One Health 19 (2024) 100851

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Seroreactivity against *Leptospira* spp. differs between community cats and privately-owned cats in Hong Kong

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ARTICLE INFO

Keywords:
Feline
Leptospiruria
Leptospira
Microagglutination testing
Serology
Prevalence

ABSTRACT

Leptospirosis is a bacterial zoonotic disease of major One Health significance and public health impact globally, with a wide host range including mammals, cetaceans and herpetofauna. This study aimed to determine *Leptospira* seroprevalence, risk factors for seroreactivity and prevalence of urinary *Leptospira* shedding among domestic cats in Hong Kong.

Microagglutination testing of 22 *Leptospira* serovars from 20 serogroups was performed on 738 sera from outdoor free-roaming "community" cats ($n = 391$) and privately-owned ($n = 347$) cats. Urine from 268 community cats was tested for pathogenic *Leptospira* DNA by qPCR targeting *lipL32*. Potential risk factors associated with exposure were assessed using logistic regression.

Overall *Leptospira* seroprevalence was 9.35%. Of 14 serogroups detected, Javanica (4.3%), Djasiman (2.3%) and Australis (1.5%) were most common. Seroreactivity was significantly higher among community (13.3%) than privately-owned cats (4.9%; OR 2.98 [95% CI 1.68–5.25], $P < 0.001$), especially to Javanica (7.65% of community cats versus 0.58% of privately-owned cats ($P < 0.001$). Antibody titres to all serogroups ranged from 1:100 to 1:6400 (median 1:200) and were highest for Javanica (median 1:800).

Leptospira DNA was detected in urine from 12/268 community cats (4.48%; median load 6.42×10^2 copies/mL urine; range 1.40×10^1–9.63×10^4). One in three seroreactive community cats with paired urine and blood samples had leptospiruria. After adjusting for source, none of breed, sex, neuter status, age, district rodent infestation rate, serum alanine transaminase or creatinine values were associated with seroreactivity.

Cats in Hong Kong are exposed to a diversity of *Leptospira* serogroups and can shed *Leptospira* silently in urine. The higher seroprevalence among outdoor free-roaming community cats highlights the importance of environmental drivers in leptospirosis transmission and risks of exposure for sympatric human populations. Gloves should be worn when handling feline urine to minimise the risk of zoonotic transmission from subclinically infected cats.

1. Introduction

Leptospirosis is a zoonotic disease of profound One Health significance and public-health impact globally. It is caused by bacterial spirochetes from the genus *Leptospira* which can infect humans, dogs, cats and most other terrestrial mammals as well as cetaceans and herpetofauna [1]. The estimated disease burden in 2015 of 1 million human cases resulting in 60, 000 deaths annually, is at risk of dramatic surges

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https://doi.org/10.1016/j.onehlt.2024.100851
Received 5 March 2024; Accepted 2 July 2024
Available online 6 July 2024

due to more frequent climate-change associated flooding events, especially in countries of lower socioeconomic status [2,3].

Leptospira transmission primarily occurs when mucous membranes or abraded skin come into direct contact with urine from an infected reservoir host, or with contaminated soil or water. Less commonly, *Leptospira* transmission occurs from being bitten by or ingesting an infected animal [4]. Acute clinical disease (leptospirosis) is most frequently reported in dogs and humans [2], whereas subclinical (asymptomatic) infections occur in many animals, including cats.

Leptospira are classified by genomic differences into 64 species that form two pathogenic [P1 (pathogenic), P2 (intermediately pathogenic)] and two saprophytic (S1, S2) subclades [5]. P1 species frequently associated with leptospirosis in humans and animals include L. *interrogans, L. kirschneri,* L. *noguchii* and *L. borgpetersenii* [6]. *Leptospira* can also be classified by their outer lipopolysaccharide antigens into >300 pathogenic serovars [5]. Strains from the same serovar may belong to different genetic species [7]. Antigenically related serovars are grouped into 30 serogroups, which can be identified using the microagglutination test (MAT).

For some *Leptospira* serovars infections in animal reservoirs are almost always subclinical and urinary shedding is persistent, including serovars Icterohaemorrhagiae in rodents, Hardjo in cattle and Canicola in dogs [8]. Rodents are an important infection reservoir in urban environments and can also persistently shed many other *Leptospira* serovars. [9]. Predation of rodents is an additional route of *Leptospira* transmission for cats.

In Hong Kong human leptospirosis is a notifiable disease with 1 to 8 cases reported annually from 2012 to 2022 [10]. While the prevalence of *Leptospira* infection in farmed pigs in Hong Kong is negligible [11], conditions are favourable for leptospirosis outbreaks due to high annual rainfall [12,13]. The proximity of rodents to humans in densely populated urban areas creates an environment further conducive to *Leptospira* transmission. Rodent *Leptospira* seroprevalence in Mainland China, which borders Hong Kong, is estimated to be 15.9% [14].

