Potentially zoonotic pathogens and parasites in opportunistically sourced urban brown rats (Rattus norvegicus) in and around Helsinki, Finland, 2018 to 2023

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Background: Brown rats (*Rattus norvegicus*) are synanthropic rodents with worldwide distribution, which are known to harbour many zoonotic pathogens and parasites. No systematic zoonotic surveys targeting multiple pathogens and parasites have previously been conducted in urban rats in Finland. **Aim:** In Helsinki, Finland, we explored the presence and prevalence in brown rats of certain pathogens and parasites (including helminths, viruses and bacteria) across potentially zoonotic taxa. **Methods:** We opportunistically received rat carcasses from pest management operators and citizens from 2018 to 2023. We searched for heart- or lungworms, performed rat diaphragm digestion to check for *Trichinella* and morphologically identified intestinal helminths. We assessed virus exposure by immunofluorescence assay or PCR, and detected bacteria by PCR (*Leptospira*) or culture (*Campylobacter*). **Results:** Among the rats investigated for helminths, no heart- or lungworms or *Trichinella* species were detected and the most common finding was the cestode *Hymenolepis nana* (in 9.7% of individuals sampled, 28/288). For some of the surveyed virus taxa, several rats were seropositive (orthopoxviruses, 5.2%, 11/211; arenaviruses, 2.8%, 6/211; hantaviruses 5.2%, 11/211) or tested positive by PCR (rat hepatitis E virus, 1.8%, 4/216). *Campylobacter jejuni* (6.6%, 17/259) and *Leptospira interrogans* (1.2%, 2/163) bacteria were also present in the rat population examined. **Conclusions:** Prevalences of potentially zoonotic pathogens and parasites in brown rats in Helsinki appeared low. This may explain low or non-existent diagnosis levels of rat-borne pathogen and parasite infections reported in people there. Nevertheless, further assessment of under-diagnosis, which cannot be excluded, would enhance understanding the risks of zoonoses.

Introduction

In urban areas, pets and domestic animals may acquire certain pathogens from humans and/or transmit them to humans [1]. This can also be the case for wild animals and, although urban environments host limited wildlife, these settings seem to be enriched in species susceptible to human pathogens [2] (but see [3]). Rodents which are often highlighted as a considerable source of human infections [4], have historically, both received zoonotic pathogens from people and transmitted such pathogens to people [5]. In the world, growing urbanisation raises the likelihood for rodents, especially species characterised as pests, to come into contact with people [6]. In addition, as the importance of urban biodiversity gains further recognition, the number of urban green spaces, which can host rodents, may increase, potentially creating more settings for possible transmission events between humans, pets and rodents [7-9].

Brown rats (*Rattus norvegicus*) are one of the most synanthropic mammals in the world and are known carriers of numerous zoonotic pathogens and parasites including helminths (nematodes and cestodes), bacteria and viruses [10]. Thus, they make an

KEY PUBLIC HEALTH MESSAGE

What did you want to address in this study and why?
Brown rats have been shown to host several zoonotic pathogens and parasites and they are believed to be important sources of human infections in various environmental and social contexts. We aimed to survey for the presence of several important potentially zoonotic parasites and pathogens in brown rats in Helsinki, Finland, from 2018 to 2023. These included worms (e.g. *Trichinella* and *Hymenolepis nana*), viruses, and bacteria (e.g. *Campylobacter jejuni* and *Leptospira interrogans*).

What have we learnt from this study?
Whereas we did not detect *Trichinella* and heart- and lung worms in the rats examined, we observed most of the zoonotic pathogens and parasites that we searched for. These included H. nana (9.7% of rats investigated), rat hepatitis E virus (1.8% of rats surveyed), as well as *C. jejuni* and *L. interrogans* (6.6% and 1.2% of rats surveyed respectively). Nevertheless, many of the prevalences in our study seemed lower than in other European cities and the reasons for this remain unexplored.

