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Capillaria hepatica (syn. Calodium hepaticum) infection and factors influencing infection carriage in rats (*Rattus* spp.) in Hong Kong

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ABSTRACT

Capillaria hepatica (syn. *Calodium hepaticum*) (Bancroft, 1893) is a nematode, which colonises the liver of a wide range of hosts including humans. The worldwide prevalence of infection in the genus *Rattus* can be as high as 100% and the Norway rat (*R. norvegicus*) and black rat (*R. rattus*) are considered the main host species. This study is the first to investigate the epidemiology of *C. hepatica* infection in wild rats trapped in various geographical locations in Hong Kong. Four species of trapped rats were identified, with 65% being *R. norvegicus*, followed by 30% *R. tanezumi* (Asian house rat), 4% *R. andamanensis* (Sikkim rat), and 1% *Niviventer huang* (South China white-bellied rat). The overall prevalence of *C. hepatica* infection was 36.7% (81/221) (95% CI 30.4–43.4) and *R. norvegicus* was the most common rat species trapped during this study, with the highest prevalence of *C. hepatica* infection. Two risk factors for host infection were skin wounds and geographical region, whilst sex, body weight, stage of development, and presence of ectoparasites were not risk factors for this infection. Gross hepatic lesions were absent in 17% of infected rats and when present, were not pathognomonic for the infection. Infected rats lacked severe hepatic inflammation or fibrosis, indicating that rats tolerate the infection well. Egg production was observed in the livers of 69% of infected rats, which emphasizes their role as reservoirs of this zoonotic parasite. Several infected rats in this study were trapped inside residential buildings, which highlights the zoonotic risk of *C. hepatica* to humans following the potential ingestion of embryonated eggs from contaminated food, water, or soil.

1. Introduction

Capillaria (C.) hepatica (syn. *Calodium hepaticum*) is a cosmopolitan zoonotic nematode with a direct life cycle [1]. Following ingestion of embryonated eggs, the worm larvae hatch in the intestine of their host, enter the liver via the portal vein system, mature into adults, with a prepatent period of 28 days, female worms lay unembryonated eggs which become encapsulated by the host's liver parenchyma associated with inflammation and fibrosis. The eggs are only released into the environment through the decay of the host's carcass or through shedding of these eggs in the faeces of a predator which has fed on an infected rat. The eggs then embryonate over a period of 5 to 8 weeks. The life cycle is completed by the ingestion of embryonated eggs by a new host, usually associated with contaminated water, food, or soil [2]. The ingestion of unembryonated eggs leads to spurious infections only [3–8].

This nematode has been reported in >80 species of murids, in marsupials, carnivores, hominids and other families of mammals [5]. Among human populations unsanitary and poor hygienic conditions and presence and frequent contact with rodents and domestic animals have been shown to increase the risk of infection with *C. hepatica* [3,9,10].

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The seroprevalence of *C. hepatica* in humans is relatively low, ranging from 0.8% to 1.8% [9,11,12]. In Southeast Asia, *C. hepatica* infection in humans is reported as either isolated cases [10,13–16] or as spurious infections [8]. Infection of rats with *C. hepatica* is widespread globally, especially in Norway rats (*R. norvegicus*), with prevalences surpassing 50% in some regions [17]. In Hong Kong, the prevalence of *C. hepatica* remains unknown, but we hypothesize that it will be within the global range, as reported in other studies.

The aims of this study conducted in Hong Kong were: (a) estimation of prevalence of *C. hepatica* infection in different rat species; (b) characterization of the gross and histological lesions associated with *C. hepatica* infection in rats, and (c) identification of risk factors for *C. hepatica* infection in rats.

2. Materials and methods

The selection of study sites was based on different ecological areas, which included 16 geographical locations, representing 3 regions and 9 districts, with 6 semi-rural and 10 urbanized residential areas of Hong Kong Special Administrative Region (SAR). Semi-rural areas located in the New Territories included 2 chicken and 2 pig farms, and 2 horse-riding schools. Urbanized residential areas were located in the Kowloon region (7) and Hong Kong Island (3). Trapping of rats was performed between October 2020 and August 2021 (Fig. 1). Consent forms for trapping rats within the private premises were obtained from the owners or their representatives. Rats were trapped using 10.5 × 5.5 × 4.5 in. aluminum cage traps (Kensizer Inc., Shenzhen, Guangdong, China) loaded with baits of dried seeds, fruits, and bacon. The geographical position system (GPS) coordinates of every trap were recorded and 8–12 traps were set at each location, these traps were checked every morning, and all the trapped rats were captured. Once a total of 15–20 rats were collected from a particular location, traps were moved to the next trapping site. Traps were placed 3–4 weeks at each location. Captured rats were individually placed in hermetic boxes, sedated with isoflurane, and transferred to the Veterinary Diagnostic Laboratory (VDL), City University of Hong Kong. Upon arrival, rats were euthanized by inhalation of 5% isoflurane followed by cervical dislocation, in accordance with American Veterinary Medical Association Council on Research protocol [18].

