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RESEARCH ARTICLE

Seroprevalence and factors associated with leptospirosis among market traders in Kudat town, Sabah: A cross-sectional study

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ABSTRACT

Leptospirosis remains a major public health concern in tropical regions, including Malaysia, where it is endemic. To date, evidence from remote and understudied areas remains limited. This study aims to assess the epidemiological evidence of leptospirosis among market traders in Sabah, addressing the limited available data for this potentially high-risk occupational group in this region. A cross-sectional study was conducted among 295 market traders in Kudat Town, Sabah. Sociodemographic, occupational, environmental and behavioural data were collected using a modified validated questionnaire. Venous blood samples were collected to test for *Leptospira* antibodies using the Microscopic Agglutination Test; a titre of 1:50 or higher was considered seropositive. Seroprevalence was estimated descriptively, while factors associated with leptospiral seropositivity were examined using univariable and multivariable logistic regression analyses. The seroprevalence of leptospirosis among market traders was 5.4% (95% CI: 3.1–8.7). Antibodies were detected against 11 *Leptospira* serovars/strains, mainly Lep175. In univariable analysis, predictors for seropositivity included trading frequency, rodent presence, and proximity to garbage piles. With only 16 cases, the initial multivariable model had a low EPV of 4, suggesting possible overfitting. After reducing the model (EPV = 8), proximity to garbage piles remained strongly associated (AOR = 52.61; 95% CI: 13.50–205.13; $p < 0.001$), while rodents at home also became significant (AOR = 5.70; 95% CI: 1.12–29.08; $p = 0.037$). This study provides the first empirical evidence of leptospirosis exposure among market traders in Sabah. Although the overall seroprevalence was low, the findings indicate a measurable risk and underscore the need for further large-scale studies and integrated One Health interventions to reduce exposure in this vulnerable population.

Keywords: Leptospirosis; seroprevalence; associated factors; environmental; occupational.

INTRODUCTION

Leptospirosis is a globally important zoonotic disease caused by pathogenic bacteria of the genus *Leptospira* and transmitted primarily through direct or indirect contact with water or soil contaminated by the urine of infected animals (Bradley & Lockaby, 2023; Md-Lasim *et al*., 2021). Globally, approximately 1 million cases of human leptospirosis occur per year, with about 60,000 deaths, and the highest burden is reported in tropical and subtropical regions such as Southeast Asia, Latin America, and the Pacific Islands (Costa *et al.*, 2015; Munoz-Zanzi *et al.*, 2020). The clinical manifestation of leptospirosis is characterised by fever, chills, headache and myalgia, sometimes with abdominal pain during the acute phase and may progress to severe forms, including Weil's syndrome and pulmonary involvement, leading to multiorgan failure or death (Rajapakse, 2022; Parra Barrera *et al.*, 2023).

In developing nations, leptospirosis poses both a public health and socioeconomic challenge, as bacterial transmission is facilitated by inadequate sanitation, waste management and drainage systems (Karpagam & Ganesh, 2020; Teles *et al.*, 2023; Muñoz-Zanzi *et al.*, 2025). The pathogenic *Leptospira* spp. survive and sometimes adapt to hostile environmental conditions, such as humid, warm, and waterlogged conditions, which can cause leptospirosis among humans, shaped by occupational, environmental, and behavioural factors (Bierque *et al.*, 2020; Bradley & Lockaby, 2023). Vulnerable occupational groups at risk of leptospirosis include sanitation or sewage workers (Baharom *et al.*, 2024), agriculture or livestock workers (Afandi *et al.*, 2023) and military personnel (Neela *et al.*, 2019; Petakh *et al*., 2025). Moreover, limited access to healthcare further increases the risk of leptospirosis and its outcomes due to delayed diagnosis, treatment, and prevention (Parra Barrera *et al.*, 2023; Muñoz-Zanzi *et al.*, 2025).

