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The Development of a Leptospirosis Screening Index for Oil Palm Plantation Workers

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ABSTRACT

Leptospirosis, a potentially fatal bacterial infection, is endemic in tropical and sub-tropical countries. Agricultural workers are at greater risk because of their increased contact with the environment. This study aimed to develop a leptospirosis screening index for oil palm plantation workers. It involved 350 oil palm plantation workers in southern Malaysia using an interviewer-guided questionnaire and serological microscopic agglutination test (MAT) with cut-off titre for seropositive at $\geq 1:100$. A new 13-point screening index was developed based on six significant leptospirosis associated factors of 'fruit collector job', 'did not wear rubber glove PPE', 'working with the presence of hand wound', 'did not wash hands with soap after work before eating or drinking', 'presence of cows in the plantation' and 'presence of a landfill site in the plantation'. The screening index has good discriminatory power of 83.9 percent. The newly-developed evidence-based screening index will likely help identify which of the many Malaysian oil palm plantation workers are most at risk of leptospirosis infection.

Keywords: Leptospirosis, screening index, microscopic agglutination test, oil palm plantation workers

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INTRODUCTION

Leptospirosis, a potentially fatal bacterial infection, is endemic in tropical and sub-tropical countries, estimated to affect tens of millions of humans annually worldwide ¹. In high-risk groups such as agriculture workers, the incidence may reach more than 100 per 100,000 population ².

An increase in reported cases and a significant number of deaths has occurred in Malaysia during the past decade ^{3, 4}. *Leptospira* survives in surface water and in warm, moist soil environments which are supported by the high rainfall in tropical climates ^{5, 6}.

Carrier animals cycle leptospires within their population, then transmit the leptospires to humans via contact with infected urine or contaminated soil or water. Leptospires enter the human body through skin lesions or mucous membranes ⁷.

Agricultural workers are at greater risk because of their increased contact with the environment ^{8, 9}. A survey of 18 occupational groups in Malaysia showed that oil palm plantation workers had the highest rate of antibodies at 32.6 percent ¹⁰. A previous animal study identified *R. tiomanicus*, the predominant rat species in oil palm plantations, as contributing 88.1 percent of the pathogenic leptospires isolated in the study ¹¹.

The palm oil industry is the chief agricultural activity and a key economic sector in Malaysia, contributing seven percent of gross domestic product in 2009, the fourth highest in the country ^{12, 13}. The industry is a major source of employment, supporting 468,056 plantation workers according to 2012 data ¹⁴.

MATERIALS AND METHOD

Study design and population

A cross-sectional study design was used involving workers on ten plantations.

Ethics

The study was granted ethical approval by the Research and Ethics Committee (Human), School of Medical Sciences, Health Campus, Universiti Sains Malaysia. All involved workers signed an informed-consent document.

Blood samples and serologic tests

Consenting workers were guided through a validated Malay-language questionnaire consisting of sociodemographic, work practice and workplace environment sections.

Venous blood samples were taken in the field and tested at the Institute of Medical Research (IMR) using a microscopic agglutination test (MAT) following standard methods ⁷. The MAT was performed with a panel of live leptospires. *Leptospira* reference cultures were obtained from the Department of Biomedical Research at the Royal Tropical Institute in Amsterdam, The Netherlands, and from IMR.

A titre of $\geq 1:100$ was used as a cut-off and considered to indicate that samples were seropositive for the leptospire bacteria ^{14,15}.

Statistical analysis

Data were recorded and analysed using IBM Statistical Program for Social Sciences (SPSS) Version 22 software for Windows ¹⁶. Confidentiality was maintained throughout the analysis. Multiple logistic regressions were used to identify the associated work practices and workplace environment risk factors of leptospirosis seropositivity among workers. The associated factors were estimated with the odds ratio calculated during logistic regression modeling. If the 95 percent confidence-interval (CI) crossed 1.0, the odds ratio was not significant at the p value ≥ 0.05 level.

Development of the screening index was based on the method described by Chang ¹⁷, by using the identified associated factors for leptospirosis through a multiple logistic-regression analysis. The first step in the development of the screening index was the division of each of the adjusted odds ratios by the smallest adjusted odds ratio in the final model of multiple logistic regressions, creating the Version II screening index. The Version II screening index was further adjusted by rounding the integers, making a final simplified 13-point screening index. The screening index was evaluated by discrimination which was further evaluated by the receiver operating characteristic area under the curve.

Our decision on the cut-off point for categorization of the screening index was based on the sensitivity and specificity of the Version III screening index at different total point levels for leptospirosis seropositive estimates calculated using Stata version 12 analysis.

