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Work Environment-Related Risk Factors for Leptospirosis among Plantation Workers in Tropical Countries: Evidence from Malaysia

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Abstract

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Background: Leptospirosis is a zoonotic disease that is recognized as a re-emerging global public health issue, especially in tropical and subtropical countries. Malaysia, for example, has increasingly registered leptospirosis cases, outbreaks, and fatalities over the past decade. One of the major industries in the country is the palm oil sector, which employs numerous agricultural workers. These laborers are at a particularly high risk of contracting the disease.

Objective: To identify the work environment-related risk factors for leptospirosis infection among oil palm plantation workers in Malaysia.

Methods: A cross-sectional study involving 350 workers was conducted. The participants were interviewed and administered a microscopic agglutination test. Seropositivity was determined using a cut-off titer of $\geq 1:100$.

Results: 100 of 350 workers tested positive for leptospiral antibodies, hence, a seroprevalence of 28.6% (95% CI 23.8% to 33.3%). The workplace environment-related risk factors significantly associated with seropositive leptospirosis were the presence of cows in plantations (adjusted OR 4.78, 95% CI 2.76 to 8.26) and the presence of a landfill in plantations (adjusted OR 2.04, 95% CI 1.22 to 3.40).

Conclusion: Preventing leptospirosis incidence among oil palm plantation workers necessitates changes in policy on work environments. Identifying modifiable factors may also contribute to the reduction of the infection.

Keywords: Leptospirosis; Workplace; Risk factors; Agglutination tests; Agricultural workers' diseases

Introduction

Leptospirosis, a disease caused by spirochetes of the genus *Leptospira*, is a globally re-emerging bacterial

zoonotic disease that affects both humans and animals. It is transmitted to humans through contact between the skin or mucous membrane and water, moist soil, vegetation, or environmental surfaces that are

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contaminated with the urine of an infected animal. Given that human-to-human transmission is virtually unknown, human leptospirosis constitutes a dead-end infection, with the human as the dead-end host.^{1,2}

Symptomatic leptospirosis usually manifests as a range of diseases, from flu-like illness to Weil's syndrome, resulting in multi-organ failure and pulmonary hemorrhage. The most severe form of the disease is Weil's syndrome, which is characterized by jaundice, renal failure, and hemorrhage with a variable clinical course; its case fatality rate ranges from 5% to 15%.¹

Leptospirosis is estimated to affect tens of millions of humans all over the world annually, with case fatality rates of 5% to 25%.³ Among high-risk individuals, incidence may reach more than 100 per 100 000 people during outbreaks.⁴ The disease is considered endemic in Malaysia, with a moderate annual incidence of 1–10 incidences per 100 000 people.⁵ The country is characterized by a tropical climate and rainfall, thereby serving as a favorable environment for long-term bacterial survival. *Leptospira* can survive in moist, warm soil and in surface water for weeks to months, thus leading to a high incidence of leptospirosis.^{6,7}

Agricultural workers are at a particularly high risk of contracting leptospirosis infection.^{8,9} A previous study conducted in the Malaysian context indicates that among occupational groups, oil palm plantation workers exhibit the highest leptospirosis seroprevalence.¹⁰ As a country rich in natural resources, Malaysia is one of the world's main palm oil exporters, currently accounting for 44% of the world's total exports and 39% of the world's palm oil production. Such production means that the country is an important player in satisfying the global need for sustainable oils and fats.¹¹ According to a 2009 statistical report, the palm oil industry has become

the fourth largest contributor to the Malaysian economy.¹² It is also a major sector of employment, with plantation operators hiring more than 400 000 individuals as field workers in 2009.^{11,13} Another study in Malaysia reports that the predominant rat species in the country's oil palm plantations are *Rattus tiomanicus*, which accounted for the majority of the overall rat pathogenic leptospire isolates identified in the study. This finding confirms the high leptospirosis risk faced by oil palm plantation workers.¹⁴

Materials and Methods

Participants and Study Design

This cross-sectional study was conducted in June 2014 involving 10 oil palm plantations in Melaka and Johor. The ethical clearance for this study was obtained from the Research and Ethics Committee (Human), School of Medical Sciences, Health Campus, Universiti Sains Malaysia, on March 10, 2014. The sampling frame was the list of plantation workers provided by plantation managers. Guided by an interviewer, the workers who agreed to participate in the research answered a validated Malay language questionnaire that consisted of questions regarding sociodemographic and work environment characteristics.