What are the implications of your findings for public health?
We found a low prevalence of parasites and pathogens in the urban brown rats that we studied, which may suggest that the risk of transmission to people in Helsinki is limited. This could be a reason for the low or non-existent reports of rat-borne pathogen and parasite infections in humans there, but under-diagnosis might also be an explanation, so this could be assessed in the future to further understand the risk posed by urban rats to humans.

interesting species to study in the context of urban pathogen spillovers. Currently, mortality and morbidity caused by rat-borne pathogens and parasites in humans is known to be concentrated in the Global South [11], whereas the same pathogen and parasite species are commonly found in the Global North but with limited public health consequences [10]. While this situation may relate to exposure, risks of zoonotic infections are poorly understood and may evolve due to anthropogenic modifications of the environment and/or climate change [12].

Finland lies on the northernmost continuous distribution area of the brown rat. At such high latitudes, few studies have focused on this species and, in this context, risks of pathogens and parasites occurring in brown rats and of their transmission to humans in Finland are poorly understood. One report from 2005 described two human cases of rat bite fever in the country, which were caused by *Streptobacillus moniliformis* and linked to rats [13]. Finnish rats, however, are likely to carry numerous potentially zoonotic parasites and pathogens.

Within a larger objective of assessing rat-related infectious disease risks in Finland and of further conducting surveys in humans targeting the most relevant underlying pathogen species, the aim of this study was to use stakeholder-collected brown rat samples in the city of Helsinki, Finland [14,15], to investigate whether the rats hosted certain pathogens and parasites, which were potentially zoonotic.

Methods

Sample collection
This study was a part of the multidisciplinary Helsinki Urban Rat Project. We acquired rat carcasses between February 2018 and April 2023 from pest management professionals and citizens who collected rats from their kill traps and brought them to our storage freezers along with information on catch date and location. Pest control interventions have previously been suggested for opportunistic sampling of rat-borne pathogens [14], and we used this approach for two main reasons. Firstly, due to the participatory nature of our project, we had extensive collaboration with our stakeholders (e.g. environmental health authorities and property managers), so involving also pest management professionals suited the set-up. Secondly, members of our research project expressed reservations about killing rats for research purposes.

All samples were collected within or next to buildings as lethal pest control is not generally conducted in city parks or other green areas. We mostly limited the samples to the Helsinki City area but also collected additional samples from a waste incineration plant where rats are assumed to arrive not only from Helsinki City but also from a larger area around it (municipalities of Espoo, Hanko, Hyvinkää, Inkoo, Järvenpää, Karkkila, Kauniainen, Kerava, Kirkkonummi, Lohja, Mäntsälä, Nurmijärvi, Pornainen, Raasepori, Sipoo, Siuntio, Tuusula, Vantaa, Vihti and partly Porvoo).

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The spatial distribution of samples across the districts of Helsinki, Finland, 2018–2023 (n = 288)
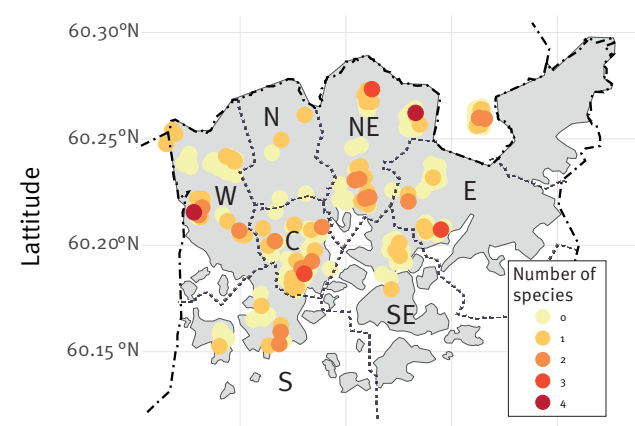
C: Central major district; E: Eastern major district; N: Northern major district; NE: North-eastern major district; S: Southern major district; SE: South-eastern major district; W: Western major district.