In Malaysia, leptospirosis remains endemic and continues to pose a significant public health challenge, with periodic outbreaks reported across several states (Philip & Ahmed, 2023; Lea *et al.*, 2025). A recent report revealed that the case of leptospirosis in this country had an incidence rate of 7.12 and a mortality rate of 0.13 per 100,000 population in 2024, highlighting its ongoing public

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health importance despite lower mortality than other communicable diseases such as tuberculosis and HIV/AIDS (MOH, 2025). The endemicity of leptospirosis in this country is attributed to its warm, humid tropical climate and frequent rainfall and monsoon-related flooding (Garba *et al.*, 2017; Mohd Yusof *et al.*, 2025). Public markets, particularly wet markets, are recognised as transmission hubs due to poor drainage systems, waste accumulation and the abundance of rodents, which favour the survival of *Leptospira* (Ally *et al.*, 2023; Noh *et al.*, 2024; Ling *et al.*, 2025). Previous studies have reported high leptospirosis seroprevalence among public or wet markets occupational groups in Peninsular Malaysia, including 46.3% in central Malaysia (Samsudin *et al.*, 2018) and 33.6% in northeastern Malaysia (Rahman *et al.*, 2018), which agrees with similar groups in Morocco (El Azhari *et al.*, 2020) and Indonesia (Widiastuti & Priyanto, 2020).

Despite these findings, data on leptospirosis in Sabah remain scarce. Although sporadic outbreaks and serological evidence suggest that the disease is endemic in the region (Jeffree *et al.*, 2020; Philip & Ahmed, 2023), no published study has focused on market traders or examined the combined effects of occupational, behavioural and environmental exposures among this population. Therefore, the present study was conducted to determine the seroprevalence of leptospirosis and to identify factors associated with infection among market traders in Kudat Town, Sabah.

MATERIALS AND METHODS

The study involved questionnaire administration, blood sample collection, laboratory testing, and statistical analysis to determine leptospirosis seropositivity and associated factors among market traders. The overall study workflow, including data collection, processing, analysis, and output generation, is summarized in Figure 1.

Study Design

A cross-sectional study was conducted among market traders in Kudat Town, Sabah, between September and October 2022. Eligible participants were asymptomatic traders aged $\geq$18 years with at least six months of trading experience who provided informed consent; incomplete responses were excluded. The sample size was estimated using the single-population proportion formula by Charan and Biswas (2013), based on a 95% confidence level, a 5% margin of error, and an expected prevalence of 33.6% from an occupational group in a similar setting (Rahman *et al.*, 2018), as follows:

$$\text{Sample size } (n) = \frac{(Z_{1-\alpha/2})^2 \cdot p_0\,(1-p_0)}{d^2}$$

N = The total sample size collected in this study (295)
$Z_{1-\alpha/2}$ = Confidence interval at 95% (1.96)
p_0 = Expected population proportion based on previous study (33.6% or 0.336)
d = Margin of error in this study = *MOE* = 5%

The required sample size was calculated to be 344.8 by substituting the values of ($Z_{1-\alpha/2}$ = 1.96, p_0 = 0.336, d = 0.05), rounded up to 345 respondents. To account for potential nonresponse, an additional 10% was added, resulting in a final sample size of N = 383 respondents, providing about 80% statistical power to detect significant associations at α = 0.05 (Charan & Biswas, 2013).

Sampling Procedure

A stratified sampling design was used, grouping market traders into strata by product type (Rahman *et al.*, 2022). Due to the absence of a complete list of eligible traders, simple random sampling could not be used; instead, every third eligible trader within each stratum was selected using stratified systematic sampling, following the fixed-interval approach described by Mweshi and Sakyi (2020). These estimates were used to allocate the sample proportionally across strata, improving representativeness and precision (Uribeetxebarria *et al.*, 2018).

Data Collection (Questionnaire)

Data were collected using a structured, interviewer-administered questionnaire adapted from previous leptospirosis Knowledge, Attitude and Practice (KAP) studies and adjusted to the local context (Nozmi *et al.*, 2018; Zahiruddin *et al.*, 2018). The tool was developed in Google Forms, enabling efficient, paperless, and cost-effective data collection with broad accessibility (Nguyen *et al.*, 2018), and is commonly used in recent KAP studies (Ali *et al.*, 2021; Chang *et al.*, 2021). Briefly, the questionnaire covered sociodemographic, occupational, environmental, and the KAP domains. Content

