The Occupational Hazard Study for Leptospirosis among Agriculture Workers

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Abstract

Introduction: Leptospirosis is a re-emerging zoonotic, occupational disease especially in tropical countries. Oil palm plantation workers are likely to be exposed to the leptospiral infection due to their manual work practices with frequent environment contact.

Objective: The aim of this study was to determine the seroprevalence of leptospirosis and to identify the work practices risk factors for leptospirosis infection among oil palm plantation workers in Malaysia.

Methods: This cross-sectional study involved 350 oil palm plantation workers in southern Malaysia using an interviewer-guided questionnaire. In addition, blood samples were taken for serological testing using a microscopic agglutination test conducted at the Institute of Medical Research; the cut-off titre for seropositive was ≥1:100.

Results: The overall seroprevalence of leptospiral antibodies was 28.6%. The significant work practices risk factors found to be associated with seropositive leptospirosis were 'did not wear rubber glove PPE' (AOR: 5.25; 95% CI: 2.88, 9.56; p<0.001), 'working with the presence of hand wound' (AOR: 3.13; 95% CI: 1.83, 5.36; p<0.001), and 'did not wash hands with soap after work before eating or drinking' (AOR: 3.97; 95% CI: 2.25, 7.02; p <0.001).

Conclusion: The high seroprevalence of leptospirosis shows that this group of workers are at high risk of Leptospira infection. The notable associated work practice factors provide a clear indication that awareness towards the risk of this disease is important and the infection can probably be prevented by stressing these modifiable factors through intervention programmes.

Keywords: Leptospirosis, risk factors, microscopic agglutination test, oil palm plantation workers

Introduction

Leptospirosis is a re-emerging, potentially fatal, zoonotic, occupational disease of worldwide importance, especially in tropical and subtropical countries. It is estimated that annually, the

disease affects tens of millions of humans worldwide, with a case fatality rate ranging from 5% to 25%[1]. Due to a lack of worldwide surveillance, the exact number of cases is not known precisely[2]; the incidence may reach over 100 per 100,000 populations in high-risk groups during outbreaks[3]. Malaysia is considered to be an endemic country for leptospirosis, with a high number of reported cases and outbreaks with a significant number of deaths over the past decade[4,5]. Malaysia's seasonal monsoon and tropical climate, characterised by high capacity rainfall, makes it favourable for bacterial survival. *Leptospira* have the ability to survive in moist, warm soil and in surface water for weeks to months, which leads to a high incidence rate of leptospirosis [6, 7].

Carrier animals cycle the leptospires within the population, and these bacteria may then be transmitted to humans directly via contact with infected urine or indirectly via contaminated soil or water. Human leptospirosis constitutes a dead-end infection with the human as the dead-end host, as human-to-human transmission is virtually unknown [8, 9].

This disease is often related to work circumstances, and agricultural workers have been noted to be one of the occupational groups at high risk of leptospirosis infection[10, 11]. In Malaysia, according to a survey for anti-*Leptospira* antibody sensitized-erythrocyte-lysis (SEL) tests conducted on 18 occupational groups, the highest antibody rates were found among oil palm plantation workers, accounting accounted for 32.6% of the cases [12].

In Malaysia, the oil palm industry is the main agriculture sector and a major source of employment; the industry supports 468,056 workers in the plantations alone[13, 14]. A previous animal study showed that the predominant rat species in oil palm plantations, *R. tiomanicus*, contributed 88.1% of the overall rat pathogenic leptospire isolates in the study, which revealed a high leptospirosis risk related to oil palm workers' activities [15].

Methodology

Study design and population

This cross-sectional study was conducted in 2014 among oil palm plantation workers in ten plantations in Melaka and Johor. Ethical approval was granted by the Research and Ethics Committee (Human), School of Medical Sciences, Health Campus, Universiti Sains Malaysia. All of the workers involved freely signed the informed consent form.

The sample size for the study was calculated based on 32.6% seroprevalence of leptospirosis among oil palm workers in Malaysia[12]. After considering a 10% non-response rate; the estimated sample size required was 374 workers. The inclusion criterion was oil palm plantation workers who had worked for at least six months; workers involved in office-related tasks were excluded.

