



ORIGINAL ARTICLE

Study on Seroprevalence and Leptospiral Antibody Distribution among High-risk Planters in Malaysia

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Abstract

Objectives: To determine the leptospirosis seroprevalence and to identify the predominant infecting serovars among oil palm plantation workers.

Methods: The cross-sectional study involved 350 asymptomatic oil palm plantation workers in Melaka and Johor. A serological test using the microscopic agglutination test was conducted in the Institute of Medical Research with a cut-off titre for seropositivity of $\geq 1:100$.

Results: The overall seroprevalence of leptospiral antibodies was 28.6%. The job category with the highest seroprevalence was the fruit collector with 59.2%. The predominant serovar identified was serovar Sarawak (Lepto 175) (62%).

Conclusion: A high seroprevalence of leptospiral antibodies was detected among oil palm plantation workers and specifically among fruit collectors. The predominant infecting serovar among the workers was serovar Sarawak (Lepto 175). The findings suggest that more studies are needed to determine the reasons for the high seroprevalence and the transmission and pathogenicity of the local serovar Sarawak (Lepto 175).

1. Introduction

Leptospirosis is a potentially fatal bacterial infection recognized as a zoonotic spirochetal disease. The disease is also considered as a re-emerging global public health issue of worldwide importance, especially in tropical and subtropical countries. The exact number of human cases in the world is not precisely known because of the lack of surveillance data worldwide [1]. Recent data have shown that Malaysia is an endemic country for leptospirosis, with an increased number of

reported cases and outbreaks and a significant number of deaths over the past decade [2,3]. The prevalence of leptospirosis in Malaysia increased from 2004 to 2009, and the case fatality rate within that period varied from 1.8% to 7.6% [4].

Leptospirosis is transmitted to humans through skin or the mucous membrane coming into contact with water, moist soil, vegetation, or environmental surfaces contaminated with the urine of an infected animal. Symptomatic leptospirosis usually manifests itself as a range of diseases from a flu-like illness to Weil's

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syndrome, with the case fatality rate ranging from 5% to 15%, and is characterized by jaundice, renal failure, and hemorrhage [5]. The past outbreaks of leptospirosis in developed countries were often related to recreational activities, whereas these outbreaks tended to be seasonal in nature, related to animal activities, and related to agricultural and occupational factors in developing countries [6,7]. A recent hospital-based study reported that the majority of leptospirosis cases in Malaysia were among agricultural workers [8].

The palm oil industry is the main agricultural sector in Malaysia, one of the world's main palm oil exporters, and it accounts for 77% of the total agricultural land and has become the fourth largest contributor to the Malaysian economy [9,10]. The available data show that the palm oil industry in Malaysia is a major sector of employment, with the industry supporting more than 1.4 million jobs and 468,056 people hired as field workers in plantations [10,11].

2. Materials and methods

2.1. Study design and population

A cross-sectional study was conducted in June 2014 involving 10 oil palm plantations in Melaka and Johor, which are southern states of Malaysia. The study was granted ethical approval by the Research and Ethics Committee (Human), School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kota Bharu, Malaysia. All the workers involved voluntarily signed the informed consent form after they were given a detailed explanation about the procedure and adequate time to make a decision.

Oil palm plantation workers have seven job categories: (1) fruit collector; (2) harvester; (3) pruner; (4) pesticide applicator; (5) fertilizer applicator; (6) driver; and (7) nursery worker.

Calculated based on a 32.6% seroprevalence of leptospirosis among oil palm workers [12], 95% confidence interval (CI) and 10% nonresponse rate, the estimated sample size required for the study was 374. The sampling frame consisted of workers who had been working in the plantation for more than 6 months. Office workers were excluded.