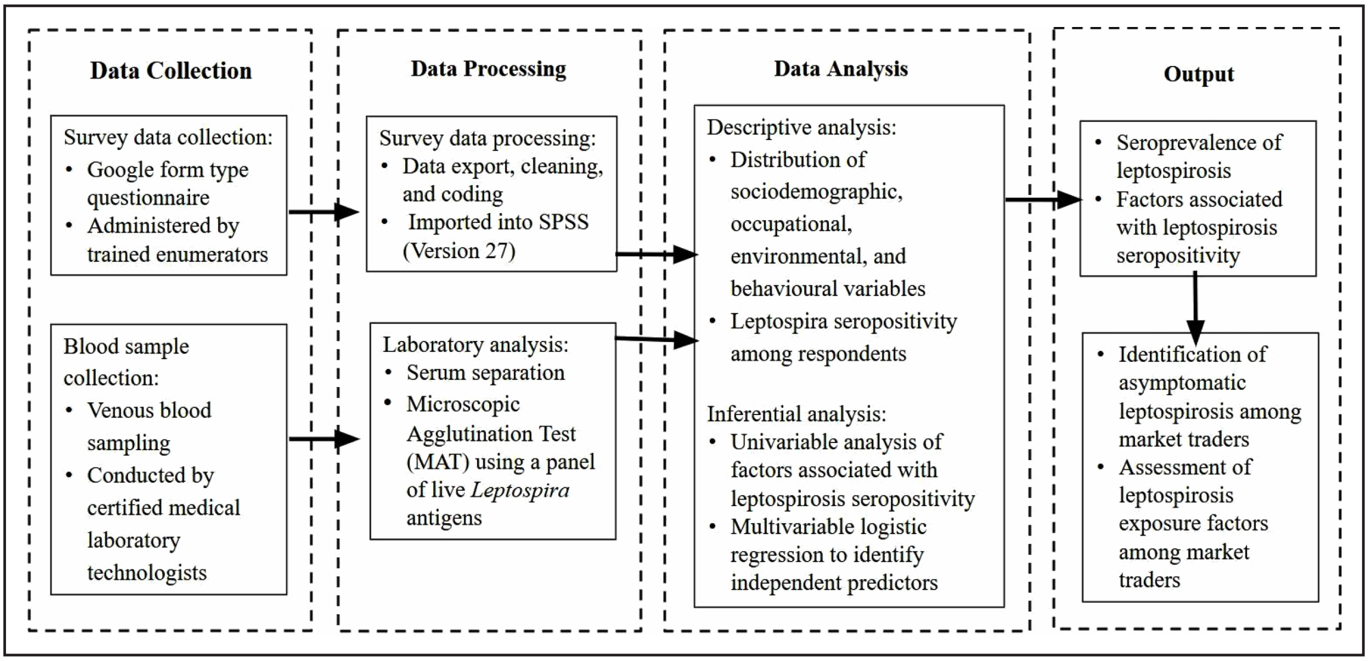

Figure 1. Study flowchart illustrating the sequence of data collection, processing, analysis and output.

validity was checked by experts, followed by a pre-test (n = 10) and pilot study (n = 30), with minor revisions for clarity. As shown in Table 1, reliability was assessed using Cronbach's alpha for both the pilot and main studies, indicating acceptable consistency across domains (Cronbach, 1951; Tavakol & Dennick, 2011; Jain & Angural, 2017; Taber, 2018). Interviews were conducted in Bahasa Malaysia by trained enumerators, but responses may be subject to recall and social desirability biases.

Table 1. Reliability Statistics of Likert-Scale Variables in Questionnaire

Study Phase	Construct	*n*	*α*	Interpretation
Pilot Study (*N* = 30)	Knowledge	24	0.951	Excellent
	Attitude	13	0.769	Acceptable
	Practice	19	0.744	Acceptable
Overall Interpretation				Good
Main Study (*N* = 295)	Knowledge	24	0.975	Excellent
	Attitude	12	0.769	Acceptable
	Practice	17	0.765	Acceptable
Overall Interpretation				Fairly High

Note. α = Cronbach's alpha coefficient; Total sample (*N*) = 295; *n* = number of items (sub-sample size). Values indicate internal consistency for each construct. Overall interpretation summarises reliability across constructs for each study phase, based on standard guidelines (Jain & Angural, 2017).

Specimen Collection and Serological Testing

Approximately 5 mL of venous blood was collected aseptically from each consenting participant, and serum samples were tested for *Leptospira* antibodies using the Microscopic Agglutination Test (MAT) following standard procedures (Goris & Hartskeerl, 2014). The MAT panel included 24 live antigens comprising WHO reference strains and locally isolated strains supplied by the Institute for Medical Research, Malaysia (Supplementary Table S1). A MAT titre of $\geq$1:50 was used to define seropositivity, consistent with sero-epidemiological studies among asymptomatic populations in endemic settings (Suut *et al.*, 2016; Jeffree *et al.*, 2020; Rajapakse, 2022).