2.1. Postmortem examination

Postmortem examination of rats was preceded by collection of their morphological data including their sex (male and female), developmental stage (subadult and adult), body weight (g), body length (the tip of the nose to the anus, cm), tail length (the anus to the tip of the tail, cm), skull length (the extremity of the nose to the back of the skull, mm), hind foot (the tip of the third phalange to the back of the heel, cm), and ear length (the notch at the base of the ear to the extremity of the ear, cm). The rats were also examined for the presence of skin wounds. During the postmortem, liver lesions were recorded, and the percentage of liver parenchyma affected was graded into 4 levels: (0) absent; (1) <25%; (2) 25–50%; (3) >50% [19–21]. After a thorough postmortem examination, the liver was collected and stored in 10% buffered formalin for histopathology, and at −80 °C for genomic rat species identification and genomic *C. hepatica* confirmation.

2.2. Histopathology of liver

Livers were fixed in 10% buffered formalin and were routinely subjected to histopathological examination. Sections were cut serially from paraffin blocks at 4 μm and stained using hematoxylin and eosin (H&E). Liver sections were assessed by a board-certified pathologist (first author). Livers were classified as infected with *C. hepatica* if any adults, larvae and/or eggs were observed. Inflammation was classified as acute if any combination of necrosis (lytic or coagulative), fibrin lakes or suppurative exudate was seen, chronic active if inflammatory exudate was non suppurative (histiocytic, lymphoplasmacytic) in combination with either fibrin lakes or necrosis, and chronic if mineralization and fibrosis only was seen. Inflammation was graded 0–3, representing absent, mild, moderate, and severe. Grade 1 inflammation had a cuff of up to 6 cells surrounding nematode segments, no fibrosis, with adjacent interstitial tissues minimally expanded by lymphocytes, plasma cells, and oedema. Grade 2 inflammation had discrete, coalescing foci of inflammation up to 10 layers surrounding nematode segments, on a background of mild fibrosis, with adjacent interstitial tissues moderately expanded by lymphocytes, plasma cells, and oedema. Grade 3 inflammation involved effacement of liver parenchyma lobules by inflammatory exudate and fibrosis.

2.3. Genomic identification of rat species

Genomic DNA was extracted from the liver of the rats. The polymerase chain reaction (PCR) was performed according to Huang et al. (2022) [22]. The PCR products (762 bp) were confirmed on 1.5% agarose gel and then sequenced by Sanger sequencing method (BGI

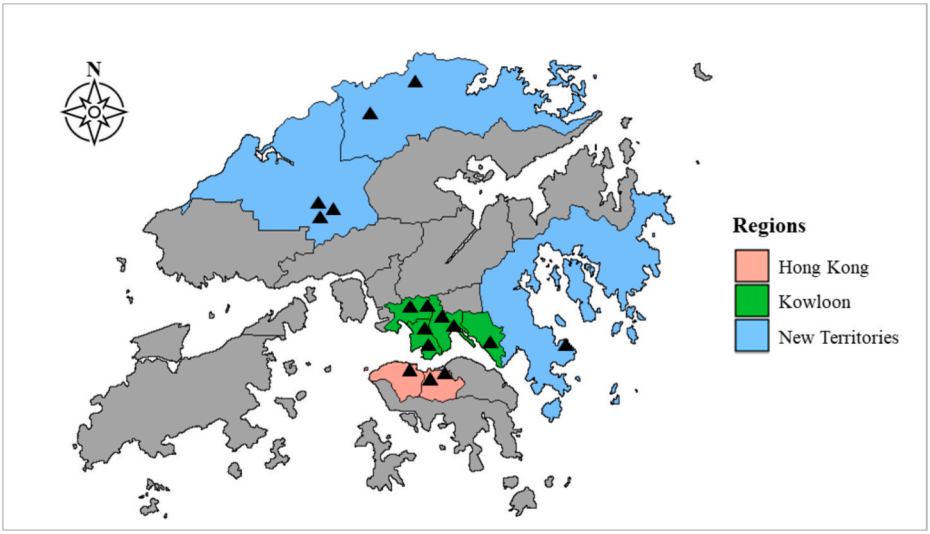

Fig. 1. Map of sixteen trapping locations (black triangles) covering three regions and nine districts of Hong Kong Special Administrative Region.