For overall pathogen and parasite diversity in the samples, red refers to the highest species richness in individual samples, whereas a lighter colour indicates lower species richness. The locations were generalised to mask the accurate locations of the sampling sites. Gray represents the land area within city limits. Samples from the incineration plant are those that fall outside of city borders, which are represented by a thick dotted line.

The rat carcasses, which were frozen at −20 °C, were then defrosted. Upon defrosting, those further included in the study, based on quality inspection, were used to obtain tissue/organs for pathogen or parasite analysis. We set no clear-cut limits for the acceptable time between rat death, and the carcass being deposited in a freezer, as, for example, during the winter-time rat collections, carcasses could be already effectively frozen due to sub-zero ambient temperatures. In general, indoor traps are checked every 24 hours or more frequently to prevent odours. We only included fresh-looking carcasses to limit the effects of decomposition on the analysis. Upon inclusion of the rats in the study, we recorded their sex and body mass.

Pathogen and parasite investigations

To be targeted by the study, pathogens and parasites had to have been mentioned in the literature as commonly rat-borne and zoonotic [10] and their detection had to be feasible within the project. Their list was as follows: (i) helminths including cestodes and nematodes or other heart-, lung- and gut worms; (ii) bacteria (*Campylobacter jejuni*; other *Campylobacter* spp.; *Leptospira interrogans*); and (iii) viruses (poxviruses, hantaviruses, arenaviruses, rat hepatitis virus (ratHEV)).

We further refer to these as 'potential' zoonoses because we do not know whether they cause actual zoonotic infections in Helsinki and whether the

pathogens or parasites (or their different strains) are the same as those causing human infections.

For each rat, we collected a piece of colon with a variable amount of faecal matter for *Campylobacter* analysis, a piece of diaphragm and/or thigh muscle for *Trichinella* analysis and tissues from the lung, heart, liver and kidney for virus and *Leptospira* analysis.

Helminths

We analysed diaphragm and thigh muscle samples by artificial digestion for *Trichinella* according to European Union Regulation 2015/1375, Annex I, as applicable, accounting for the small size of samples [16]. We inspected the heart, lungs and the whole gastrointestinal tract from the stomach to the large intestine under the microscope for the presence of nematodes, cestodes or other helminths. We morphologically identified any observed helminths to the species level whenever possible. We did not fix or stain tissues. The helminth species were cross-referenced with lists of known brown rat parasites [17] and identified with reference to previous studies [18-20].

Bacteria

We homogenised the colon samples using a cotton swab dipped in sterile buffered peptone water and cultured them directly on *Campylobacter*-selective charcoal-cefoperazone–deoxycholate agar (CCDA) plates (Oxoid Ltd., Basingstoke, United Kingdom) that were incubated under microaerobic conditions (5% O_2, 10% CO_2, ≤10% H_2, balanced with N_2; Anoxomat System, Mart Microbiology, the Netherlands) at 41.5 °C for 48–72 hours. One typical colony was confirmed per sample as *Campylobacter* spp. or *C. jejuni* using Gram-stain and genus- and species-specific PCR. We used SsoAdvanced Universal SYBR Green real-time PCR (RT-PCR) Supermix (Bio-Rad, Hercules, California, United States) according to the manufacturer's instructions with primers 16S-CampyF1 and 16S-CampyR1 [21] or JH0039 and JH0040 [22] (metabion, Planegg, Germany), respectively. We measured fluorescence intensity using the CFX96 Touch RT-PCR Detection System (Bio-Rad) and CFX Maestro Software v.2.3. We considered a sample positive when the quantification cycle was below 30 and a specific melt curve with peak temperature between 78.5 and 79.5 °C was observed.