An integrated One-Health approach is critical to inform public health policy to minimise the risk of leptospirosis regionally and within certain occupation groups, such as veterinarians and farmers. Since feline *Leptospira* infections are always subclinical and urinary shedding occurs for up to 8 months, an increased understanding of the epidemiological role of cats in *Leptospira* carriage and shedding is desirable. [15].

Hong Kong has a sizable population of outdoor, free-roaming street cats, also known as “community cats”, whose human contact is limited to intermittent feeding by the public. Although exact numbers are not known, the Hong Kong SPCA has neutered >75, 000 community cats since the year 2000. In comparison, most privately-owned cats live alongside their owners in high-rise apartments with no outdoor access. The distinct cat populations and environmental conditions in Hong Kong present a unique opportunity to investigate the role of cats in *Leptospira* transmission and the impact of lifestyle and environment on feline *Leptospira* exposure.

The aims of this study were to determine *Leptospira* seroprevalence, risk factors for seropositivity and the prevalence of urinary *Leptospira* shedding among cats in Hong Kong.

2. Materials and methods

2.1. Ethics statement

Ethical approvals for this study were granted by the Animal Ethics Committee of City University of Hong Kong, approval numbers A-0478, A-0709 and A-0696. Licenses for sampling community cats were granted by The Government of the Hong Kong SAR, Department of Health, license numbers 20–164 to 20–179 and 22–6 to 22–8.

2.2. Sample collection

2.2.1. Community cats

Whole blood was collected from free-roaming, outdoor community cats presented to a trap-neuter-return program from January to May 2021 and January to August 2022. Serum was separated and stored at −80 °C until batch testing. Urine samples were also collected between January to August 2022 by manual bladder expression or cystocentesis. Urine (2.5 mL) was mixed with phosphate buffered saline (PBS, 1 mL) to prevent degradation of *Leptospira* DNA [7]. PBS-buffered urine samples were stored at 4 °C for up to 48 h or at −80 °C until DNA extraction. Signalment (age, breed, sex, neuter status) and capture location of each cat was recorded. The mean of monthly district rodent infestation rates, reported by the Food and Environmental Hygiene Department [16], was calculated to give annual district rodent rates between January 2020 to June 2022. Samples from a subset of 90 community cats were submitted to the CityU Veterinary Diagnostic Laboratory (VDL) to determine serum creatinine values.

2.2.2. Privately-owned cats

Residual diagnostic sera (>0.5 mL) were obtained from cats presented to CityU Veterinary Medical Centre (VMC) or from VDL between January 2020 and April 2022. Signalment, serum alanine transferase (ALT) and creatinine values were recorded where available.

A flow chart depicting recruitment and processing was created using draw.io (draw.io AG, Zurich, Switzerland).

2.3. Microagglutination testing of sera

Sera underwent MAT against a panel of 22 *Leptospira* serovars from 20 serogroups at the World Health Organisation Collaborating Centre for Reference and Research on Leptospirosis, Queensland Health Forensic and Scientific Services, Brisbane, Australia (Table 1). Serovars were selected to represent a wide range of serogroups, informed by serovars present in neighbouring regions [17–21]. Sera were tested at dilutions from 1:50 to 1:6400. MAT titres ≥1:100 were considered positive (reactive), in accordance with World Organisation for Animal Health guidelines [22].

Table 1
List of *Leptospira* strains representing 22 *Leptospira* serovars used for MAT in this study, and their respective serogroup and species [41].

Species	Serogroup	Serovar	Strain
L. borgpetersenii	Ballum	Ballum	Mus 127
L. borgpetersenii	Javanica	Javanica	Veldrat Batavia 46
L. borgpetersenii	Tarassovi	Tarassovi	Perepelitsin
L. borgpetersenii	Mini	Mini	Sari
L. interrogans	Australis	Australis	Ballico
L. interrogans	Autumnalis	Autumnalis	Akiyami A
L. interrogans	Bataviae	Bataviae	Swart
L. interrogans	Canicola	Canicola	Hond Utrecht IV
L. interrogans	Icterohaemorrhagiae	Copenhageni	M20
L. interrogans	Djasiman	Djasiman	Djasiman
L. interrogans	Sejroe	Hardjo	Hardjoprajitno
L. interrogans	Hebdomadis	Hebdomadis	Hebdomadis
L. interrogans	Pomona	Pomona	Pomona
L. interrogans	Pyrogenes	Pyrogenes	Salinem
L. interrogans	Serjoe	Saxkoebing	Mus 24
L. interrogans	Australis	Pohnpei	PRK12
L. kirschneri	Cynopteri	Cynopteri	3522C
L. kirschneri	Grippotyphosa	Grippotyphosa	Moskva V
L. noguchii	Panama	Panama	CZ 214
L. santarosai	Shermani	Shermani	1342 K
L. weilii	Celledoni	Celledoni	Celledoni
L. weilii	Sarmin	Sarmin	Sarmin