Blood Samples and Serologic Tests

For the microscopic agglutination test (MAT), blood samples collected at the field were tested at the Institute of Medical Research (IMR) Kuala Lumpur. The MAT was conducted with a panel of live leptospire reference cultures, which were 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 for WHO

For more information on leptospirosis among butchers in Jamaica see

<http://www.theijoem.com/ijoem/index.php/ijoem/article/view/69>



serovars (Australis, Autumnalis, Bataviae, Canicola, Celledoni, Grippytyposa, Ictero-haemorrhagiae, Javanica, Pomona, Pyrogenes, Hardjoprajitno, Patoc, Tarassovi, and Djasiman) and from the IMR for local serovars (Melaka, Terengganu, Sarawak, Lai, Hardjobovis, and Copenhageni). Live leptospire cell suspensions that represent 20 serovars were added to serially diluted serum specimens in a well of microtiter plates and incubated at 30 °C for 2 hours. Agglutination was examined by dark field microscopy. If the approximate number of free leptospire was <50% relative to the control well, then a sample was regarded as positive for infection. A cut-off titer of $\geq 1:100$ is used in most laboratories for seroprevalence research to identify past exposure to an illness.^{15,16} In the current work, therefore, this cut-off value was adopted for definition of leptospirosis seropositivity.

Statistical Analyses

Data were entered and analyzed using the IBM Statistical Program for Social Sciences ver 22 for Windows®.¹⁷ Confidentiality was maintained throughout the analysis. All continuous variables were described using means and SDs. Categorical variables were presented in frequencies and percentages. Leptospirosis seroprevalence was calcu-

lated with a 95% CI. The associations between exposure to workplace environment risk factors and leptospirosis seropositivity were examined by univariate analysis. The odds ratios (ORs) of the associations, as well as their 95% CIs, were also calculated. Variables with a p value <0.25 upon univariate analysis were included in multivariate logistic regression models; a stepwise backward elimination method was used to identify the final model of the association between work environment risk factors and leptospirosis seropositivity. The final model was checked for interaction and multi-collinearity. Crude and adjusted ORs were calculated. A p value <0.05 was considered statistically significant.

Results

Sociodemographics

The initial sample for the survey was 374, but 24 workers withdrew from participation because of previously scheduled commitments and other reasons, thus leaving us with a final sample of 350 workers, hence, a response rate of 93.6%. Table 1 presents the sociodemographic characteristics of the participants. The mean age of participants was 31.4 (SD 9.7, range 18 to 65) years. In terms of citizenship, 285 (81.4%) of the respondents were non-Malaysians. Most of the respondents (71.1%) had either primary or secondary school education; 101 (28.9%) of them had no formal education. Half of the respondents had been working in their respective oil palm plantations for more than two years.

Seroprevalence

Among the 350 respondents, 100 (28.6%, 95% CI 23.8% to 33.3%) were seropositive for leptospiral antibodies. In terms of serovar distribution among the positive samples, the highest positive serovar was Sarawak (62%). For the other local *Leptospira*

TAKE-HOME MESSAGE

- We determined the seroprevalence of leptospirosis, a re-emerging zoonotic disease, in Malaysia.
- Oil palm plantation workers are at a high risk of leptospiral infection.
- Presence of cows in plantations and the presence of a landfill site in plantations were independent risk factors for leptospirosis seropositivity.
- Environment policies should be established to prevent leptospirosis incidence among oil palm plantation workers.

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

| Variables | n (%) |
|---------------------|------------|
| 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 yr | 74 (21.1) |
| 1–2 yrs | 101 (28.9) |
| >2 yrs | 175 (50.0) |
| Education | |
| No formal education | 101 (28.9) |
| Primary school | 144 (41.1) |
| Secondary school | 105 (30.0) |

serovars, the positive samples identified were Copenhageni (1%) and Terengganu (1%). For the WHO *Leptospira* serovars, the positive samples identified were Patoc (42%), Celledoni (7%), Javanica (3%), Australis (3%), Autumnalis (2%), and Pyrogenes (1%), (Table 2).

Univariate Analysis

As determined from the univariate analysis, the workplace environment characteristics that were significantly associated with seropositive leptospirosis were the presence of cows in plantations (OR 5.03, 95% CI 2.92 to 8.65), rat sightings in plantations (OR 3.27, 95% CI 1.72 to 6.19), and the presence of a landfill site in plantations

Table 2: Serovar distribution among 100 plantation seropositive workers. As the number of seropositive workers is 100, the number of serovars is equivalent to its percentage. frequency Workers tested may be positive for one or more serovars.

| Serovars tested | n |
|-----------------|----|
| Sarawak | 62 |
| Patoc | 42 |
| Celledoni | 7 |
| Javanica | 3 |
| Australis | 3 |
| Autumnalis | 2 |
| Pyrogenes | 1 |
| Copenhageni | 1 |
| Terengganu | 1 |

(OR 2.28, 95% CI 1.41 to 3.70). No statistically significant association was found between the presence of a river or swamp in plantations, the use of river or swamp water, and the presence of buffalos, goats, or pigs in plantations (Table 3).