We detected *Leptospira* by PCR from the kidney samples. We extracted DNA using the Nucleospin Tissue mini kit (Macherey-Nagel, Düren, Germany), followed by quantitative PCR (qPCR) targeting the secY gene of *Leptospira* as previously described [23]. We performed the qPCR using the Agilent Technologies' AriaMx RT-PCR system and melted the amplified product at 70–94 °C to confirm the identity of the amplified product.

Characteristics of the opportunistic rat sample (*Rattus norvegicus*) within and around Helsinki, Finland, 2018–2023 (n = 288)

Group	Sample size
Weight category	
Juvenile (< 100 g)	170
Adult (> 100 g)	110
Unknown[a]	8
Sex	
Male	146
Female	116
Unknown[a]	26
Collection period of the year	
January–March	122
April–June	109
July–September	20
October–December	37
Year of collection[b]	
2018	20
2019	103
2020	48
2021	44
2022	15
2023	52
Location of collection	
City of Helsinki	245
Southern major district	17
Western major district	53
Central major district	72
Northern major district	4
North-eastern major district	31
South-eastern major district	22
Eastern major district	46
Incineration plant	43
Total	**288**

[a] Some individuals could not reliably be sexed due to small size, whereas some could not be reliably weighed, as they were missing a substantial (but for the purposes of this study non-considerable) part of the body.

[b] For six rats, the year of collection was not recorded.

Viruses

We tested ratHEV RNA from the liver samples that were homogenised using glass beads and sand in 1 mL TRIzol reagent (Invitrogen, ThermoFisher Scientific, Waltham, Minnesota, United States) with MagNa Lyser (Roche Diagnostics, Rotkreuz, Switzerland). We extracted RNA from the samples with the TRIzol reagent following product instructions and amplified ratHEV RNA in two steps: a hepevirus specific broad-spectrum PCR as a first step, and then a nested PCR protocol, targeting a conserved region of open reading frame (ORF)1 as the second step. For the hepevirus RT-PCR we used Superscript III Platinum One-Step qRT-PCR Kit without carboxyrhodamine (ROX; Invitrogen, Thermo Fisher Scientific) with primers HEV-cs and HEV-cas [24]. Subsequently, the nested PCR used the Platinum Taq DNA polymerase (Invitrogen) with the primers HEV-csn mod and the primer HEV-casn [25]. We performed the PCR reactions with either a ThermoScientific Arktik Thermal Cycler or an MJ Research PTC-200 Peltier Thermal Cycler. For a second approach we confirmed the presence of rat-specific HEV with a higher sensitivity by using a specific RT-qPCR for ratHEV [26] and adapting the protocol to TaqMan Fast Virus 1-Step Master Mix (4X) (Thermo Fisher Scientific). To target the region 5,214–5,286 in the rat/Mu/0685/DEU2010 sequence, we used primers rHEV-F and rHEV-R2 with a rHEV-P2 probe [26] labelled with 6-carboxyfluorescein (6-FAM) at the 5' end and Black Hole Quencher (BHQ) was used at the 3' end. We performed RT-PCR using the Agilent Technologies AriaMx RT-PCR system.

We screened samples for antibodies to orthopoxviruses, arenaviruses, and hantaviruses using immunofluorescence assays (IFA), as previously described [27-29]. These assays are developed for specific viruses (Puumala hantavirus, PUUV; Dobrava-Belgrade hantavirus, DOBV; lymphocytic choriomeningitis virus, LCMV; and coxpow virus), but cross-react with other closely related viruses, which is useful when we do not know which particular orthopoxvirus, arenavirus, or hantavirus is present in the samples. As PUUV and DOBV represent different serogroups, we can detect all possible rodent-borne hantaviruses by performing assays on both [27]. The LCMV assay cross-reacts with all Old-World arenaviruses [27,29], and similarly, the cowpox virus assay is highly cross-reactive across orthopoxviruses [28].

Whereas the morphological surveys of helminths, *Campylobacter* culture and *Leptospira* and ratHEV PCR detect acute infections, IFA used for viruses is indicative of past infection.