2.4. Detection of Leptospira DNA in urine

2.4.1. DNA extraction

Refrigerated and frozen PBS-buffered urine samples were centrifuged at 6000 xg for 10 mins and at 13,000 x g for 5 mins, respectively. Supernatant was discarded except for 200 μL which was used to resuspend the pellet. Samples were stored at 4 °C for up to 24 h before DNA extraction using the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) with the following modification; for each reaction, 2 μL of VetMAX Xeno™ DNA Control (Thermo Fisher Scientific, Austin, United States of America) was added to the lysis solution, serving as an exogenous extraction internal positive control (IPC). DNA was eluted in 80 μL of Buffer AE (Qiagen GmbH). DNA concentration (ng/μL) was measured with the NanoDrop™ OneC Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific) and DNA extracts were kept at 4 °C for up to 48 h or at -80 °C before PCR.

2.4.2. Internal positive control

To confirm integrity of extracted DNA, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene was amplified by PCR [23]. Samples testing negative for GAPDH were tested for the IPC by qPCR using the VetMAX™ Xeno™ IPC assay (Thermo Fisher Scientific). A DNA extract obtained from 200 μL of deionized-water containing 2 μL XENO DNA IPC was used as no-inhibition DNA control during each run. A sample was deemed inhibited when a shift in Ct value of >1.5 cycles was observed when compared to the no-inhibition DNA control. Samples failing to amplify the XENO DNA IPC were excluded from further analyses.

2.4.3. Detection of leptospiral lipL32 gene

To detect pathogenic *Leptospira* spp. qPCR targeting *lipl32* was performed [24,25]. A pMK-T plasmid containing the *lipL32* fragment was synthesized (Thermo Fisher Scientific). After in-house transformation and cloning into competent *E. coli* JM109 Strain (Promega) cells, plasmid DNA was purified and quantified. A standard curve from 10^8 to 10^1 target copies per reaction was generated using serial tenfold dilutions of the *lipL32* plasmid. Plasmid dilutions were prepared in Tris–EDTA (TE) with 5 μg/mL salmon sperm DNA (Thermo Fisher Scientific).

To identify a 123-bp fragment of the *lipL32* gene, forward *lipL32-F* (5'-CTGTGATCAACTATTACGGATA-3′) and reverse *lipL32-R* (5'-GAACTCCCATTTCAGCGAT-3′) primers, together with *lipL32*-P *TaqMan* probe (6-FAM-5'-AAAGCCAGGACAAGCGCCG-3'-BHQ1) were used [24]. Each PCR reaction contained 200 nM of the *lipL32* primers and probe in a final volume of 25 μL (iQ Supermix; Bio-Rad Pacific Limited, Hong Kong SAR, China). Template DNA (10 μL) was added to each PCR reaction, with a final template concentration ranging from 4 to 100 ng per reaction. The amplification was performed on a Bio-Rad CFX96 Touch with a thermal cycling profile of 95 °C for 3 min, followed by 42 cycles of denaturation at 95 °C for 10 s and annealing-extension at 60 °C for 30 s.

No-template negative controls contained nuclease-free water and 5 μg/mL of salmon sperm DNA in TE. Samples and standards were run in triplicate and results were reported as mean values. A sample was considered positive if at least 3 target DNA copies per reaction were detected in at least two of three replicates.

Data analysis for *lipL32* qPCR was performed with CFX Maestro Software. Validation parameters of $R^2 \geq 0.98$ for assay linearity and 90 to 110% for PCR efficiency per run were used.

2.4.4. Statistical analysis

Analyses were conducted using Stata v18 (StataCorp LLC, College Station, Texas, USA). The proportions of positive samples of tested serovars ("seroprevalence") were graphed and compared between the two sources of cats using tests of proportions. Serum ALT and creatinine levels were categorized as "elevated" or "normal" based on reference intervals. Scatter plots comparing mean district-level rodent rate and the percentage of seropositive cats per district for all serogroups and specifically for serogroup Javanica were created.