Genomics, Hong Kong). Rat species were identified using sequence data from the National Center for Biotechnology Information (NCBI) nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/).

2.4. Characteristics of captured rats

A total of 221 rats were captured among which 111 were male rats and 110 were females, from all 16 trapping locations. Fifty-three percent (117/221) of rats were captured from the 6 semi-rural areas (New Territories), followed by 33% (73/221) from 7 urbanized residential centres in Kowloon, and 14% (31/221) from 3 urbanized residential centres on Hong Kong Island. Adult rats represented 59% of the captured rats and 41% were subadults and among female rats, 26/110 rats (24%) were pregnant. Morphological data from all rats revealed mean body weight of 143.6 g (Interquartile range (IQR): 40–216 g), mean body length was 15.4 cm (IQR: 10.9–19.4 cm), mean tail length was 15.3 cm (IQR:11.6–19.0 cm), mean skull length was 46.8 mm (IQR: 38.4–55.8 mm), mean ear length was 2 cm (IQR:1.9–2.1 cm) and mean hindfoot length 3.5 cm (IQR:3.0–4.2 cm). Forty-six rats (20.8%) had at least one skin wound, and 167 rats (75.6%) had at least one ectoparasite, however, the species identification of ectoparasites was not conducted in this study. Four species of rats were identified through PCR with 65% (144/221) being *R. norvegicus*, followed by 30% *R. tanezumi* (67/221), 4% *R. andamanensis* (8/221) and 1% *N. huang* (2/221).

2.5. Genomic confirmation of Capillaria hepatica

Liver from one infected rat from 12 of the 16 trapping locations was randomly selected for PCR, targeting the highly conserved 18S ribosomal RNA gene of *C. hepatica*. Frozen liver samples were thawed, 25 mg of tissue with visible lesions was manually homogenized in phosphate-buffered saline and DNA was extracted using DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the manufacturer instructions. The amplification of the target gene was performed using TaKaRa *taq*™ DNA polymerase (TaKaRa Bio Inc., Shiga, Japan) and premixed with two published primers (233F: 5'-CGG TTC GCT GTT CAG TTG TT-3′ and 436R: 5'-TGC TGC CTT CCT TGG ATG TA-3′) [23]. The synthetic *C. hepatica* DNA (GenBank acc. no. LC425008) [23] and sterile nuclease-free water were used as positive and negative controls, respectively. The amplification conditions were denaturation at 95 °C for 3 mins; 30 cycles of 30 s at 95 °C, 30 s at 52 °C, and 40 s at 72 °C; and final extension at 72 °C for 5 mins. The PCR products (204 bp) were confirmed on a 2% agarose gel electrophoresis.

2.6. Statistical analysis

For statistical analysis, the outcome variable was *C. hepatica* infection (infected and non-infected). The explanatory variables were categorized into 2 groups: host and environmental factors. The host factors were rat species, sex, body weight, stage of development (subadult and adult), presence of skin wound, and presence of ectoparasites. The environmental factors included region (Hong Kong Island, Kowloon, and New Territories) and type of trapping area (urban or semi-rural). Pearson's chi-squared ($\chi 2$) test was performed to affirm that the explanatory variables were all independent. The relationship between the outcome and explanatory variables was examined using logistic regression. Any variables in univariate models associated with $P < 0.2$ were included in the multivariate analysis [21]. The statistical significance of the multivariate model was set at $P < 0.05$. The best fitted model was chosen based on Akaike's Information Criterion (AIC). All statistical analyses were performed in R v. 4.1.1. (R Development Core Team, Vienna, Austria).

3. Results

A total of 81 rats, belonging to three distinct rat species (*R. norvegicus*, *R. tanezumi*, and *R. andamanensis*), were found to be infected by *C. hepatica*. The prevalence of *C. hepatica* infection in trapped rats was 36.7% and these infected rats were captured from 12 of the 16 trapping locations (four locations failed to detect infected rats). *Capillaria hepatica* infection was determined based on the histological presence of the parasite (eggs, larvae and/or adults) and confirmed via PCR (Fig. 2). Liver from one infected rat representing each of the 12 districts produced a 204 bp band on 2% agarose gel electrophoresis, confirming *C. hepatica* species. Two additional rat livers (rat 71 and 238) were randomly chosen from the non-infected rats to act as additional negative controls.