Statistical analyses

While the rat carcass sample size was not small, potential statistical analyses were limited by the number of parasite and pathogen species: modelling individual species could easily have led to multiple testing, which in turn reduces the power of analysis. To infer general patterns, we performed one generalised linear mixed model where we used the number of parasite and pathogen species in a sample as a response variable, whereas weight, sex, season and district were used as explanatory variables and year and site nested within district as random variables. We used the lme4 package in R for statistical testing [30]. As lme4 does not report p-values due to the difficulties in estimating degrees of freedom in mixed-effects models, we report Wald t values, where values higher than 1 or lower than −1 indicate substantial differences from zero. We tested the spatial variation in *Campylobacter* presence with the x^2 test [31].

Results

We received a total of 288 rat carcasses that were of adequate quality to conduct at least some analyses

TABLE 2

Pathogen and parasite prevalences in urban brown rats (*Rattus norvegicus*) within and around Helsinki, Finland, 2018–2023 (n = 288)

Pathogens or parasites		All rats			Rats within city of Helsinki										
		Numbers		% (95%CI)	Juvenile			Adults			Both				
		+	Successfully tested		Numbers		% (95%CI)	Numbers		% (95%CI)	Numbers		% (95%CI)		
					+	Successfully tested		+	Successfully tested		+	Successfully tested			
Viruses	Poxviruses	11	211	5.2 (2.6–8.7)	1	111	0.9 (0–3.6)	10	84	11.9 (5.7–20.5)	11	195	5.6 (2.8–9.5)		
	Hantaviruses	11	211	5.2 (2.6–8.7)	2	111	1.8 (0.2–5.2)	9	84	10.7 (4.9–18.9)	11	195	5.6 (2.8–9.5)		
	Arenaviruses	6	211	2.8 (1.0–5.6)	1	111	0.9 (0–3.6)	5	84	6.0 (1.9–12.49)	6	195	3.1 (1.1–6.0)		
	Rat hepatitis E virus	4	216	1.8 (0.5–4.1)	1	114	0.9 (0.0–3.5)	2	96	2.1 (0.2–5.6)	3	200	1.5 (0.3–3.7)		
Bacteria	Campylobacter jejuni	17	259	6.6 (3.8–10.1)	2	136	1.5 (0.1–4.2)	13	84	15.4 (8.2–25.1)	14	220	6.4 (3.5–10.1)		
	Other *Campylobacter* spp.	2	259	0.8 (0.1–2.2)	2	136	1.5 (0.1–4.2))	0	84	0 (0–1.5)	2	220	0.9 (0–2.6)		
	Leptospira interrogans	2	163	1.2 (0.1–3.5)	0	82	0 (0–1.5)	2	65	3.1 (0.3–8.9)	2	147	1.4 (0.1–3.9)		
Cestodes	Hymenolepis nana	28	288	9.7 (6.3–13.3)	11	151	7.3 (3.6–12.2)	13	94	13.7 (7.3–22.4)	24	245	9.8 (6.3–14.1)		
	Hymenolepis diminuta	1	288	0.3 (0.0–1.3)	0	151	0 (0–0–8)	0	94	0 (0–1.3)	0	245	0 (0–0.5)		
	Heterakis spumosa	15	288	5.1 (2.8–8.0)	4	151	2.6 (0.7–5.9)	11	94	11.7 (5.8–19.7)	15	245	6.1 (3.4–9.6)		
	Syphacia sp.	11	288	3.7 (1.9–6.3)	5	151	3.3 (1.1–6.9)	5	94	5.3 (1.7–11.0)	10	245	4.1 (1.9–7.0)		
Nematodes	Nippostrongylus sp.	5	288	1.7 (0.6–4.2)	0	151	0 (0–0.8)	5	94	5.3 (1.7–11.0)	5	245	2.0 (0.6–4.2)		
	Trichinella spp.	0	257	0 (0–0.5)	0	138	0 (0–0.9)	0	84	0 (0–1.5)	0	222	0 (0–0.6)		
	Angiostrongylus spp. or other heart and lung worms	0	288	0 (0–0.4)	0	151	0 (0–0.8)	0	94	0 (0–1.3)	0	245	0 (0–0.5)		

CI: confidence intervals; max.: maximum; +: positive.