Univariable associations between *Leptospira* seropositivity and each independent variable of interest (source, sex, age, breed, neuter status, serum ALT and creatinine values) were evaluated using simple logistic regression. Age was categorized into three groups (<12, 12–83, and > 83 months) to enable meaningful comparisons. Odds ratios (OR) and their corresponding 95% confidence intervals (95% CI) were estimated. Independent variables with a conservative $P < 0.2$ were considered for inclusion in a multivariate logistic regression model [26]. Pairwise correlations between the independent variables with $P < 0.2$ were assessed using Chi-square tests and where collinearity was present, a theoretical causal web guided the selection of the most meaningful variable(s) for the final multivariable model.

3. Results

3.1. Study population

The final study population comprised 773 cats, of which 426 were community cats and 347 were privately-owned (Fig. 1). Sera from 738 cats underwent MAT, while 268 urine samples from community cats were tested for *Leptospira* DNA. Paired urine qPCR and serum MAT results were available for 233 community cats. Serum creatinine and ALT results were available for 437 and 297 cats, respectively.

Community cats were all domestic shorthairs (DSH), were 0.5 to 9 (median 2) years-old and comprised of 48% males and 52% females, with 97.4% sexually-intact and 2.6% neutered. Privately-owned cats were aged 0.5 to 21 years (median 10.6), with 62.2% males and 89.8% cats were neutered. Of 27 breeds represented, the most common were DSH (40.9%), British shorthair (19.9%) and Exotic shorthair (10.1%).

3.2. Seroprevalence

Overall *Leptospira* seroprevalence was 9.3% (69/738) with MAT titres of 1:100–1:6400 (median 1:200). Community cats were more likely to be seroreactive (13.3%, 52/391) than privately-owned cats (4.9%, 17/347) (OR = 2.98, $P < 0.001$). Seroreactivity was detected against 15 serovars from 14 serogroups (Table 2), most commonly serogroups/serovars Javanica (4.3%, 32/738), Djasiman (2.3%, 17/738) and Australis (1.5%, 11/738).

The most frequently detected serogroup/serovar in community cats was Javanica (7.65%) and in privately-owned cats was Djasiman (2.59%) (Fig. 2). Exposure to Javanica was significantly higher in community cats (7.65%, 30/392) than privately-owned (0.57%, 2/347) ($P < 0.001$). Half of the cats seroreactive for serogroup Javanica (16/32) had titres ≥1:800.

Antibodies to 2–5 serogroups were detected in 11/69 (15.9%) seropositive cats (Supplementary Materials 1). Three cats had antibodies against both serovars of serogroup Australis (Australis and Pohnpei), while three others were reactive to serogroups Mini and Hebdomadis. Serogroup Javanica had the highest antibody titre in 5/11 (45.5%) cats reactive to multiple serogroups.

3.3. Urinary shedding of Leptospira

Leptospira DNA was detected in urine of 12/268 (4.48%) community cats (Table 3), with a median of 6.41×10^2 copies/mL of urine and median cycle quantification (C_t) value of 33.9. All cats with $>10^2$ copies of *lipL32*/mL of urine were seroreactive (Fig. 3).

Of the community cats with paired sera and urine, 10.7% (25/233) were positive by at least one test. Seven (28.0%) were seroreactive and shedding *lipL32*, 14 (56%) were seroreactive but not shedding, while four (16%) were seronegative and had leptospiruria (16%, 4/25). A third of seroreactive cats (7/21) were shedding *Leptospira* DNA in urine,