Statistical Analysis

Data were cleaned and analysed using IBM SPSS Statistics version 27.0. Descriptive statistics were summarised as frequencies and percentages. Associations between potential sociodemographic, occupational, environmental and behavioural factors and leptospirosis seropositivity were examined using univariable logistic regression and variables with $p \leq 0.25$ were entered into a multivariable logistic regression model using the backward likelihood ratio method (Hosmer *et al*., 2013). Adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were reported, with $p \leq 0.05$ considered statistically significant; model goodness-of-fit statistics are shown in Supplementary Table S2.

Ethical Considerations

Ethical approval was obtained from the Medical Research Ethics Committee of Universiti Malaysia Sabah, and permission for fieldwork was granted by the Lembaga Bandaran Kudat. Written informed consent was obtained from all participants, participation was voluntary, and confidentiality and anonymity were maintained throughout the study.

RESULTS

Sample Size and Response Rate

The planned sample size was 383 to achieve ~80% statistical power to detect significant associations at $\alpha = 0.05$. However, only 295 traders were included in the final analysis, yielding a response rate of 77.0% (295/383 × 100), thereby reducing the study's statistical power to approximately 74% (an informal post hoc estimate, not a formal post hoc analysis). The exact margin of error (MOE) for the current study was 5.4%, which is slightly higher than the target of 5.0%, indicating reduced precision. Therefore, precision and uncertainty were reported using the MOE and 95% confidence interval, which better reflects the reliability of the findings.

Seroprevalence and serovar/strain distribution

The seroprevalence of leptospirosis in this study was 5.4% (16/295; 95% CI: 3.1–8.7). As shown in Supplementary Table S3, seropositive respondents reacted to 11 *Leptospira* serovars/strains, with MAT titres ranging from 1:50 to 1:800 and no single serovar predominating, indicating heterogeneous exposure within the population. The most common serovars/strains were HOS and Lep 175 (each 1.4%), with titres ranging from 1:50 to 1:800, although most were low (mainly 1:50). Other serovars/strains (BIR, PAT, BIG, Lep 1, Lep 115, BOV, GRI, PRAV) showed minimal reactivity. Most cases showed single serovar reactivity (75.0%), while 25.0% showed dual reactivity, suggesting possible co-exposure or cross-reactivity.

Sociodemographic factors

Leptospiral seropositivity by sociodemographic characteristics is shown in Table 2. Lower seropositivity was observed among respondents aged $\geq$49 years (4.5%) compared with those aged <49 years (6.4%), while females showed slightly higher seropositivity (6.1%) than males (4.1%). Married respondents had higher seropositivity (6.4%) than single or widowed respondents (1.7%), whereas similar proportions were observed among Malaysian (5.4%) and non-Malaysian respondents (5.3%). Higher seropositivity was observed among participants from households with $\geq$4 persons (6.0%) compared with those from smaller households (4.2%). Respondents with basic or higher education showed lower seropositivity (4.1%) than those with less than basic education (9.2%), while those with a monthly household income $\geq$RM800 had slightly higher seropositivity (6.1%) than those earning <RM800 (4.8%). No sociodemographic variables were significantly associated with seropositivity in univariable analysis; however, marital status and education level met the screening criterion ($p < 0.25$) and were included in multivariable modelling.

Occupational factors

Leptospiral seropositivity by occupational characteristics is shown in Table 3. Respondents trading for $\geq$27 months showed lower seropositivity (4.0%) than those trading for <27 months (6.9%). Seropositivity was lower among respondents operating from mobile or temporary premises (3.1%) than among those operating from permanent premises (4.5%), while seropositivity was higher among those trading from ground-based premises (11.3%). Respondents selling fresh animal-based products showed higher seropositivity (8.2%) than those selling fresh plant-based (4.9%) or processed products (6.5%), whereas non-food product sellers had lower seropositivity (2.0%). Higher seropositivity was also observed among respondents who traded $\geq$4 days per week (7.5%) compared with those who traded <4 days per week (2.5%). No occupational variables were statistically significant in univariable analysis; however, type of premise, trading frequency and type of product sold met the screening criterion ($p < 0.25$) and were considered for inclusion in subsequent multivariable modelling.