Multivariate Analysis

Multivariate logistic regression analyses revealed a statistically significant association between leptospirosis seropositivity and work environment-related risk factors. These factors were the presence of cows in plantations (adjusted OR 4.78, 95% CI 2.76 to 8.26) and the presence of a landfill site in plantations (adjusted OR 2.04, 95% CI 1.22 to 3.40) (Table 4). The OR of the relationship between leptospirosis seropositivity and rat sightings in plantations was not statistically significant (adjusted OR 1.90, 95% CI 0.96 to 3.79).

The fitness of the preliminary model was validated; no interaction or multi-collinearity was detected. On this basis, this model was accepted as the final model.

Table 3: Association between workplace environment risk factors with seropositive leptospirosis as determined by simple logistic regression (n=350)

| Variables | Seropositive n=100, n (%) | Seronegative n=250, n (%) | Crude OR (95% CI) |
|------------------------------|------------------------------|------------------------------|---------------------|
| Presence of river or swamp | | | |
| No | 6 (30.0) | 14 (70.0) | 1 |
| Yes | 94 (28.5) | 236 (71.5) | 0.88 (0.35 to 2.49) |
| Using river or swamp water | | | |
| No | 56 (29.3) | 135 (70.7) | 1 |
| Yes | 44 (27.7) | 115 (72.3) | 0.92 (0.58 to 1.50) |
| Cows in plantation* | | | |
| No | 21 (12.8) | 143 (87.2) | 1 |
| Yes | 79 (42.5) | 107 (57.5) | 5.03 (2.92 to 8.65) |
| Buffalos in plantation | | | |
| No | 66 (27.6) | 173 (72.4) | 1 |
| Yes | 34 (30.6) | 77 (69.4) | 1.16 (0.71 to 1.90) |
| Goats in plantation | | | |
| No | 55 (29.6) | 131 (70.4) | 1 |
| Yes | 45 (27.4) | 119 (72.6) | 0.90 (0.57 to 1.44) |
| Pigs in plantation | | | |
| No | 90 (28.8) | 223 (71.2) | 1 |
| Yes | 10 (27.0) | 27 (73.0) | 0.92 (0.43 to 1.97) |
| Rat sightings in plantation* | | | |
| No | 13 (13.7) | 82 (86.3) | 1 |
| Yes | 87 (34.1) | 168 (65.9) | 3.27 (1.72 to 6.19) |
| Landfill site in plantation* | | | |
| No | 55 (23.0) | 184 (77.0) | 1 |
| Yes | 45 (40.5) | 66 (59.5) | 2.28 (1.41 to 3.70) |

*Variables with p<0.25 included in multivariate analysis

The validation of the assumptions in the multivariate logistic regression showed that all the assumptions were supported

by the data.

The fitness of the final model was then determined using the Hosmer-Lemeshow goodness-of-fit test. The model was non-significant (p=0.997), indicating fitness with a small discrepancy between observed and expected probabilities. Model fitness was also supported by the classification table and receiver operating characteristic curve. The area under the curve was 0.72 (95% CI 0.66 to 0.78), indicating that the model can accurately discriminate 72% of the cases. Overall, the model correctly classified the cases at 72.9% accuracy. The satisfaction of these criteria indicates the fitness of the final model.

Discussion

The seroprevalence results on the oil palm plantation workers suggest that exposure to work environment risk factors and leptospirosis reinfection with different serovars may occur even though the respondents had developed the antibodies needed against a certain strain from a previous infection.^{6,18}

Leptospirosis poses an important occupational hazard to agricultural manual laborers. A recent hospital-based study conducted in Kelantan, Malaysia, for which the MAT was used, showed that leptospirosis seroprevalence is highest among agricultural workers.⁹ The relatively high level of seroprevalence (28.6%) among the plantation or agriculture workers probably related to their daily occupational activities. Their natures of work heavily involve manual labor that increases the workers' contact with the environment that might be contaminated with *Leptospira* infected urine in the plantation compared to other job categories.

Leptospirosis seroprevalence among oil palm plantation workers is also higher than that observed in the general population of Malaysia (8.4%), as reported in

a 2013 study.⁹ An investigation into the context of India also revealed higher seroprevalence among workers in high-risk occupations than among the general population.¹⁹ These results support the argument that oil palm plantation workers are at a comparably high risk of leptospirosis infection.

The findings of the present research showed that the highest positive serovar was Sarawak (Lepto 175). A limited number of studies have been devoted to *L. interrogans* serovar Sarawak (Lepto 175), which is the local strain in Malaysia. A local study found that Sarawak (Lepto 175) is predominant in wild animals, especially squirrels and bats.²⁰ Information regarding pathogenicity and reservoir animals that harbor the serovar is minimal. In the current work, we could not speculate on reservoirs of infection because animal surveys have not been carried out in this area. Further research on these local serovars can advance our understanding of infection sources and transmission routes, as well as contribute to the development of prevention programs.