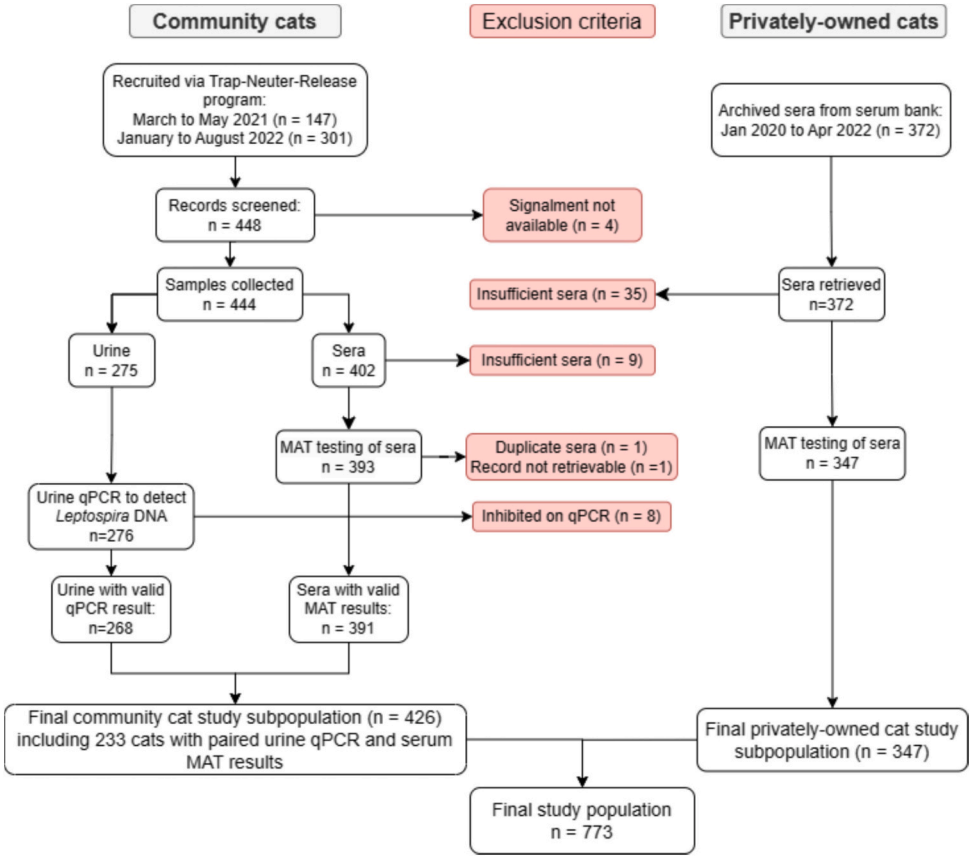

Fig. 1. Flow chart describing sample recruitment and refinement of the study population, which comprised of community cats and privately-owned cats. Samples excluded due to "inhibition on qPCR" contained PCR inhibitors that prevented DNA amplification.

Table 2
Frequency distribution of antibody titres against each serovar of *Leptospira* spp. by microscopic agglutination test (MAT) in sera from cats of Hong Kong.

Serovar*	Antibody Titre							Total
	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	
Javanica	4	5	7	6	4	3	3	32
Djasiman	14	3	0	0	0	0	0	17
Australis	6	4	0	0	0	0	0	10
Bataviae	2	2	1	1	1	0	0	7
Pohnpei	3	0	0	1	0	0	0	4
Hebdomadis	0	1	0	0	0	1	1	3
Mini	0	1	0	0	2	0	0	3
Celledoni	1	1	0	0	0	0	0	2
Sarmin	1	1	0	0	0	0	0	2
Autumnalis	2	0	0	0	0	0	0	2
Canicola	0	0	1	0	0	0	0	1
Pomona	0	0	1	0	0	0	0	1
Ballum	1	0	0	0	0	0	0	1
Copenhageni	1	0	0	0	0	0	0	1
Pyrogenes	1	0	0	0	0	0	0	1
Total	36	18	10	8	7	4	4	87**

* Serovars Cynopteri, Grippotyphosa, Hardjo, Panama, Saxkoebing, Shermani and Tarassovi were tested but not included in the table as no antibodies were detected against them.

** As antibodies against multiple *Leptospira* serovars were detected in some cats, the total number of antibody titres reported ($n = 87$) is greater than the number of seropositive cats detected ($n = 69$).

with seroreactivity to serogroups Javanica, Bataviae Hebdomadis and/or Mini (Table 3).

3.4. Rodent infestation rate

Annual district rodent infestation rates ranged from 1.6% (Islands) to 5.2% (Yau Tsim Mong) (median 2.9%). No association was observed between rodent infestation rate and *Leptospira* seroprevalence in community cats (Supplementary Materials 2 and 3).

3.5. Univariable associations

Leptospira seroprevalence in privately-owned cats with elevated ALT values was 4.4% (1/23) compared to 5.8% (15/258) in cats with non-

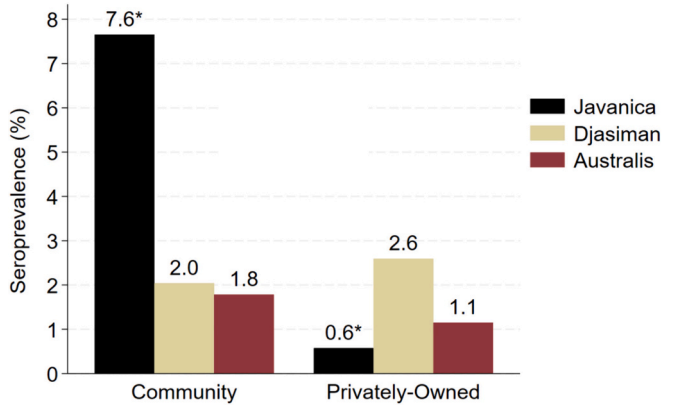

Fig. 2. Seroprevalence of the three most common serogroups of *Leptospira* spp. (Javanica, Djasiman and Australis) detected from 391 community and 347 privately-owned cats in Hong Kong. '*' indicates a statistically significant difference in the seroprevalence of serogroup Javanica between the two sources of cats ($P < 0.001$).