Gross lesions were visible in 83% (68/81) and were absent in 17% (14/81) histologically infected rats. The lesions appeared as twisted/tortuous linear to coalescing, slightly raised or flat tan discolorations of the liver parenchyma, graded from 0 to 3 (Fig. 3). Grade 1 lesions were seen in 64% (52/81) of infected rats (42 *R. norvegicus* rats; 9 *R. tanezumi* rats and the single *R. adamanensis* rat); grade 2 lesions in 16% (13/81) of infected rats (9 *R. norvegicus* rats, 4 *R. tanezumi* rats) and grade 3 lesions in 3% (2/81) of infected rats (both *R. norvegicus* rats). An additional 43 rats had gross liver lesions either suspicious for *C. hepatica* (as described above, but no parasites were seen histologically) for which either no obvious cause or had identifiable causes including infection with *Taenia taenieformis* (rats 34, 41, 80), a septic abscess (rat 102) or neoplasia (rat 58).

Histopathological examination revealed that *C. hepatica* was present in different life cycle stages within the 81 infected rats, with egg production seen in 69% (56/81). *Capillaria hepatica* eggs were observed in almost 80% of *R. norvegicus* rats (44/56) and 21.4% (12/56) were seen in *R. tanezumi*. Eggs were identified more commonly in adult rats (73.2%; 41/56). Eggs were barrel-shaped, unembryonated, with a striated shell, often in clusters surrounding a female worm, entrapped in thin layer of fibrous tissue. Larvae and/or adults with no egg production were seen in 28% (23/81) of infected rats. *Rattus norvegicus* livers harbored 78.3% (18/23) of the larvae and/or adults, followed by 17.4% and 4.4% in *R. tanezumi* (4/23) and *R. andamanensis* (1/23), respectively. The worms with no eggs were spotted equally in adult (11/23) and subadult rats (12/23). The remaining 2 infected rats (rats 106 and 56) only had mineralized concrements with cuticular remnants, surrounded by layers of fibrosis. Adult worms had a smooth cuticle, intestinal tract and female worms had prominent uterus often containing ova. Histologically, grade 0 inflammation was seen in 16/81 infected rats, associated with visible eggs (11/81) or larvae/adults (3/81), and in 2 rats, mineralized foci were associated with parasite remnants (Fig. 4). Histological degeneration was characterized by any combination of eosinophilic pallor, mineralization, or disruption of the parasite structure by infiltrating inflammatory cells. Degeneration in any part of the parasite life stage of *C. hepatica* triggered either grade 1 or 2 inflammation (65/81 infected rats). No grade 3 inflammation was seen in any of the infected rat livers. Inflammation was characterized as acute only in 1 rat (rat 159, *R. norvegicus*), associated with a worm track containing a large segment of nematode, surrounded by a layer of pus, adjacent to a zone of hepatocellular coagulative necrosis. Chronic active, non-suppurative hepatitis accompanied by necrosis was seen in 56/81 rats, associated with either viable or degenerative eggs, larvae, or adults. Chronic hepatitis characterized by mineralization and fibrosis was seen in 8/81 rats, associated with degeneration of parasitic stages.

Notably, the *C. hepatica* infection was identified solely in one individual of the *R. andamanensis*. Due to this low number of infected *R. andamanensis,* only data from *R. norvegicus* and *R. tanezumi* ($n = 211$) was subjected to statistical analysis. A total of 61.2% of infected rats were trapped from the urbanized residential regions; 21.2% (17/80) from Hong Kong Island and 40% (32/80) from Kowloon, whilst the semi-rural areas (New Territories) had 38.8% (31/80). *Rattus norvegicus* and *R. tanezumi* accounted for 80% (64/80) and 20% (16/80) of *C. hepatica* infected rats, respectively. Univariate analysis showed the region, rat species, body weight, and the presence of skin wound were