RESULTS AND DISCUSSION

Sociodemographic

The respondent workers were relatively young. The majority were male non-Malaysians (Table 1). Half the respondents had been working for more than two years.

Univariable analysis

Variable selection was based on p -value, which was less than 0.25. Table 2 displays the univariable analysis of the associated work-practice risk factors among the respondents with leptospirosis seropositivity.

Table 1: Socio demographic 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)
Duration of work	
< 1 year	74 (21.1)
1 to 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: Association of work practice characteristics of the respondents with seropositive leptospirosis (n=350)

Variables	Seropositive n=100 Freq. (%)	Seronegative n=250 Freq. (%)	Crude OR	95%CI	Wald (df)	P value
Job category						
Fertilizer applicator (Yes) ^b	11 (22.9)	37 (77.1)	0.71	0.35, 1.46	0.87 (1)	0.352
Fruit collector (Yes) ^b	29 (59.2)	20 (40.8)	4.70	2.51, 8.81	23.25 (1)	<0.001
Harvester (Yes) ^b	23 (24.5)	71 (75.5)	0.75	0.44, 1.30	1.06 (1)	0.304
Pesticide applicator (Yes) ^b	13 (24.5)	40 (75.5)	0.78	0.40, 1.54	0.50 (1)	0.480
Pruner (Yes) ^b	15 (23.4)	49 (76.6)	0.72	0.39, 1.36	1.01 (1)	0.316
Nursery worker (Yes) ^b	4 (21.1)	15 (78.9)	0.65	0.21, 2.02	0.55 (1)	0.459
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
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

Reference group: ^aYes, ^bNo

Fruit collectors were significantly leptospirosis-seropositive with ($P<0.25$). Workers who did not wear rubber gloves were more commonly seropositive (79.0 percent) and the risk factor was associated with seropositivity at the univariate analysis ($P<0.25$).

Forty-five percent of workers who incurred hand wounds while working were seropositive for leptospirosis. We noted this risk factor was significantly associated with the disease along with the presence of foot wounds ($P<0.25$).

The majority of the seropositive workers (70 percent) did not practice hand-washing after work and before eating or drinking. We also noted this risk factor to be significantly associated with the infection ($P<0.25$).

Workplace environment factors found to be significant at the univariable level analysis ($P<0.25$) included the presence of cows on a plantation, rat sightings in the workplace, and the presence of a landfill site in the plantation (Table 3).

Table 3: Association of workplace environment factors with seropositive leptospirosis (n=350)

Variables	Seropositive	Seronegative	Crude OR	95% CI	Wald (df)	P value
	n=100	n=250				
	Freq. (%)	Freq. (%)				
Presence of river, trench or swamp						
(Yes) ^b	94 (28.5)	236 (71.5)	0.88	0.35, 2.49	0.02 (1)	0.884
Cow in plantation						
(Yes) ^b	79 (42.5)	107 (57.5)	5.03	2.92, 8.65	34.04 (1)	<0.001
Goat in plantation						
(Yes) ^b	45 (27.4)	119 (72.6)	0.90	0.57, 1.44	0.19 (1)	0.901
Rat sightings in plantation						
(Yes) ^b	87 (34.1)	168 (65.9)	3.27	1.72, 6.19	13.15 (1)	<0.001
Landfill site in plantation						
(Yes) ^b	45 (40.5)	66 (59.5)	2.28	1.41, 3.70	11.15 (1)	0.001

Reference group: ^bNo

Multivariable analysis

After controlling the confounder with multiple logistic regression, six variables were shown to be significantly associated with seropositive leptospirosis in the preliminary main-effect model ($P<0.05$). These variables were the fruit collector job category, not wearing rubber gloves as personal protective equipment, working with a wounded hand, not washing hands after work with soap before eating or drinking, the presence of cows in the plantation and the presence of a landfill site in the plantation.

We then checked the preliminary model for fitness. No interaction or multicollinearity were detected. As shown in Table 4, we then accepted the preliminary model 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 ($P = 0.471$), indicating that the model was fit with a small discrepancy between the observed and expected probability. Model fitness was also supported by the Classification Table and Receiver Operating Characteristics (ROC) curve. The area under the ROC curve was 84.1 percent (95 percent CI: 0.80, 0.89) which means the model can accurately discriminate 84.2 percent of the cases. The

overall correctly-classified percentage was good at 79.7 percent. After meeting the required criteria, the final model was considered fit.