Table 2. Leptospirosis seropositivity of respondents by sociodemographic characteristics

Variable	Category	Total, n (%)	MAT Serodiagnosis		Crude OR	COR 95% CI	P-value
			Positive, n (%)	Negative, n (%)			
Age	< 49 years	141 (47.8)	9 (6.4)	132 (93.6)	1.00	–	–
	≥ 49 years	154 (52.2)	7 (4.5)	147 (95.5)	0.70	0.25–1.93	0.488
Gender	Male	97 (32.9)	4 (4.1)	93 (95.9)	1.00	–	–
	Female	198 (67.1)	12 (6.1)	186 (93.9)	1.50	0.47–4.78	0.493
Marital status	Single/Widowed	60 (20.3)	1 (1.7)	59 (98.3)	1.00	–	–
	Married	235 (79.7)	15 (6.4)	220 (93.6)	4.02	0.52–31.08	0.182
Nationality	Non-Malaysian	19 (6.4)	1 (5.3)	18 (94.7)	1.00	–	–
	Malaysian	276 (93.6)	15 (5.4)	261 (94.6)	1.03	0.13–8.28	0.975
Household size	< 4 persons	95 (32.2)	4 (4.2)	91 (95.8)	1.00	–	–
	≥ 4 persons	200 (67.8)	12 (6.0)	188 (94.0)	1.45	0.46–4.63	0.528
Education level	Less than basic	76 (25.8)	7 (9.2)	69 (90.8)	1.00	–	–
	Basic or higher	219 (74.2)	9 (4.1)	210 (95.9)	0.42	0.15–1.18	0.099
Monthly household income	< RM 800	147 (49.8)	7 (4.8)	140 (95.2)	1.00	–	–
	≥ RM 800	148 (50.2)	9 (6.1)	139 (93.9)	1.30	0.47–3.57	0.618

Note. Categorical data are presented as counts (percentages). Crude odds ratios (CORs) were estimated using univariable logistic regression. Reference categories are indicated accordingly; "No" responses were coded as 0. Education level was classified according to UNESCO (2012): less than basic (no formal education or pre-school), basic (primary or lower secondary), and intermediate or higher (upper secondary or tertiary). P-values were calculated within groups; values ≤ 0.25 (in bold) were considered for inclusion in multivariable analysis.

Table 3. Leptospirosis seropositivity of respondents by occupational characteristics

Variable	Category	Total, n (%)	MAT Serodiagnosis		Crude OR	COR 95% CI	P-value
			Positive, n (%)	Negative, n (%)			
Duration of trading	< 27 months	145 (49.2)	10 (6.9)	135 (93.1)	1.00	–	–
	≥ 27 months	150 (50.8)	6 (4.0)	144 (96.0)	0.56	0.20–1.59	0.278
Type of premise	Permanent (stall-based)	177 (60.0)	8 (4.5)	169 (95.5)	1.00	–	–
	Mobile/temporary (table/cart/trolley)	65 (22.0)	2 (3.1)	63 (96.9)	0.67	0.14–3.24	0.619
	Ground-based (mat/canvas)	53 (18.0)	6 (11.3)	47 (88.7)	2.70	0.89–8.16	0.079
Type of product sold	Fresh animal-based (fish/seafood/livestock meat)	61 (20.7)	5 (8.2)	56 (91.8)	1.00	–	–
	Fresh plant-based (vegetables/ fruits /plants)	123 (41.7)	6 (4.9)	117 (95.1)	0.57	0.17–1.96	0.376
	Processed/ prepared foods (dried/cooked foods/herbs/plants)	62 (21.0)	4 (6.5)	58 (93.5)	0.77	0.20–3.03	0.711
	Non-food products (cloth/textiles/handicraft)	49 (16.6)	1 (2.0)	48 (98.0)	0.23	0.03–2.07	0.191
Trading frequency	< 4 days per week	121 (41.0)	3 (2.5)	118 (97.5)	1.00	–	–
	≥ 4 days per week	174 (59.0)	13 (7.5)	161 (92.5)	3.18	0.89–11.40	0.076

Note. Categorical data are presented as a number (percentage). Crude odds ratios (CORs) were estimated using univariable logistic regression. P-values were calculated within groups; values ≤ 0.25 (in bold) were considered for inclusion in multivariable analysis.

Environmental factors

Leptospiral seropositivity by environmental characteristics is presented in Table 4. Higher seropositivity was observed among respondents with rodents at home (8.4%) than among those without, whereas no significant association was observed for the presence of animals at the market. Respondents living near garbage piles (<200 m) showed markedly higher seropositivity (13.8%) compared with those without nearby garbage piles, with a strong association observed in univariable analysis (COR 53.24; 95% CI: 14.05–201.67; $p < 0.001$). Seropositivity did not differ significantly according to flood-affected housing, proximity to rivers, farms, or forests, or participation in outdoor water-related activities. Based on the prespecified screening criterion ($p < 0.25$), the presence of rodents at home and proximity to garbage piles were included in subsequent multivariable modelling.