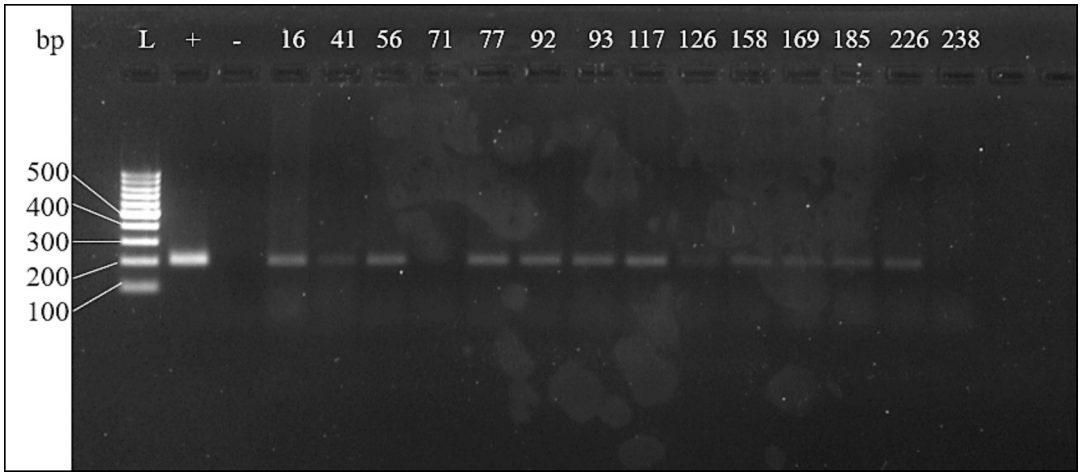

Fig. 2. Gel electrophoresis results of PCR performed on DNA extracted from 14 rat livers (12 infected and 2 uninfected) randomly selected from 12 geographical trapping sites. The PCR targeted the 18S ribosomal RNA gene of *Capillaria hepatica*. L: DNA ladder 100 bp; +: positive control; −: negative control; Rats 71 and 238; non-infected liver samples controls. All other rats: specific band at 204 bp is present, confirming *Capillaria hepatica* infection.

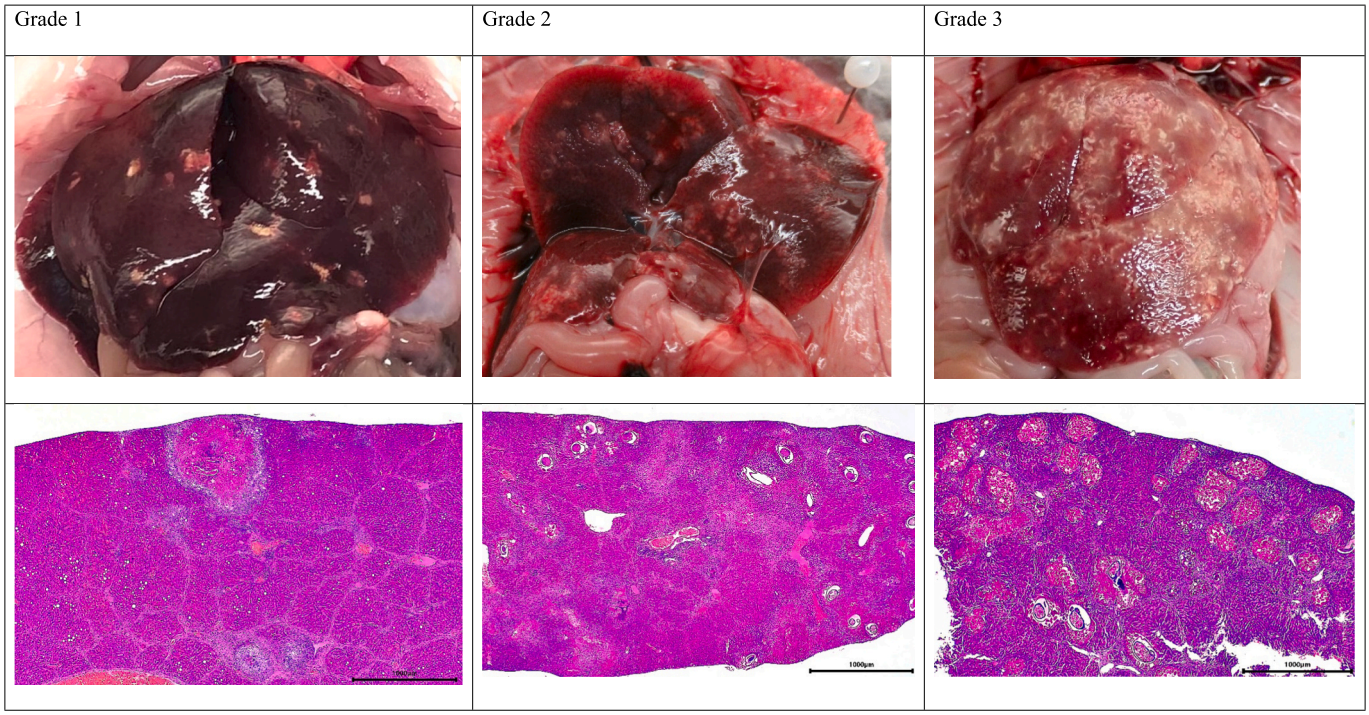

Fig. 3. Gross and histological examples of the grading scheme for *Capillaria hepatica* hepatic lesions in three rats (grade 0 is not demonstrated). Gross lesions are defined by irregular tan flat to slightly raised tortuous tracks or coalescing foci.
Grade 1: <25% of the liver parenchyma infected. Rat 10, *Rattus tanezumi.*
Grade 2: Between 25 and 50% of the liver parenchyma infected; Rat 63, *Rattus norvegicus*.
Grade 3: >50% of the liver infected. Rat 180, *Rattus norvegicus*.

significant variables ($P < 0.05$) of *C. hepatica* infection within individual rat, while the type of trapping area, sex, stage of development, and presence of ectoparasites did not influence *C. hepatica* infection (Table 1).