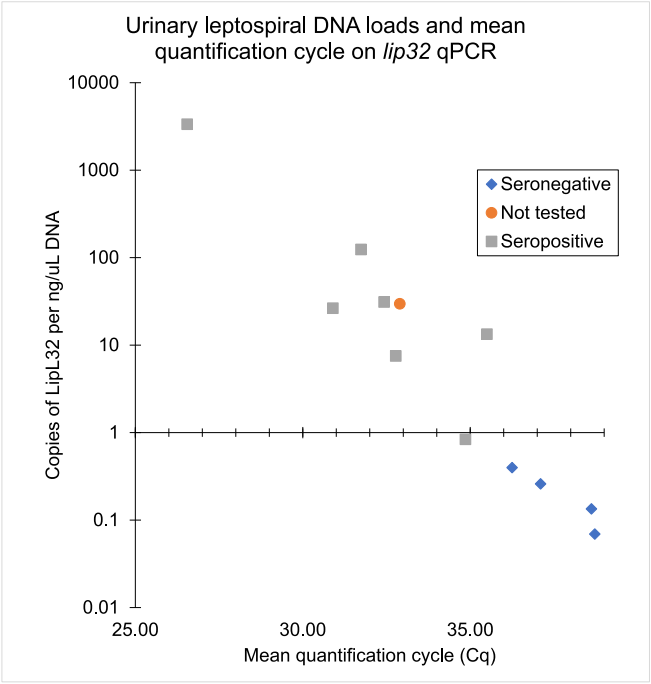

Fig. 3. Scatter plot of leptospiral urinary DNA loads in copies of leptospiral gene *lipL32* per ng/ μL DNA, and mean quantification cycle (C_t) on quantitative PCR for cats shedding Leptospira in urine ($n = 12$), with different markers representing serological status.

elevated levels. Results from univariable logistic regressions indicated significant association between *Leptospira* seropositivity and the source, breed, age, and neuter status of the study cats (Table 4). However, there were strong, significant ($P < 0.001$) correlations between "source" and the other three independent variables. Therefore, as the most important factor which theoretically preceded and dictated the distribution of the other three variables, "source" was chosen for multivariable logistic regression modelling, along with sex ($P < 0.2$). In multivariable modelling, sex was not significant ($P = 0.363$) and was removed from the final model. As a result, the final model became equivalent to the simple logistic model presented for source in Table 4. The odds of *Leptospira* seroreactivity in community cats was 2.98 [95% CI: 1.68–5.25] times higher than that in privately-owned cats in this study ($P < 0.001$).

4. Discussion

The overall *Leptospira* seroprevalence of 9.35% in cats in our study is comparable with global estimates (11.7%) [27] and similar to nearby Taiwan (9.3%) [18]. *Leptospira* seroprevalence of community (13.3%) and privately-owned cats (4.3%) in Hong Kong was also similar to that in stray (10.7%) and privately-owned cats (5.7%) in Taiwan [18]. We found outdoor community cats were almost three times as likely to be seropositive than privately-owned cats, similar to the findings of a global meta-analysis in which outdoor cats had 2.74-times higher odds of seroreactivity than indoor cats [27]. Whilst we did not collect data on the domicile of privately-owned cats, 90.5% of Hong Kong people live in apartments, thus most privately-owned cats likely have no outdoor access and limited rodent exposure [28]. The few seroreactive privately-owned cats (4.90%) could have been adopted community cats, had outdoor access, or been exposed indoors.

In accordance with findings from meta-analyses that rodent contact is associated with human but not feline *Leptospira* exposure, we found no relationship between rodent infestation rates and feline seropositivity [27,29]. Although low numbers of cats in some districts and the indirect method used to estimate rodent infestation rates may have hampered our ability to detect an association, environmental exposure to *Leptospira* is the most plausible explanation for the substantially higher seroreactivity in community cats. Waterlogged soil is an ideal environment for pathogenic *Leptospira* to replicate, yet is often overlooked as a reservoir of infection [13,30].

Direct comparisons of feline *Leptospira* seroprevalence between studies is difficult, since MAT titres as low as 1:20 have been considered seropositive [21,31]. Reducing the MAT cut-off from 1:100 to 1:50 in our study would have increased the overall seroprevalence from 9.35% to 17.3%, including 20.7% of community cats and 13.5% of privately-owned cats, largely driven by serogroup Djasiman, which was responsible for 54.2% (52/96) of the 1:50 titres. However, this lower cut-off

Table 3
Signalment and test results for 12 community cats from Hong Kong with *Leptospira* DNA detected in their urine by *lipL32* qPCR.