Prevalences are given in percentages with 95% CIs. Data from the waste incineration plant are not shown, as the sample size is low (max. n=35). The spatial distribution of individual pathogens is shown in Figure 2.

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The occurrence in urban brown rats (*Rattus norvegicus*) of each pathogen or parasite plotted on a map of Helsinki, Finland, 2018–2023
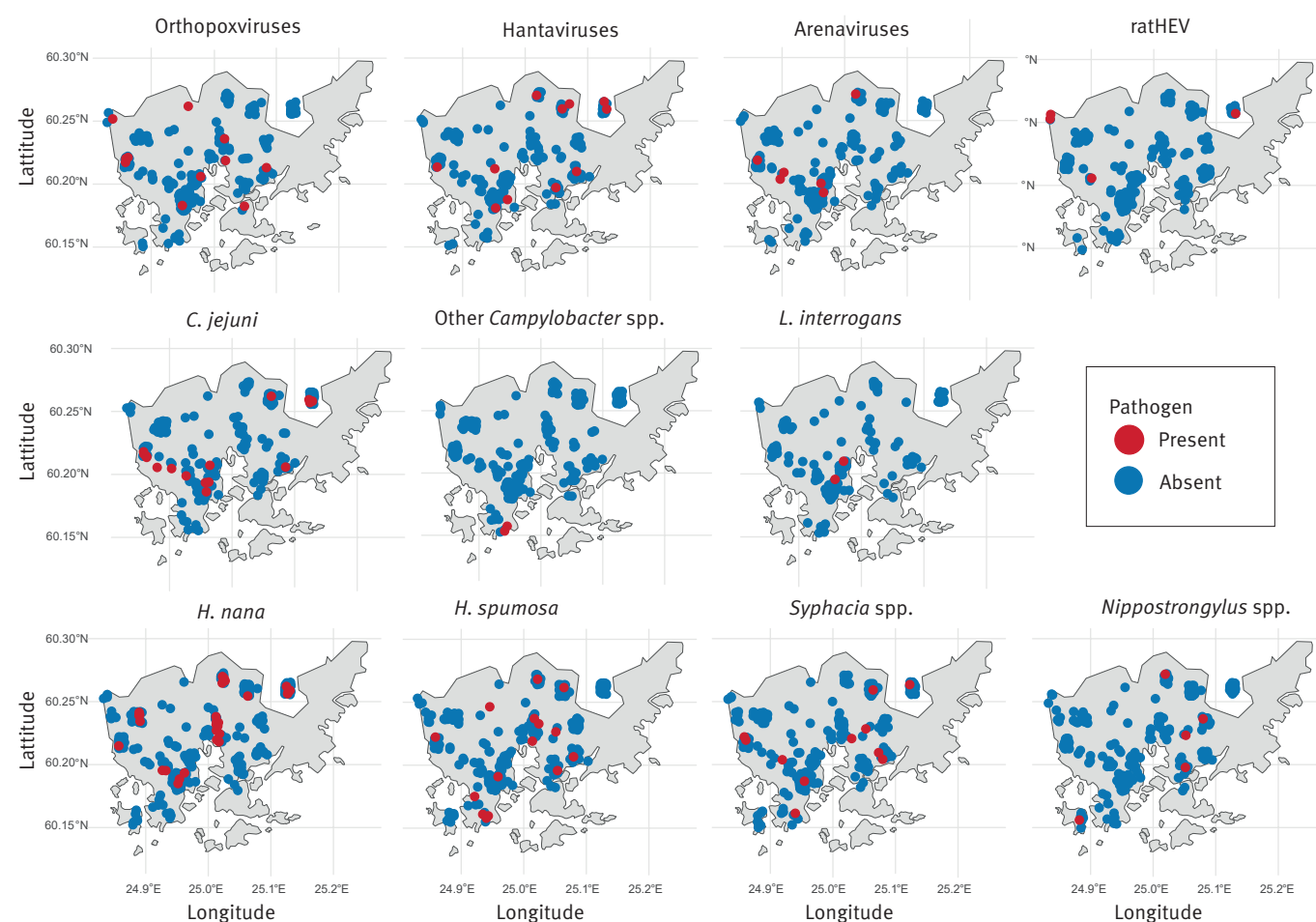
C. jejuni: *Campylobacter jejuni*; *H. nana*: *Hymenolepis nana*; *H. spumosa*: *Heterakis spumosa*; *L. interrogans*: *Leptospira interrogans*; ratHEV: rat hepatitis E virus.