However, multivariate analysis and logistic regression model did not find the type of trapping area, body weight, and stage of development as significant contributors to *C. hepatica* infection (Table 2). The odds of being infected by *C. hepatica* was higher in rats caught in Kowloon (odds ratio (OR) = 3.00, 95% confidence interval (CI) = 1.49–6.22) and Hong Kong Island (OR = 2.97, 95% CI = 1.27–7.15) compared to those rats trapped in New Territories. *Rattus norvegicus* (OR = 3.48, 95% CI = 1.71–7.50) were more commonly infected compared with *R. tanezumi*. Rats exhibiting skin wounds were statistically more likely to be infected with *C. hepatica* than rats lacking wounds (OR = 2.74, 95% CI = 1.34–5.72). This model was the best-fit model which generated the lowest AIC score of 260.9 compared to other models.

4. Discussion

This is the first epidemiological study of *C. hepatica* infection in *Rattus* species in Hong Kong. The prevalence of *C. hepatica* infection in 221 trapped rats is consistent with previous prevalence data within wild

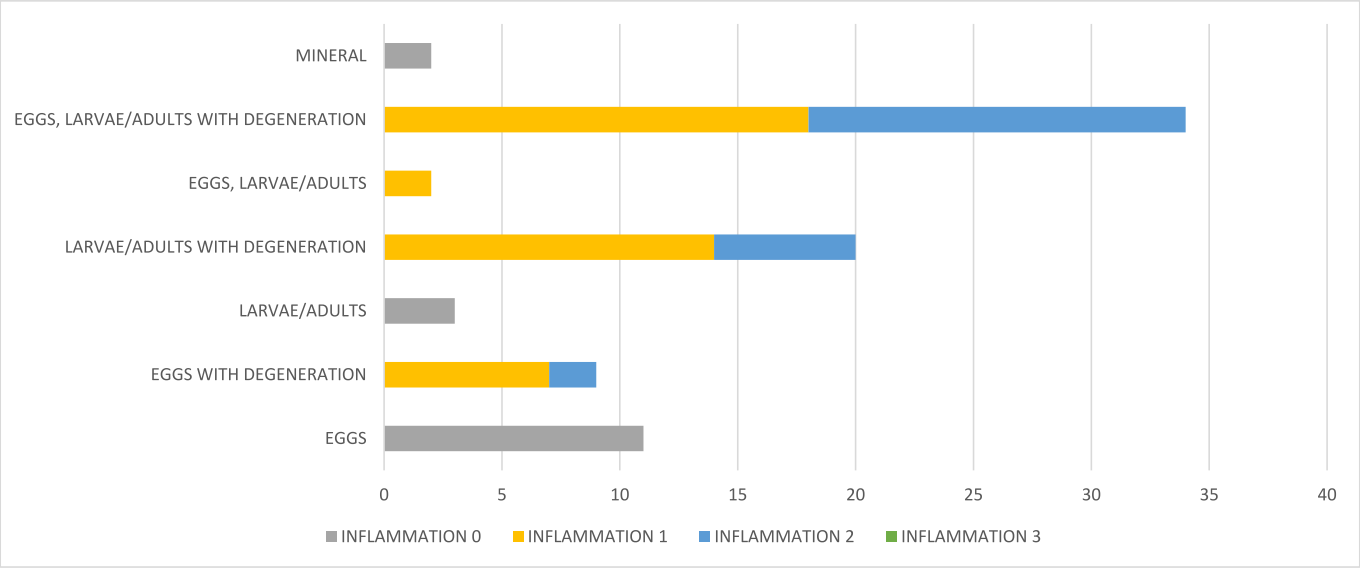

Fig. 4. Histologically graded liver inflammation (graded 0–3), caused by different growth stages and viability status of *Capillaria hepatica* in 81 rats.
Grade 0: No inflammation.
Grade 1: Inflammation centered around nematode segments, creating a zone of inflammation, up to 6 cells thick, on a background of subtle fibrosis, with adjacent interstitial tissues minimally to mildly expanded by inflammatory cells.
Grade 2: Discrete, coalescing foci of inflammation centered around nematode segments, with broad cuffs of inflammatory cells up to 10 layers thick, embedded in thin layers of collagen fibres and plump fibroblasts. The interstitial tissues extending away from infection contained moderate numbers of predominantly lymphocytes and plasma cells with mild/moderate oedema.
Grade 3: Effacement of liver parenchyma by inflammatory exudate and fibrosis.