Table 4: Associated factors for seropositive leptospirosis by multiple logistic regression analysis (n=350)

Variables	Beta	SE	Adjusted OR	95% CI	df	P Value
Fruit collector						
No			1			
Yes	1.55	0.39	4.69	2.20, 10.05	1	<0.001
PPE: Rubber gloves						
Yes			1			
No	1.52	0.32	4.58	2.41, 8.69	1	<0.001
Working with hand wound						
No			1			
Yes	1.18	0.30	3.26	1.80, 5.89	1	<0.001
Washing hands after work with soap before eating or drinking						
Yes			1			
No	1.10	0.32	2.98	1.60, 5.53	1	0.001
Cows in plantation						
No			1			
Yes	0.82	0.32	2.27	1.20, 4.30	1	0.011
Landfill site						
No			1			
Yes	0.71	0.30	2.04	1.13, 3.70	1	0.019

Hosmer and Lemeshow Test p-value = 0.471,

No multicollinearity and no interaction found

Classification Table overall correct percentage = 79.7%

Area under ROC curve = 84.1% (95% CI: 0.80, 0.89)

Development of screening index

Development of the screening index and its simplification were based on the multiple logistic regression results among the associated factors of leptospirosis seropositivity. Table 5 shows how the screening index was simplified by dividing each of the adjusted odds ratios by the smallest adjusted odds ratio in the final model of multiple logistic regression, thus creating the Version II screening index. Version II screening index points were further adjusted by rounding the integers: index points higher than two were rounded to three, and index points higher than one were rounded to two. That became the Version III screening index. This resulted in a final 13-point screening index with a maximum point-value of 13.

Based on the ROC curve (ROC 0.839), the Version III screening index maintained good discriminatory power. That means the model can accurately discriminate 83.9 percent of cases.

Table 5: Simplification and development of Version III screening index

Variables	Version I Adj. OR	Version II Points	Version III Points
Fruit collector	4.69	2.30	3.00
Did not wear rubber gloves as PPE	4.58	2.25	3.00
Working with hand wound	3.26	1.60	2.00
Washing hands after work with soap before eating or drinking	2.98	1.46	2.00
Presence of cows in the plantation	2.27	1.11	2.00
Presence of landfill site in the plantation	2.04	1.00	1.00
ROC (95% CI)	0.842 (0.80, 0.89)	0.842 (0.80, 0.89)	0.839 (0.79, 0.89)

Cut-off point

In deciding the cut-off point for separating workers into high and low-risk groups, the sensitivity and specificity value of each level of the total points were assessed (Table 6). The screening index with six total points had sensitivity of 83.0 percent and specificity of 69.6 percent. Raising the total points to seven improved the specificity to 79.2 percent but reduced sensitivity to 73.0 percent. As this study focused on the risks toward leptospirosis exposure, and the newly-developed screening index was meant for use in the field to prompt inclusion of exposed workers in future intervention programmes, we cut off the point score at six which had the receiver operating characteristic (ROC) curves furthest into the top left corner (Figure 1) and the largest combination of sensitivity and specificity, possibly reading off the curve^{18, 19}.

Table 6: Sensitivity and specificity of the Version III screening index at different total point levels for leptospirosis seropositive estimates

Points	Sensitivity (%)	Specificity (%)	Correctly classified	LR+	LR-
0	100.0	0.0	28.6	1.00	
1	99.0	12.0	36.9	1.13	0.08
2	98.0	14.4	38.3	1.14	0.14
3	96.0	25.6	45.7	1.29	0.16
4	95.0	45.6	59.7	1.75	0.11
5	89.0	56.0	65.4	2.02	0.20
6	83.0	69.6	73.4	2.73	0.24
7	73.0	79.2	77.4	3.51	0.34
8	57.0	89.2	80.0	5.28	0.48
9	47.0	94.0	80.6	7.83	0.56
10	30.0	98.0	78.6	15.0	0.71
11	15.0	99.6	75.4	37.5	0.85
12	9.0	99.6	73.7	22.5	0.91
13	2.0	100.0	72.0		0.98