Behavioural factors

Leptospiral seropositivity by behavioural characteristics is presented in Table 5. Seropositivity was observed among respondents with low knowledge levels (6.1%), negative attitudes (5.6%) and poor practices (7.8%). In univariable analysis, lower odds of seropositivity were observed among respondents with low knowledge (COR 0.57; 95% CI: 0.16–2.07) and poor practices (COR 0.48; 95% CI: 0.18–1.34), although these associations were not statistically significant. Attitude

Table 4. Leptospirosis seropositivity of respondents by environmental characteristics

Variable	Category	Total, n (%)	MAT Serodiagnosis		Crude OR	COR 95% CI	P-value
			Positive, n (%)	Negative, n (%)			
Presence of animals at market	Rodents	266 (90.2)	16 (6.0)	250 (94.0)	–	–	–
	Cats	135 (45.8)	7 (5.2)	128 (94.8)	0.92	0.33–2.53	0.868
	Dogs	148 (50.2)	9 (6.1)	139 (93.9)	1.30	0.47–3.57	0.618
	Other animals	179 (60.7)	11 (6.1)	168 (93.9)	1.45	0.49–4.30	0.499
Presence of animals at home	Rodents	166 (56.3)	14 (8.4)	152 (91.6)	5.85	1.31–26.22	0.021
	Cats	198 (67.1)	13 (6.6)	185 (93.4)	2.20	0.61–7.92	0.227
	Dogs	44 (14.9)	2 (4.5)	42 (95.5)	0.81	0.18–3.68	0.781
	Other animals	10 (3.4)	0 (0.0)	(100.0)	–	–	–
House surrounding	Affected by flood	64 (21.7)	4 (6.3)	60 (93.8)	1.22	0.38–3.91	0.742
	Near river (<200 m)	77 (26.1)	4 (5.2)	73 (94.8)	0.94	0.29–3.01	0.918
	Near paddy field (<200 m)	26 (8.8)	2 (7.7)	24 (92.3)	1.52	0.33–7.08	0.595
	Near animal farm (<200 m)	14 (4.7)	0 (0.0)	14 (100.0)	–	–	–
	Near forest (<200 m)	70 (23.7)	3 (4.3)	67 (95.7)	0.73	0.20–2.64	0.632
	Near garbage pile (<200 m)	34 (11.5)	13 (38.2)	21 (61.8)	53.24	14.05–201.67	<0.001
Outdoor water-related activities	Gardening	102 (34.6)	4 (3.9)	98 (96.1)	0.62	0.19–1.96	0.412
	Fishing	55 (18.6)	2 (3.6)	53 (96.4)	0.61	0.13–2.76	0.520
	Swimming	42 (14.2)	2 (4.8)	40 (95.2)	0.85	0.19–3.90	0.838

Note. Categorical data are presented as a number (percentage). Crude odds ratios (CORs) were estimated using univariable logistic regression. P-values were calculated within groups; values ≤ 0.25 (in bold) were considered for inclusion in multivariable analysis.

Table 5. Leptospirosis seropositivity of respondents by behavioural characteristics

Variable	Category	Total, n (%)	MAT Serodiagnosis		Crude OR	COR 95% CI	P-value
			Positive, n (%)	Negative, n (%)			
Knowledge	Low level	212 (71.9)	13 (6.1)	199 (93.9)	0.57	0.16–2.07	0.396
Attitude	Negative	71 (24.1)	4 (5.6)	67 (94.4)	0.95	0.30–3.04	0.929
Practice	Poor	116 (39.3)	9 (7.8)	107 (92.2)	0.48	0.18–1.34	0.162

Note. Categorical data are presented as a number (percentage). Crude odds ratios (CORs) were estimated using univariable logistic regression. P-values were calculated within groups; values ≤ 0.25 (in bold) were considered for inclusion in multivariable analysis.

showed no meaningful association with seropositivity (COR 0.95; 95% CI: 0.30–3.04). Based on the prespecified screening criterion ($p < 0.25$), practice was included in subsequent multivariable modelling, whereas the remaining behavioural variables were not.