Table 1
The associations of factor variables and *Capillaria hepatica* infection in 211 rats trapped in Hong Kong Special Administrative Region between October 2020 and August 2021.

	Variable		Number of rats (n = 211) (%)	*C. hepatica* infection		*P*-value
				Absent (n = 131) (%)	Present (n = 80) (%)	
Environmental factor	Region	Hong Kong Island	31 (14.7)	14 (10.7)	17 (21.2)	0.01*
		Kowloon	72 (34.1)	40 (30.5)	32 (40.0)	
		New Territories	108 (51.2)	77 (58.8)	31 (38.8)	
	Type of trapping area	Semi-rural	116 (55.0)	79 (60.3)	37 (46.2)	0.07
		Urban	95 (45.0)	52 (39.7)	43 (53.8)	
Host factor	Rat species	*R. norvegicus*	144 (68.2)	80 (61.1)	64 (80.0)	0.01*
		R. tanezumi	67 (31.8)	51 (38.9)	16 (20.0)	
	Sex	Male	105 (49.8)	61 (46.6)	44 (55.0)	0.30
		Female	106 (50.2)	70 (53.4)	36 (45.0)	
	Weight	≤ 100 g	104 (49.3)	73 (55.7)	31 (38.8)	0.02*
		> 100 g	107 (50.7)	58 (44.3)	49 (61.2)	
	Stage of development	Subadult	86 (40.8)	59 (45.0)	27 (33.8)	0.14
		Adult	125 (59.2)	72 (55.0)	53 (66.2)	
	Skin wound	Present	44 (20.9)	19 (14.5)	25 (31.2)	0.01*
		Absent	167 (79.1)	112 (85.5)	55 (68.8)	
	Ectoparasites	Present	159 (75.4)	101 (77.1)	58 (72.5)	0.56
		Absent	52 (24.6)	30 (22.9)	22 (27.5)	

* $P < 0.05$.

rats worldwide, which confirms our hypothesis [20,24–26]. The Norway rat (*R. norvegicus*) was the most common rat species identified, followed by *R. tanezumi* which confirms that both rat species are common in southern China. Prevalence findings in this study should be interpreted with caution due to the lack of data on the individual rat species populations in Hong Kong.

Rattus norvegicus from urbanized residential areas (Kowloon and Hong Kong Island) with skin wounds, were identified at risk for *C. hepatica*. The majority of infected rats were *R. norvegicus*, followed by *R. tanezumi* and *R. andamanensis*, consistent with global data indicating *Rattus* species are a common host [19,27], and Norway rats are the main host species for this nematode [11,17,19–21,26,28–31]. Most infected rats were captured from urbanized residential regions (Kowloon and Hong Kong Island), which may be attributed to poor waste management and sanitation due to high population density landscapes within these areas [32,33]. However, high numbers of *R. norvegicus* could be a confounder because it was the dominant species trapped in Hong Kong Island and New Territories. The presence of skin wounds was retained in the final multivariate model, which is consistent with the findings of Rothenburger et al. [30]. The presence of skin wounds can be attributed to social dominance within the rat colony. Dominance can be established through behaviors such as fighting, controlling access to food and water, or even crawling over subordinate rats [34]. The associations between bite wounds and infection may suggest that infected rats may be more vulnerable to socially dominant rats [30].

Sex, body weight, stage of development, and presence of

Table 2
Unadjusted and adjusted odds ratio with 95% confident interval for the association of *Capillaria hepatica* infection and variables in 211 rats caught in Hong Kong Special Administrative Region from October 2020 to August 2021.