Blood samples and serologic tests

The respondents who consented to take part in the study were interviewed for sociodemographic and work practice characteristics using a validated Malay version of a questionnaire. Venous blood samples were tested at the Institute of Medical Research (IMR) for the presence of anti-leptospiral antibodies with a microscopic agglutination test (MAT), following standard methods [16]. The MAT was performed with a panel of live leptospires. *Leptospira* reference cultures were obtained from Biomedical Research Royal Tropical Institute Amsterdam and from IMR. Agglutination was observed by observing free leptospires in each well. Sera with approximate numbers of free leptospires <50% in the control well were considered positive. A titre of ≥1:100, indicating past exposure to leptospira bacteria, [5, 17]was used as the cut-off titre for leptospirosis seropositive in this study.

Statistical analysis

Data were entered and analysed using IBM Statistical Program for Social Sciences (SPSS) Version 22 software for Windows (IBM, 2014) [18]. Confidentiality was maintained throughout the analysis. All continuous variables were described using mean and standard deviation (SD), and frequencies and percentages were used for categorical variables. Seroprevalence of leptospirosis was described with a 95% confidence interval (CI). Multiple logistic regressions were used to identify the associated work practice risk factors of leptospirosis seropositivity among the respondents.

Results

Sociodemographic

The response rate of the study was 93.6%, with 350 workers participating out of the 374 who were recruited. Table 1 presents the sociodemographic characteristics of the oil palm plantation workers who took part in this study. The respondents were relatively young, with a mean age of 31.38 years (SD 9.68); the majority were males (84.6%). In terms of citizenship, the majority, 285 (81.4%) respondents, were non-Malaysian. Half of the respondents had been working in their respective oil palm plantations for more than two years, and 101 (28.9%) of them had no formal education.

Seroprevalence

The overall seroprevalence of leptospirosis among the oil palm plantation workers was 28.6% (95% CI: 0.24, 0.33) (Table 2).

Univariate analysis

Table 3 displays the univariate analysis of the associated work practice risk factors among the respondents with leptospirosis seropositivity. 'Did not wear rubber gloves while working' was found to be more common among workers who were seropositive to leptospirosis (79.0%), and it was shown to be associated with seropositivity by univariate analysis (OR: 4.01; 95% CI: 2.33, 6.89; $p<0.001$). Use of the other personal protective equipment (PPE) items was not found to be significant by univariate analysis. Forty-five percent of workers who worked with a wounded hand were found to be seropositive to leptospirosis, and the risk factor was significantly associated with the disease by univariate analysis (OR: 3.83; 95% CI: 2.35, 6.23; $p<0.001$). Working with a foot wound was also found to be significantly associated with leptospirosis seropositivity by univariate analysis. The majority of the seropositive workers did not practice hand washing after work and before eating or drinking (70%), and that risk factor was found to be significantly associated with the infection (OR: 3.94; 95% CI: 2.39, 6.49; $p<0.001$). Smoking while working and animal contact were not found to be significant by univariate analysis. Variable selection was based on *p*-value, which was less than 0.25.

Multivariate analysis

Multiple logistic regression analyses revealed that the work practice risk factors significantly associated with leptospirosis seropositivity were 'did not wear rubber glove PPE' (AOR: 5.25; 95% CI: 2.88, 9.56; $p<0.001$), 'working with the presence of hand wound' (AOR: 3.13; 95% CI: 1.83, 5.36; $p<0.001$), and 'did not wash hands with soap after work before eating or drinking' (AOR: 3.97; 95% CI: 2.25, 7.02; $p<0.001$) (Table 4).

The preliminary final model was checked for model fitness, and no interaction for this preliminary final model or multicollinearity was detected. Based on the findings, this preliminary model was accepted as the final model. The assumptions in multiple logistic regression were also checked, and all the assumptions were met.

Fitness of the preliminary final model was determined using the Hosmer–Lemeshow goodness-of-fit test. It was found to be not significant ($p=0.778$), indicating that the model was fit, with a small discrepancy between the observed and expected probabilities. Model fitness was also supported by the classification table and receiver operating characteristics (ROC) curve. The area under the ROC curve was 79.1% (95% CI: 0.73, 0.85), indicating that the model could accurately discriminate 79.1% of the cases. The overall correctly classified percentage was good, with 79.1%. After meeting the criteria required, the final model was considered fit.