Study ID	Sex	Neuter status	Age (months)	qPCR C_t value	qPCR copies/mL urine*	Reciprocal MAT titre	MAT positive serovar
E	Male	Entire	24	26.55	9.63×10^4	3200 1600	Hebdomadis, Mini
N	Female	Entire	24	31.74	4.48×10^3	100	Bataviae
O	Female	Neutered	72	32.43	2.38×10^3	400	Javanica
P	Male	Entire	36	32.90	2.09×10^3	Not tested	Not tested
Q	Male	Entire	36	30.90	9.86×10^2	400	Javanica
R	Male	Entire	48	35.50	1.52×10^3	200	Bataviae
S	Female	Entire	12	32.78	2.81×10^2	200	Bataviae
T	Male	Entire	36	34.86	2.98×10^2	200	Javanica
U	Male	Entire	60	36.25	7.84×10^1	Negative	Negative
V	Male	Entire	6	37.10	3.78×10^1	Negative	Negative
W	Female	Entire	24	38.62	1.40×10^1	Negative	Negative
X	Male	Entire	36	38.72	1.45×10^1	Negative	Negative

* Copies per reaction were converted to copies per mL urine considering volumes of urine used for DNA extraction, eluate and DNA template per reaction.

Table 4
Results of logistic regressions assessing univariable associations between seropositivity to *Leptospira* spp. and independent variables of interest for the 738 studied cats.

Variable	Categories	No. of seronegative cats	No. of seropositive cats	OR[b]	95% CI[c]	*P*-value
Source	Privately-owned	330	17	–	–	–
	Community	339	52	**2.98**	1.68–5.25	**< 0.001**[d]
Breed	Purebred	194	11	–	–	–
	Domestic Shorthair (DSH)	475	58	**2.15**	1.11–4.19	**0.024**
Sex	Male	371	32	–	–	–
	Female	298	37	1.44	0.87–2.37	0.151
Neuter status	Neutered	302	14	–	–	–
	Entire	367	55	**3.06**	1.69–5.53	**< 0.001**
Age* (months)	< 12	59	5	–	–	–
	12–83	360	51	1.67	0.64–4.36	0.294
	> 83	250	13	0.61	0.21–1.79	0.371
Creatinine (μmol/L)	Normal	312	23	–	–	–
	Elevated	98	4	0.55	0.19–1.64	0.286

* Overall *P*-value for age = 0.006; follow-up pairwise comparisons (with Bonferroni adjustment) indicated a significant difference between age categories 12–83 months and > 83 months ($P = 0.005$).
[b] OR Odds Ratio
[c] CI Confidence Interval
[d] Bolded values are statistically signficant

would have also reduced the odds ratio of infection in community cats from 2.98 to 1.66 (95% CI: 1.10–2.52), suggesting reduced specificity.

In our study there was high *Leptospira* serovar diversity with 15/22 serovars (68.2%) detected compared to 3/20 (15%), 6/25 (24%) and 7/24 (29.2%) in cats from Malaysia, Vietnam and Thailand, respectively [17,20,21]. However, these results are not directly comparable since serovar panels were not identical.

We detected serogroup Canicola in one cat (1:400) for the first time in a felid in East and Southeast Asia. We also detected serovar Pohnpei, originally isolated from rodents in Micronesia but not tested for before in Asian cats [32]. However, some cats seroreactive for Pohnpei also reacted to serovar Australis in the same serogroup, indicating possible cross-reactivity. Cats seroreactive to serogroup Mini also reacted to the heterologous serogroup Hebdomadis, which can result from antigenic similarities in *rfb* locus genes [33].

Overall, cats in Hong Kong were most commonly exposed to serogroup Javanica (4.33%), which was also the most common serogroup detected among cats in Okinawa island, Japan and in Vietnam [19,20]. By contrast, in Taiwan, which is geographically closer to Hong Kong, Shermani was the most common serogroup (4.2%) detected in cats [18]. It was also the most common cause of human leptospirosis cases there and was detected in cattle, swine and stray dogs [18,34]. However, serogroup Shermani was not detected in any cats in our study. These differences in serogroup frequency among cats in regions neighbouring Hong Kong could be due to multiple differences in *Leptospira* transmission drivers, such as reservoir species diversity, land use and population density despite relative geographic proximity. Geographically weighted logistic regression models and Bayesian, hierarchical mixed-model frameworks incorporating transmission drivers such as climatic data (e.g. precipitation, temperature), distance to rivers, livestock density (e.g. pigs, cattle), and residential setting (e.g. urban, periurban, rural) and poverty rates, have been used to inform a precision-medicine targeted approach to leptospirosis preparedness in countries such as Fiji [35,36]. Such models would be further strengthened by incorporation of direct measures of *Leptospira* carriage and shedding in animals, using an enhanced One-Health approach.