Multivariable logistic regression

Multivariable logistic regression results are shown in Tables 6 and 7. With only 16 seropositive cases, the initial model with four predictors had a low events-per-variable (EPV) of 4, suggesting possible overfitting, where proximity to garbage piles remained strongly associated with seropositivity (AOR = 49.88; 95% CI: 12.03–206.83; p < 0.001), while other variables showed higher but not significant odds (Table 6). To improve stability, the model was reduced to two predictors (Table 7), raising the EPV to 8 (near recommended 10 EPV), where proximity to garbage piles remained strongly associated (AOR = 52.61; 95% CI: 13.50–205.13; p < 0.001) and rodent presence at home became significant (AOR = 5.70; 95% CI: 1.12–29.08; p = 0.037). Although confidence intervals remain wide due to small events in this revised model, the estimates are more stable and indicate a clear environmental risk pattern; results should be interpreted with caution.

Table 6. Multivariable logistic regression analysis of factors associated with leptospirosis seropositivity

Predictor	AOR	95% CI	p-value
Lower education level	—	—	0.997
Trading frequency >3 days/week	3.67	0.79–17.11	0.099
Presence of rodents at home	4.07	0.74–22.46	0.107
House near garbage pile	49.88	12.03–206.83	<0.001

Note. AOR: adjusted odds ratio; CI: confidence interval. Variables with $p \leq 0.25$ in univariable analysis were included in the multivariable model. Statistical significance was set at $p \leq 0.05$. Dashes indicate estimates that were not interpretable.

Table 7. Reduced multivariable logistic regression model for factors associated with leptospirosis seropositivity

Predictor	AOR	95% CI	p-value
Presence of rodents at home	5.70	1.12–29.08	0.037
House near garbage pile	52.61	13.50–205.13	<0.001

Note. AOR: adjusted odds ratio; CI: confidence interval. Adjusted odds ratios were estimated using multivariable logistic regression. The reduced model retained two epidemiologically relevant predictors to improve model stability because of the limited number of seropositive cases. Statistical significance was set at $p \leq 0.05$.

DISCUSSION

This study shows leptospiral exposure among market traders in Kudat, Sabah, with a seroprevalence of 5.4%, indicating measurable but low exposure. The prevalence is much lower than the reference prevalence of 33.6% in a similar occupational group (Rahman *et al*., 2018) . The small number of positive cases limits the ability to detect associations but does not invalidate the study. The prevalence is comparable to that reported in other community and occupational studies in endemic areas, where exposure is often subclinical and under-recognised (Galan *et al*., 2021; Silva *et al*., 2023). The study was planned for ~80% power at $\alpha = 0.05$ (Charan and Biswas, 2013), but the smaller sample (n = 295) reduced the achieved power to ~74%, slightly increasing the risk of Type II error (Serdar *et al*., 2020). Thus, results should be interpreted with caution, focusing on confidence intervals and effect sizes rather than only statistical significance, in line with current epidemiological guidance (Amrhein *et al*., 2019; Wasserstein *et al*., 2019).

In this study, the younger age group (below 49 years) showed higher seropositivity. Although seropositivity often increases with age due to cumulative exposure (Mirambo *et al.*, 2022; de Oliveira *et al.*, 2024), several studies have reported higher infection rates among younger adults in settings where occupational and environmental exposures are concentrated (Felzemburgh *et al.*, 2014; Baveja *et al.*, 2022; Ally *et al.*, 2023). Females showed slightly higher seropositivity than males; however, this difference was not statistically significant and may reflect the sex distribution of traders rather than biological susceptibility, as sex-specific patterns vary across occupational contexts (Parra Barrera *et al.*, 2023; Teles *et al.*, 2023). Basic or higher education was linked to lower seropositivity in univariable analysis, but not after adjustment, suggesting education alone may not independently influence leptospirosis risk (Siti Sara *et al*., 2020; Palma *et al*., 2022; Galan *et al*., 2023).

Several occupational characteristics showed plausible but non-significant patterns. Higher seropositivity among traders working ≥4 days per week and using ground-based premises may reflect greater exposure to contaminated wet market environment, as prolonged exposure in humid, poorly drained market settings has been associated with leptospiral transmission (Widiastuti & Priyanto, 2020; Ling *et al*., 2025). Similarly, occupational groups in wet markets, particularly those handling fresh animal-based products, support existing evidence that contact with wet organic matter, and animal products may increase leptospiral exposure (Rahman *et al*., 2018; Widiastuti & Priyanto, 2020; Baharom *et al*., 2024). However, the lack of statistical significance after adjustment suggests that occupational exposure alone may not fully explain infection risk in this setting, particularly when environmental contamination is widespread (Palma *et al.*, 2022; Baharom *et al.*, 2024; de Oliveira *et al.*, 2024).

