similar finding, in which the seroprevalence among high-occupation-risk subjects was higher than the general population [22].

The MAT is the gold standard for confirming leptospirosis. However, the cut-off was not standardized. Instead, the cut-off usually depends on the baseline of the community in a particular geographical area [19] and varies among laboratories [18]. In Malaysia, there is no consensus regarding a standard cut-off titer for the seroprevalence of leptospirosis in high-risk groups. However, a cut-off of ≥ 100 is generally accepted [18] and frequently used in other seroprevalence studies [29] [30] [31]. Other researchers recommend a titer of ≥ 50 for serosurveys in asymptomatic high-risk groups [32]. Accordingly, the seroprevalence of the subjects in the present study might be underestimated if other cut-offs were applied. Another study performed in Brazil using the MAT with different cut-offs ≥ 100, ≥ 50, and ≥ 25 demonstrates seroprevalence rates of 18%, 23%, and 30% respectively [33].

In this study, the most common reaction obtained with the sera tested by the MAT was against a strain of *Leptospira* spp. isolated from Terengganu, Malaysia (38.3%) followed by *L. biflexa* serovar Patoc (35.2%). The actual infecting pathogenic serovar is likely to have evolved from the local saprophytic strains and is unrelated to the pathogenic serovars included in the test panel. Therefore, the local endemic pathogenic serovars in this area need to be studied further.

Non-pathogenic *L. biflexa* serovar Patoc was the second most common serovar identified in this study. A study in Turkey reports a similar finding [34]. *L. biflexa* serovar Patoc was a saprophytic strain that cross-reacts with human antibodies generated by several pathogenic serovars in rare cases or perhaps with a strain that was currently unknown in the area concerned [16]. The finding of non-pathogenic serogroups was consistent with the notion that a finding of non-pathogenic serogroups suggests the circulation of other pathogenic serogroups that may not be included in that particular study [16]. Thus, this highlights the importance of validating MAT cut-offs for the specific epidemiological situation in which a seroprevalence survey was being performed.

The importance of identifying specific serogroups was prompted by the findings by previous studies suggesting distinct clinical features were associated with specific serogroups [35]. However, a more thorough long-term study rejected this hypothesis [36]. Several serovars were strongly associated with certain occupational risk groups [37]. However, these associations could not be demon-

strated in the present study.

The importance of asymptomatic infection and subclinical presentation was not well understood, and efforts to determine their significance were limited [38]. However, it was generally believed that serovar-specific antibodies were protective, and that person will immune to re-infection by the same serovar if the concentration (*i.e.*, titer) of specific antibodies was sufficiently high [16] [39]. In view of those findings, asymptomatic infections may play a role in population immunity (*i.e.*, "herd immunity") against leptospirosis. Therefore, seroprevalence studies in high-risk groups may reflect exposure but not necessarily overt disease.

Severe complications may occur after the course of the illness in a small proportion of patients [40]. In unfortunate cases, there were clinical presentations of multiple organ involvement. Case fatality rates in different regions worldwide were reported to range from <5% - 30% [40]. Death remains significant and was mainly due to delayed diagnosis owing to a lack of infrastructure as well as adequate clinical suspiciousness [1]. Leptospirosis was recognized as an emerging potentially epidemic disease representing a significant public health challenge [41].

5. Conclusion

The seroprevalence of leptospirosis among army personnel in Northeastern Malaysia was 16.2%. High seroprevalence of leptospirosis among the respondents indicated that army personnel were occupational risk groups for leptospirosis. The most common reaction obtained with the sera tested by the MAT was against a strain of *Leptospira* spp. isolated from Terengganu (38.3%) followed by *L. biflexa* serovar Patoc (35.2%). The findings from this study could be used for prevention and control of leptospirosis among high risk groups. The top management of the army should take all the practicable steps and appropriate measures to promote a safe and healthy workplace. Involvement of the top management can ensure the sustainability of the program. Good data from the seroprevalence study among the army personnel can help to demonstrate how serious the problem in the respective place.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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