2.2. Blood samples and serologic tests

The consenting respondents were interviewed for sociodemographics, job category, and duration of employment. Venous blood samples were tested for the presence of antileptospiral antibodies using microscopic agglutination test (MAT) at the Institute of Medical Research (IMR) following standard methods [13]. The MAT was performed with a panel of live leptospire reference cultures obtained from the Royal Tropical Institute (World Health Organization/Food and Agriculture Organization of the United Nations

Collaborating Centre for Reference and Research on Leptospirosis) in Amsterdam, The Netherlands (Australis, Autumnalis, Bataviae, Canicola, Celledoni, Grippotyposa, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Hardjoprajitno, Patoc, Tarassovi, and Djasiman) and from the IMR (Melaka, Terengganu, Sarawak, Lai, Hardjobovis, and Copenhageni).

Live leptospire cell suspensions representing 20 serovars were added to serially diluted serum specimens in 96-wells microliter plates and were incubated at 30°C for 2 hours. Through dark field microscopy, agglutination was examined at a magnification of 100 times. Using the control well for comparison, agglutination was examined by observing free leptospire in each well. The MAT were considered positive if the free leptospire approximate numbers were < 50% in the control well. A titer of $\geq 1:100$ was used as the cut-off titer for leptospirosis seropositive in this study. The level of titer indicated previous exposure to the leptospira bacteria [3].

2.3. Statistical analysis

Data were entered and analyzed using IBM SPSS version 22 for Windows [14]. All continuous variables were described using means and standard deviations. Frequencies and percentages were presented for categorical variables. Seroprevalence of leptospirosis was described with a 95% CI.

3. Results

Among the 374 total respondents recruited, there were 350 participants in this study, resulting in a response rate

Table 1. Sociodemographic characteristics of the respondents ($n = 350$).

Variables	Frequency (%)	Mean (SD)
Age (y)		31.4 (9.68)
Sex		
Male	296 (84.6)	
Female	54 (15.4)	
Citizenship		
Malaysian	65 (18.6)	
Non-Malaysian	285 (81.4)	
Marital status		
Married	229 (65.4)	
Single/widower	121 (34.6)	
Income		
<RM1,000	135 (38.6)	
RM1,000–2,000	201 (57.4)	
>RM2,000	14 (4.0)	
Education		
No formal education	101 (28.9)	
Primary school	144 (41.1)	
Secondary school	105 (30.0)	

RM = Malaysian Ringgit.

Table 2. Seroprevalence of leptospirosis seropositivity according to job category ($n = 350$).

Job category	n	MAT \geq 1:100	
		Frequency (%)	95% CI
Driver	23	5 (21.7)	3.5, 39.9
Fertilizer applicator	48	11 (22.9)	10.6, 35.3
Fruit collector	49	29 (59.2)	44.9, 73.5
Harvester	94	23 (24.5)	15.6, 33.3
Pesticide applicators	53	13 (24.5)	12.6, 36.5
Pruner	64	15 (23.4)	12.7, 34.1
Nursery worker	19	4 (21.1)	0.9, 41.2
Total	350	100 (28.6)	23.8, 33.3

CI = confidence interval; MAT = microscopic agglutination test.

Table 3. Serovar distribution among 100 plantation workers determined by positive microscopic agglutination test (titer \geq 1:100).*

Serovars tested	Frequency	%
Sarawak	62	62
Patoc	42	42
Celledoni	7	7
Javanica	3	3
Australis	3	3
Autumnalis	2	2
Pyrogenes	1	1
Copenhageni	1	1
Terengganu	1	1

*Workers tested may be positive to one or more serovars.

of 93.6%. Table 1 shows the sociodemographic characteristics of the oil palm plantation workers. The respondents were workers who were relatively young, with a mean age of 31.38 (standard deviation, 9.68) years and the majority of the workers were men (84.6%). According to citizenship, 285 (81.4%) of the respondents were non-Malaysians. Most of the respondents (71.1%) had either primary or secondary school experience and 101 (28.9%) had no formal education.

The overall seroprevalence of leptospirosis was 28.6% (95% CI: 0.24, 0.33). Among the respondents, fruit collectors showed the highest seroprevalence of 59.2%, followed by harvesters and pesticide applicators at 24.5%, respectively (Table 2).