Discussion

The seroprevalence of leptospirosis among oil palm plantation workers was found to be high at 28.6% and comparable to previous studies. A high seroprevalence for anti-*Leptospira* antibodies using the SEL test was reported among oil palm plantation workers (32.6%), and in fact, it was

the highest prevalence among all 18 occupational groups studied, which included, among others, hospital staff (25.5%), rubber estate workers (23.2%), and town cleaning labourers (17.9%) [12]. A recent study conducted in a hospital in Kelantan, Malaysia, found that the prevalence of leptospirosis was highest among agriculture workers [11]. Another local study conducted among healthy paddy planters in north-eastern Malaysia using the SEL test reported 24.2% seroprevalence [19]. A previous study that adopted a similar MAT cut-off titre of ≥1:100 reported a lower seroprevalence rate (24.8%) of leptospirosis among town service workers compared to other high-risk occupational groups in Malaysia [5]. A study in Peru involving military recruits after a training exercise in a jungle reported a high leptospirosis seroprevalence rate of 28.0% [20].

The seroprevalence of leptospirosis among oil palm plantation workers in this study was also noted to be higher than that of the general population. In a hospital-based cross-sectional study conducted in Malaysia, seroprevalence of leptospirosis among the general population was 8.4% [11]. That finding was supported by a study in India, in which the seroprevalence among high-risk-occupation subjects was also higher than that of the general population [21]. These findings indicate that oil palm plantation workers are comparably at high risk of leptospira infection.

The reason for the high positivity in seroprevalence of leptospirosis among the workers in this study is probably related to rats, which are known to be carriers of leptospires. The rats can be found anywhere in oil palm plantations because they are attracted to fresh oil palm fruit [15, 19]. In addition, due to the favourable tropical climate and surface environment conditions in the plantations, the pathogenic leptospires are able to survive for long periods of time, thereby increasing the risk of disease transmission [7].

Oil palm plantation workers mainly use their hands in carrying out their manual work. For instance, the fruit collectors, who pick up loose fruits from the ground, have direct contact with a possibly contaminated environment, and transmissions of leptospires are made easier without the protection of rubber gloves. Also noted in this study was that only 42.9% of the workers used rubber gloves while working. The fact that a large percentage of workers do not wear protective rubber gloves further heightens the risk of leptospirosis infection. Without rubber gloves, the bacteria are able to enter the blood circulatory system through broken skin barriers. In a leptospirosis outbreak following a major flood in Australia, none of the confirmed cases of infection used protective gloves during their exposure to flood waters [22].

After the multivariate analysis was completed, it was found that the risk factor of working with the presence of wound(s) was also significantly associated with leptospirosis when adjusted for other significant factors. Cuts and wounds are common among oil palm plantation workers, as they work with thorny fruits and leaves, and leptospires enter the human body through skin wounds, abrasions, and mucous membranes, such as the conjunctiva [8]. The result in this study is supported by the findings of a study conducted in Germany [23]. In that study, the risk of leptospirosis was found to increase each day when strawberry farm workers worked in the rain with the presence of hand wounds. Similarly, a case–control study conducted in Nigeria among kennel workers also reported a significant association between the presence of wound(s) on either the hands or legs and contracting leptospirosis [24]. A study in India also found a significant association between the presence of wounds on the body while working and leptospirosis infection. The results showed that the presence of wounds while working increases the chances

of leptospira infection six-fold compared to subjects without wounds [25]. However, that study did not specify the sites of the wounds, whether they were found on the hand, foot, or any other part of the body.

A serologic survey in Thailand following an outbreak of leptospirosis noted a significant association between the presence of more than two wounds and seropositivity [26]. While previous studies have shown that the presence of wounds and an increased number of breaks in the skin are associated with leptospirosis infection, very few studies have reported on the effects of specific broken skin areas or suggested an increased infection risk from working with a hand wound. The results of the current study suggest that the probable mode of disease transmission among the oil palm plantation workers was contact between hand wounds and contaminated water or soil or direct contact with carrier animals in the plantation.