Community cats were significantly more likely to be exposed to serogroup Javanica (7.65%) than privately-owned cats (0.58%) in our study. In addition, three of the seven seroreactive cats shedding *Leptospira* were reactive to serogroup Javanica. The combination of high frequency of exposure at titres to serogroup Javanica and urinary shedding among cats seroreactive to Javanica suggests active circulation of this serogroup among outdoor free-roaming cats in Hong Kong.

In Okinawa island, cats and black rats (*Rattus rattus*) were infected with the same multilocus sequence type of serovar Javanica, suggesting an infection cycle between rats and cats [19]. Serovar Javanica commonly infects many rodent species including *R. rattus, R. exulans, R. lose, R. norvegicus* and the greater bandicoot rat (*Bandicota indica*) in Japan, Taiwan and the Philippines [37]. Although feline *Leptospira* serosurveillance data in Mainland China have not yet been reported, the two most common serovars identified in rats in China are Icterohaemorrhagiae and Javanica. *Leptospira* serovars circulating in rodents in Hong Kong have not been investigated. Beyond cats and rodents, *Leptospira* from serogroup Javanica have been detected in bovine urine, canine blood and soil in India, Malaysia and Japan [38–41] and can cause severe disease in humans [42,43], demonstrating the regional specificity, diversity of infection sources and One Health importance of *Leptospira*.

The prevalence of leptospiruria in our study (4.48%) was similar to the global point prevalence in cats (3.7%) [27]. While presence of DNA does not indicate organism viability, *Leptospira* have previously been cultured from feline urine [44].

Neither our study nor Shropshire et al. found a significant relationship between elevated creatinine and *Leptospira* seroreactivity in cats [45]. Two previous reports investigating association between seroreactivity and chronic kidney disease (CKD) in cats had contradictory results [15,46]. Future studies could use other CKD biomarkers such as serum symmetric diemethylarginine to increase sensitivity of detection [47,48]. While periods of high rainfall are associated with *Leptospira* outbreaks and environmental contamination [49–51], the effect of seasonality in our study was not evaluated since samples from community and privately-owned cats were not collected year-round.

5. Conclusions

Cats in Hong Kong are exposed to a diverse array of *Leptospira* serogroups, especially Javanica. The almost three-times higher risk of exposure in free-roaming outdoor community cats, which was not associated with rodent infestation rates, highlights the importance of environmental sources of *Leptospira* as transmission drivers of leptospirosis. Contact with urine of subclinically infected cats shedding *Leptospira* is an unrecognised occupational risk for veterinarians and,gloves should be worn when handling feline urine to minimise zoonotic risk.

Funding

This work was supported by internal grants from City University of Hong Kong of one of the authors (VRB), namely a DON_RMG grant (Project No. 9229086) and an SGP grant (Project No. 9380113).

CRediT authorship contribution statement

Wing Yan Jacqueline Tam: Data curation, Formal analysis, Project administration, Software, Writing – original draft. **Omid Nekouei:** Data curation, Formal analysis, Methodology, Software, Writing – review & editing. **Francesca Rizzo:** Investigation, Methodology, Validation, Writing – review & editing. **Lok See Tiffany Cheng:** Formal analysis, Methodology, Writing – review & editing. **Yan Ru Choi:** Methodology, Project administration, Writing – review & editing. **Megan Staples:** Formal analysis, Methodology, Writing – review & editing. **Stefan Hobi:** Investigation, Methodology, Writing – review & editing. **Jane Gray:** Investigation, Methodology, Resources, Writing – review & editing. **Fiona Woodhouse:** Investigation, Methodology, Writing – review & editing. **Patricia Yi Man Shuen:** Investigation, Methodology, Writing – review & editing. **Ying Fei Chai:** Investigation, Methodology, Writing – review & editing. **Julia A. Beatty:** Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Vanessa R. Barrs:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article. Internal funding from City University of Hong Kong had no influence on the study design, results, interpretation or publication of findings. Generative AI tools were not used in the writing of our manuscript.

Data availability

All data supporting the conclusions of the study are available in the text, as supplementary material or through accessing the full dataset deposited in the OSF repository here: https://osf.io/r4n6t

Acknowledgments

The authors thank all staff at the SPCA Wan Chai Hong Kong that assisted with sample collection for this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100851.

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