Table 3 shows the distribution of serovars among 100 seropositive cases determined by the positive MAT titer \geq 1:100. The most common reaction obtained in the sera tested was the *leptospira spp.* strain isolated from the serovar Sarawak (Lepto 175) at 62.0%, followed by serovar Patoc at 42% and serovars Pyrogenes, Copenhageni, and Terengganu at 1%, respectively.

4. Discussion

This study revealed a high seroprevalence rate of 28.6% obtained from the scientific laboratory MAT, consistent with other studies. This finding is supported by that of a survey on anti-*Leptospira* antibodies using the sensitized-erythrocyte-lysis test conducted on 18 occupational groups in West Malaysia: the highest antibody rate of 32.6% was found among oil palm plantation workers [12]. Another local study conducted among healthy paddy planters in Northeastern Malaysia using the sensitized-erythrocyte-lysis test also reported 24.2% seroprevalence [15]. Using similar methods and a cut-off value of the MAT titer for comparison, Shafei et al [3] reported a low seroprevalence (24.8%) of leptospirosis among town service workers in Kelantan, which are considered another high-risk occupational group for the infection. This finding shows that oil palm plantation workers are comparably at a high risk of leptospiral infection.

The high positivity in the seroprevalence of leptospiral antibodies in this study may be related to rats, which are the main leptospire carrier and can be found in abundance in oil palm plantations because rats are attracted to fresh oil palm fruit [15,16]. Moreover, the pathogenic leptospire are able to survive for long periods of time in the oil palm plantation environment, which is characterized by a favorable tropical climate and moist and warm soil and surface water conditions [17].

The highest seropositivity of anti-*leptospira* antibodies was detected among fruit collectors (29, 59.2%), followed by harvesters (23, 24.5%), pesticide applicators (13, 24.5%), and pruners (15, 23.4%). Compared with the other job categories, fruit collectors are the oil palm plantation workers with the most contact with the surface environment of soil and water, and are the most at risk to obtain wounds on their hands. This condition is due to the nature of their job that consists of collecting loose fruits from the ground and manually lifting the thorny fruits into the cart. The higher frequency of serovars detected in this workers group may also be due to their poor personal protective equipment practices. As observed during the study, only a few fruit collectors wore gloves while working, and this practice further contributes to the high exposure to the leptospire-contaminated environment.

The predominant pathogenic serovar noted was Sarawak (62%), followed by Patoc (42%). Currently, no information on the pathogenicity of the serovar Sarawak and its endemicity in Malaysia is available. An on-going study is conducted by the IMR Kuala Lumpur on the serovar Sarawak [18]. Similar to the finding of the current study, a seroprevalence survey conducted in Selangor, Malaysia, among town service workers, who also fall in the leptospirosis high-risk occupational group, found that the highest local

serovar was Sarawak at 37.0% [19]. Moreover, a leptospirosis study involving wild animals in Sarawak, East Malaysia, found that 72% of the seropositive samples from monkeys, rats, bats, squirrels, and mongooses were also positive for the serovar Sarawak (Lepto 175) antibody [18]. Local animal sources of particularly identified infecting serovars should be determined to ascertain the transmission pathway. In relation to our study, we could not speculate on animal reservoirs as surveys involving local animals have not been conducted in these areas.

The seroprevalence study in this high-risk occupational group of workers may reflect exposure but not necessarily the overt disease. The reason is that serovar-specific antibodies are protective, and the person is considered immune to reinfection with the same serovar, even if reinfection involving different serovars may still occur [19,20].

The high seroprevalence indicates that oil palm plantation workers are the occupational risk group for leptospiral infection. The workers become exposed in the possibly leptospire-contaminated workplace through manual work practices that put them at risk of leptospiral infection.

Sarawak (Lepto 175) is the predominant infecting serovar detected among the seropositive oil palm plantation workers. As this study did not include leptospirosis among animal reservoirs, we could not conclude the pattern and interaction between humans and animal hosts. We recommend further studies on local animal reservoirs to provide important information on predominant serovars.

Conflicts of interest

We declare that we have no conflict of interest in the publication of this paper.

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