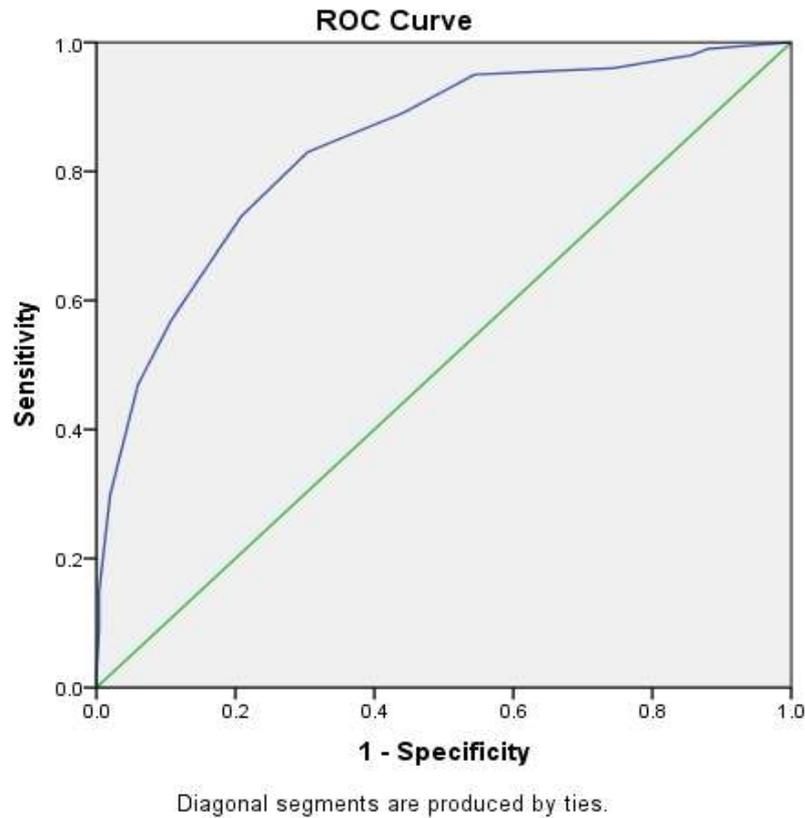


Figure 1: Receiver operating characteristic curve of the version III screening index total point score

Leptospirosis Screening Index for Oil Palm Plantation Workers

Worker's ID No: _____

Place a checkmark "√" next to each leptospirosis risk factor that applies to the worker.

No	Risk Factor	checkmark "√" if yes	Point
1	Job category of fruit collector		3
2	Did not wear PPE gloves		3
3	Working with the presence of hand wound		2
4	Did not wash hands with soap after work before eating or drinking		2
5	Presence of cows in plantation		2
6	Presence of landfill in plantation		1
		<u>Total point</u>	<u>/13</u>

If the total point of the worker is **six or more**, the worker is consider high risk for leptospirosis infection.

Figure 2: Proposed Leptospirosis Screening Index for Oil Palm Plantation Workers

The cut-off point sorted workers with total screening index points of six or more into the high-risk group, while those who had fewer than six were categorized in the low-risk group.

Table 7 shows significant association between the risk categories based on *p* value 0.05. The high-risk group were 11 times more likely to be leptospirosis seropositive than the low-risk group.

Table 7: Relationship of risk category based on the screening index with leptospirosis seropositive status

Variables	Seropositive n=100 Freq. (%)	Seronegative n=250 Freq. (%)	Crude OR	95% CI	Wald (df)	P value
Risk category						
Low risk	17 (8.9)	174 (91.1)	1			
High risk	83 (52.2)	76 (47.8)	11.18	6.22, 20.11	64.91 (1)	<0.001

DISCUSSION

Associated risk factors

Fruit collectors were 4.69 times more likely to be seropositive than other workers. Their job, which consists of picking up thorny fruit from the ground and placing it into carts, has the greatest risk of hand wounds and environmental exposure to leptospires.

Transmission of leptospires is more likely without the protection of rubber gloves. According to an outbreak report following a major flood in Australia, none of the infected individuals had used protective gloves when exposed to the flood²⁰.

Leptospires enter the human body through wounds, abrasions or mucous membranes such as the conjunctiva²¹. Transmission may also occur through ingestion², therefore washing hands with soap after work is one of the most important preventive measures. Workers who did not wash their hands with soap after work before eating or drinking were 2.98 times more likely to be seropositive compared to those workers who did wash, when adjusted for other factors. Chlorine or iodine in detergent or soap is considered lethal to the bacteria²².

The majority of the workers mentioned the presence of cows in the plantation. Cows are commonly seen in oil palm plantations, and more susceptible to leptospirosis than other animals such as goats²³.

Landfills attract rats which are the main leptospires reservoir²⁴. Reservoir animals may contaminate the surrounding area with leptospire-laden urine, exposing nearby workers.

Newly developed leptospirosis screening index

Our newly-developed screening index can be used to decide which oil palm plantation workers have a high-risk of leptospirosis exposure, enabling intervention programmes to be directed where needed.

However, this screening index should be used with caution. Those categorized in the low-risk group should not be wholly dismissed if there is other compelling evidence such as direct contact with known infected animals. The categorized screening index was noted to have negative predictive value of 91.1% (174/191) and positive predictive value of 52.2% (83/159) (table 6). Despite this limitation, the screening index can be considered a good tool in light of the high ROC value (0.839).