The transmission of leptospira bacteria can occur through ingestion and through invasion of the mucous membrane [3]; therefore, washing the hands with soap after working is one of the most important preventive measures against leptospira infection. Close to half of the workers in this study (46.6%) stated that they washed their hands with soap after working before eating or drinking. It was found that the workers who did not wash their hands with soap after working before eating or drinking had significantly higher odds (2.98%) of being seropositive for leptospirosis compared to the workers who washed their hands when adjusted for other factors. A similar finding was noted in the 2008 study among town service workers in Kelantan, which found washing hands with soap after working to be a significantly protective factor against leptospira infection [27]. This is a clear indication that dirty work practices increase the risk of infection, and that washing hands with soap after working before eating or drinking should be made compulsory to prevent leptospirosis. It is also worth noting that chlorine and iodine in detergents and soaps are considered to be lethal to the survival of the bacteria [28].

This seroprevalence study of oil palm plantation workers might reflect exposure, but not necessarily overt disease, as leptospirosis reinfection involving different serovars can still occur even if the individual has developed the antibody needed against a certain strain from the previous infection [6, 29].

Conclusion

The finding of high seroprevalence shows that oil palm plantation workers are at high risk for leptospiral infection. The manual work practices of the workers expose them to the surface soil and water environment in the plantation, which is most likely contaminated with the urine of infected animals.

The knowledge of associated work practice factors suggests that safe work practices should be highlighted in leptospirosis prevention programmes among oil palm plantation workers in future.

Conflict of interest statement

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

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

Variables	Frequency (%)	Mean (SD)
Age (years)		31.4 (9.68)
Gender		
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)	
Duration of work		
<1 year	74 (21.1)	
1–2 years	101 (28.9)	
>2 years	175 (50.0)	
Education		
No formal education	101 (28.9)	
Primary school	144 (41.1)	
Secondary school	105 (30.0)	

Table 2: Prevalence of leptospirosis seropositivity using a microscopic agglutination test (n=350)

Seropositivity status	Frequency	%	95% CI
Positive	100	28.6	23.8, 33.3
Negative	250	71.4	66.7, 76.2

Table 3: Association of work practice characteristics of the respondents with seropositive leptospirosis by simple logistic regression (n=350)

Variables	Seropositive n=100 Freq. (%)	Seronegative n=250 Freq. (%)	Crude Odds Ratio	95%CI	Wald (*df*)	*p* value
Wore PPE: rubber boots						
(No)[a]	47 (30.7)	106 (69.3)	1.21	0.76, 1.92	0.61 (1)	0.433
Wore PPE: rubber gloves						
(No)[a]	79 (39.5)	121 (60.5)	4.01	2.33, 6.89	25.29 (1)	<0.001
Wore PPE: long pants						
(No)[a]	3 (50.0)	3 (50.0)	2.55	0.51, 12.83	1.28 (1)	0.257
Wore PPE: long-sleeved shirt						
(No)[a]	40 (31.3)	88 (68.7)	1.23	0.76, 1.98	0.71 (1)	0.400
Working with hand wound						
(Yes)[b]	63 (45.0)	77 (55.0)	3.83	2.35, 6.23	29.12 (1)	<0.001
Working with foot wound						
(Yes)[b]	49 (34.5)	93 (65.5)	1.62	1.02, 2.59	4.09 (1)	0.043
Washing hands after work with soap before eating or drinking						
(No)[a]	70 (42.9)	93 (57.1)	3.94	2.39, 6.49	29.03 (1)	<0.001
Smoking						
(Yes)[b]	61 (29.5)	146 (70.5)	1.11	0.69, 1.79	0.20 (1)	0.655
Animal contact						
(Yes)[b]	21 (31.8)	45 (68.2)	1.21	0.68, 2.16	0.42 (1)	0.517

Reference group: [a]Yes, [b]No

Table 4: Association of work practice characteristics of the respondents with seropositive leptospirosis by multiple logistic regression (n=350)

Variable	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	*p* value
Wore PPE: rubber gloves			
Yes	1.00	1.00	
No	4.01 (2.33, 6.89)	5.25 (2.88, 9.56)	<0.001
Working with hand wound			
No	1.00	1.00	
Yes	3.83 (2.35, 6.23)	3.13 (1.83, 5.36)	<0.001
Washing hands after work with soap before eating or drinking			
Yes	1.00	1.00	
No	3.94 (2.39, 6.49)	3.97 (2.25, 7.02)	<0.001