Variable		Unadjusted		Adjusted	
		OR	95% CI	OR	95% CI
Region	New Territories	Ref.			
	Kowloon	8.44	1.72–63.14	3.00	1.49–6.22**
	Hong Kong Island	10.06	1.53–90.64	2.97	1.27–7.15*
Type of area	Semi-rural	Ref.			
	Urban	0.33	0.04–1.72	–	–
Rat species	*R. tanezumi*	Ref.			
	R. norvegicus	2.76	1.28–6.20	3.48	1.71–7.50***
Weight	≤ 100 g	Ref.			
	> 100 g	1.42	0.63–3.27	–	–
Stage of development	Adult	Ref.			
	Subadult	0.93	0.43–2.06	–	–
Skin wound	Absent	Ref.			
	Present	2.40	1.10–5.31	2.74	1.34–5.72**

Ref. = reference category to which each other category in that variable is compared, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

ectoparasites were not risk factors. The presence of ectoparasites should be interpreted carefully as the quantity and species of ectoparasites were not characterized further in this study. Sex of rats was also not a risk factor in previous studies, [19,20,35] and both body weight and life stage of rats have been previously identified as risk factors [27,30,36]. However, in these studies only larger rat species were examined, whereas in our study smaller rat species were also included, which possibly explains these differences. Season has been identified as a potential risk factor for *C. hepatica* infection in a Canadian study [30]. The effect of season was omitted from the analysis in the current study because – for logistics reasons – the different areas were not sampled simultaneously but consecutively. Also, sampling disruptions due to COVID-19 restrictions introduced some inconsistencies in seasonality. However, we do not expect seasonal differences like those observed in the Canadian study, as Hong Kong has a subtropical climate with mild temperatures throughout the year.

The degree of liver infection observed in the current study is similar to parasite burdens observed in rats in the Philippines [20]. Frequent heavy infection involving >50% of the hepatic mass has been reported in mice [3,37], zoo housed primates [38] and humans [10,39] although many human cases report focal or incidental lesions [23,40]. Gross lesions of *C. hepatica* infection in rat livers appeared as twisted/tortuous linear to coalescing tan discolourations of the liver parenchyma, similar to those previously described [19,20,24,31,41], but these are not pathognomonic lesions. In some infected rats, *C. hepatica* infection was detected only via histopathology as no gross lesions were present. On the other hand, the absence of parasites on histology in rats with gross hepatic lesions suspicious for *C. hepatica* infection, could be explained by the unintentional exclusion of focal lesions with parasites from histological examination.

Histologically, inflammation was predominantly associated with degeneration of parasites and most rats with only viable parasites exhibited no accompanying inflammation, consistent with a previous report [42]. Minimal inflammation associated with viable egg packets has also been reported in cats [2] and primates [38], reflecting the overall low pathogenicity of this parasite in various host species [9,23,40]. Chronic active inflammation was the most common pattern seen, which included macrophages often with multinucleated giant cells. Lymphocytes, plasma cells, neutrophils, and eosinophils with lytic or coagulative hepatocellular necrosis and fibrin lakes, accompanied by portal lymphoplasmacytic infiltrates with subtle interstitial/septal fibrosis was observed. This pattern of inflammation is similar to that reported in rats [19,20,30,41], mice [37], zoo primates [38], and dogs [43] but differs from the granulomatous inflammation with eosinophils reported in cats [2] and humans [3,4,15,23]. Larval tracks stimulating acute necrosis were identified in only one rat and was similar to lesions described in mice [37]. Fibrosis was never a prominent histopathological change in infected rats which differs to some human infections where fibrosis is prominent [44]. Egg production was noted in 69% of infected rats, highlighting the important role of rats as a reservoir of infection, and when eggs appeared viable, inflammation was either absent (grade 0) or mild (grade 1) attributable to immune evasion tactics utilized by the nematode to avoid host immune attack [45,46].

5. Conclusion

This is the first study on *C. hepatica* infection in Hong Kong and our results indicate that it is a common infection in the rat population, highlighting the zoonotic risk of *C. hepatica* infection within Hong Kong. A one health approach towards understanding the rat population dynamics, habitats and behavior around human dwellings is important to better estimate the risks to human health from the rat-borne *C. hepatica.* Although rat population control measures mainly by extensive use of rodenticide baits are in place in Hong Kong, it seems to facilitate the release of *C. hepatica* eggs through the decay of rat carcasses and hence the dissemination of infection. Therefore, alternatives to the use of poison baits should be explored to reduce the risk of release of *C. hepatica* eggs and at the same time consider effective rodent control, human safety, and animal welfare.

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Ethical approval

This study was approved of by the Animal Research Ethics Sub-Committee of the City University of Hong Kong (Internal Ref: A- 0380).

CRediT authorship contribution statement

Jeanine Sandy: Data curation, Formal analysis, Visualization, Writing – original draft. **Theethawat Uea-Anuwong:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Lam Hoi Kiu:** Investigation, Project administration. **Lisa K.F. Lee:** Investigation, Writing – review & editing. **Swaid Abdullah:** Formal analysis, Methodology, Writing – review & editing. **Ioannis Magouras:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest in this study.

Data availability

No data was used for the research described in the article.

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