The proposed leptospirosis screening index for oil palm plantation workers is shown in figure 2. Proper use of the screening index will assist occupational health practitioners in selecting high-risk groups of oil palm plantation workers with a view to developing more focused intervention-programmes.

CONCLUSION

Work practice and workplace environment factors were associated significantly with leptospirosis seropositivity. This suggests that safe work practices and environmental policy changes should be considered to prevent leptospirosis among oil palm plantation workers. This study developed a new evidence-based screening index by selecting factors which will likely identify which of the many Malaysian oil palm plantation workers are most at risk of leptospirosis infection.

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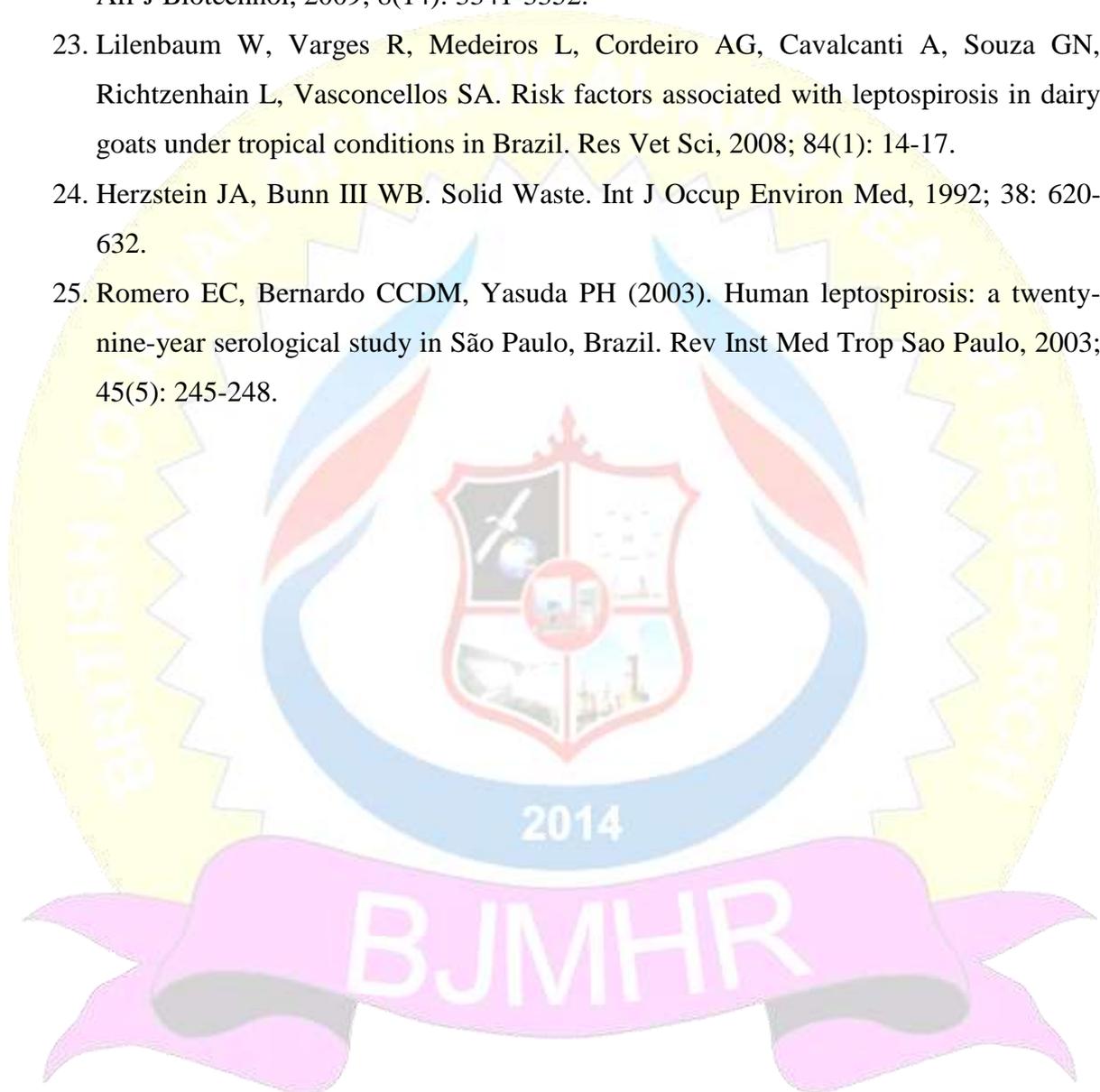
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