Close to half of the oil palm plantation workers (45.4%) use the river, trench or swamp water available in the plantations where they work—a practice that poses a risk of leptospirosis infection. Although these areas are not used as main water sources, the workers use them for swimming, bathing, and washing their hands, feet, and work equipment. They also mix the water with the pesticides used in the plantations.

An epidemiological investigation of a leptospirosis outbreak in Sabah in 1999 indicated that infection was contracted primarily by swimming in a creek that was most probably contaminated by the urine of infected animals from the surrounding area.²¹ The study, however, found no association between the presence and use of river, trench, or swamp water with leptospirosis seropositivity.

Table 4: Association of workplace environment risk factors with seropositive leptospirosis as determined by multiple logistic regression analysis (n=350)

| Variable | Crude OR (95% CI) | Adjusted OR (95% CI) |
|-----------------------------|---------------------|----------------------|
| Cows in plantation | | |
| No | 1 | 1 |
| Yes | 5.03 (2.92 to 8.65) | 4.78 (2.76 to 8.26) |
| Landfill site in plantation | | |
| No | 1 | 1 |
| Yes | 2.28 (1.41 to 3.70) | 2.04 (1.22 to 3.40) |

spiroserositivity.

Cows are frequently found in oil palm plantations, with a number of plantation managers/owners allocating a portion of the lands as grazing areas to cattle rearers.²² The integration of livestock rearing in oil palm plantations is a common practice, with proven economic benefits in terms of supplying meat and milk to plantations, improving land fertility, reducing pesticide costs, easing crop management, and increasing crop yield.²² Cows also noted to be more susceptible to leptospirosis than other common domestic animals in oil palm plantation, as goats.²³

The incidence of leptospirosis infection in cows can be classified into two groups. The first consists of contraction from a serovar type (eg, Hardjo) that is carried by and well adapted to cows. This serovar type is unaffected by regional factors or rain patterns. The second group comprises incidental infection from serovars carried by other animals in surrounding areas. These serovars are affected by surrounding environmental factors and breeding practices. The second group of leptospirosis infection commonly occurs in tropical countries.²³ In this study, serovar Hardjo types Hardjobovis and Hardjoprajitno were among the 20 serovars examined, but none of the workers tested positive for these serovars. This

finding probably suggested that the cows in the oil palm plantation area in this study (a factor significantly associated with the workers' leptospirosis seropositivity) became carriers by incidental infection from serovars carried by other animals that later contracted to the seropositive workers either direct or indirectly.

The univariate analysis indicated rat sightings as a significant factor. After controlling other variables in the multivariate analysis, this factor was found to be non-significant. Rats are main reservoir animals of *Leptospira* and are regarded as one of the most important leptospirosis disease transmission sources.²⁴ Among the participants, 255 (72.9%) reported rat sightings in their worksites—an unsurprising finding given that rats feed on fresh fruit and are therefore abundant in oil palm plantations.²⁵ Previous studies that identified *R. tiomanicus* as the major pests in Malaysian oil palm plantations showed an association between the presence of rats and leptospirosis infection.²⁶ This species was also noted as commonly carrying pathogenic leptospires.¹⁴ A study in Brazil notes that sighting five or more rats is associated with leptospirosis, which suggest potential dose-related exposure and seropositivity.²⁷

Among the respondents in the current research, 111 (31.7%) reported the presence of a landfill in the plantations where they worked. Landfills attract rat species that are primary *Leptospira* reservoirs. The presence of such sites also contributes to the proliferation of rat colonies. These carrier animals feed, breed, and multiply in uncollected solid waste, rotting piles of garbage, and open dumping areas, thereby, posing a major health risk to humans that reside or work near these surroundings.^{28,29} On top of this problem, domestic animals (eg, cows, goats, and dogs) are also present at most open dumping sites, thus further increasing the likelihood of

leptospirosis infection on the animals, which may later transmit the infection to human.

When other factors were adjusted, the workers who reported the presence of a landfill in their worksites exhibited a two-fold likelihood of seropositivity compared with the levels generated for those working in plantations with no landfill sites (Table 4). Reservoir animals in landfill sites may contaminate surrounding areas with urine containing leptospires. The oil palm plantation workers may be exposed towards leptospirosis through contact with a contaminated environment.

In conclusion, the seroprevalence results showed that oil palm plantation workers are at a high risk of leptospiral infection. The presence of cows in plantations and the presence of a landfill site in plantations were significantly associated with leptospirosis seropositivity. These findings point to the need to improve work environment policy to prevent leptospirosis incidence among oil palm plantation workers in the future.

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