In the current study, no significant association was observed between knowledge, attitude, practice (KAP) and leptospiral seropositivity, consistent with previous studies reporting that KAP dimensions are inconsistently associated with leptospiral infection or seropositivity. A systematic review highlighted that the relationship between knowledge, attitude and preventive practice toward leptospirosis is often misaligned, with strong knowledge not always leading to effective preventive behaviour (Hassan *et al.*, 2023). Meanwhile, empirical studies similarly reported the unaligned association within the internal KAP domains, where knowledge did not consistently reflect into attitude or preventive practice and reflect the infection risk (Nozmi *et al.*, 2018). Studies showed that KAP levels are associated with leptospirosis, but the link between knowledge-attitude-practice is inconsistent (Palma *et al.*, 2022; Anand *et al.*, 2023; Hassan *et al.*, 2023). Nevertheless, a case-control study showed reverse association, where infected groups showed better KAP, which reflect the increase of post-infection awareness (Fatema *et al.*, 2023).

Environmental exposure was the main determinant of seropositivity. Proximity to garbage piles strongly increased the risk of infection, reflecting environmental contamination and rodent activity (Khalil *et al.*, 2021; Baharom *et al.*, 2024; Murray *et al.*, 2024). Rodent presence is also a key factor for human leptospirosis across diverse settings due to their high survival and adaptability in various environmental settings (Boey *et al*., 2019; Tantia Nova *et al*., 2020; Sluydts *et al.*, 2022). *Leptospira* can persist in moist soil and water, especially in areas with organic waste (Casanovas-Massana *et al.*, 2018; Bradley & Lockaby, 2023). Although the estimates were large, wide confidence intervals suggest low precision due to small case numbers and an EPV H” 8, not the absence of an effect. Overall, the strong and significant association (p < 0.001) is consistent with previous studies linking poor waste management to higher leptospirosis risk in endemic urban and peri-urban areas (Jeffree *et* *al.*, 2020; Krystosik *et al.*, 2020; Sutiningsih *et al.*, 2024), supporting a clear and biologically plausible environmental risk factor.

Collectively, these findings suggest that environmental contamination is the primary driver of leptospiral exposure among market traders in Kudat, rather than solely sociodemographic, occupational or behavioural factors, consistent with the principles of the One Health framework. (Amuasi *et al.*, 2020; Lea *et al.*, 2025). Despite its importance, the effectiveness of education may be limited without concurrent improvements in sanitation, waste management and infrastructure (Kabir *et al.*, 2021; Scriptore Moreira *et al.*, 2024). Structural interventions, including regulated waste disposal, drainage improvement and market sanitation upgrades, are therefore essential components of leptospirosis prevention in endemic urban settings (Felzemburgh *et al.*, 2014; Casanovas-Massana *et al.*, 2018; de Azevedo *et al.*, 2025).

The sample size for this study (n = 295) was smaller than intended. This limitation may reduce statistical power, potentially affecting the reliability and generalizability of the findings (Barnett & Glasziou, 2021; Cao *et al.*, 2024). Few seropositive cases (n = 16) resulted in a low event-per-variable (EPV) ratio, reducing precision and producing wide confidence intervals, though model reduction improved stability (Peduzzi *et al.*, 1996; Zabor *et al.*, 2022). The single-site, cross-sectional design limits external validity and cannot show causation (Maier *et al.*, 2023; Baumeister *et al.*, 2026). MAT serology cannot separate past and recent infections and may overestimate exposure in endemic areas (Rajapakse, 2022; Valente *et al.*, 2024). Self-reported data may be subject to recall bias (Bong *et al.*, 2024), while the lack of molecular testing and environmental sampling limits the detection of active transmission (Sykes *et al.*, 2022; Bradley & Lockaby, 2023). Still, the results provide useful baseline data for leptospirosis among market traders in Sabah and support future larger studies (Philip & Ahmed, 2023; Lea *et al.*, 2025).

CONCLUSION

In conclusion, leptospirosis among the market traders in Kudat town is associated with environmental exposures, particularly in environments with poor sanitation. This study indicates the need for integrated One-Health interventions, including proper environmental management, efficient rodent control and targeted educational programs, to reduce leptospiral transmission among similar or high-risk occupational groups in Sabah and Malaysia.

Conflict of Interest

The authors declare no conflicts of interest during the time of study.

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