For individual pathogens and parasites red shows presence and blue shows absence. The city area is shown in grey. On the axes, N and E respectively indicate the latitude (north) and longitude (east). The locations were generalised to mask the accurate locations of the sampling sites. Samples from the waste incineration plant are those that fall outside of city borders.

(Table 1; Figure 1). Juvenile rats (<100 g) were more common than adult rats. The median juvenile and adult weights were 54 g and 195 g, respectively, with rat weights ranging from 13.4 g to 368 g. Substantially more samples collected over the study period were from the first half of the year (January–June: n = 231) than from the second half (n = 57), but the sex ratio (146 male/116 female rats) was close to uniform.

We observed most of the pathogens and parasites under survey, except for *Trichinella* and *Angiostrongylus* or other lung- and heart worms (Table 2). As the sample size from the waste incineration plant (i.e. 'outside' of the city of Helsinki) was smaller, the prevalences of the two locations cannot be reliably compared. The only *H. diminuta* infected individuals were found from a rat at the waste incineration plant.

When considering both acute infections (i.e. helminths, *Campylobacter*, *Leptospira* and ratHEV) and past infections detected by IFA (orthopoxviruses, arenaviruses, and hantaviruses), 7.1% (21/295) of all rat individuals had or had had multiple infections (Figure 1), with extreme cases where two individuals had four different parasites and pathogens each: one had detected *H. nana*, *H. spumosa*, cultured *C. jejuni* and seropositivity for hantavirus, and the other had detected *H. nana*, cultured *C. jejuni* and seropositivity for arenavirus and hantavirus.

The heavier rats were more likely to have parasites and pathogens, whereas neither sex nor season had a significant effect on richness of parasites and pathogens (Table 3). Rats from the Northern and South-eastern districts were less likely to have parasites and pathogens than those from other districts, whereas rats from the North-eastern and Southern districts were more likely to be infected (Table 3; Figure 1).

Estimates from the mixed effects modelling for parasite and pathogen richness in urban brown rats (*Rattus norvegicus*) sampled within and around Helsinki, Finland, 2018–2023 (n = 288)

Variable	Categories	Estimate	Standard Error	Wald t value[a]
Intercept	NA	−0.118	0.238	−0.50
Weight	NA	0.003	0.001	6.07
Sex	Female	0.087	0.220	0.39
	Male	0.137	0.213	0.64
Season	Spring	0.052	0.140	0.37
	Summer	0.064	0.192	0.33
	Winter	0.054	0.135	−0.40
Location	Eastern district	0.085	0.136	0.62
	Northern district	−0.489	0.476	−1.03
	North-eastern district	0.247	0.151	1.63
	Southern district	0.217	0.189	1.15
	South-eastern district	−0.271	0.174	−1.56
	Western district	0.117	0.122	0.97
	Incineration plant	−0.049	0.137	−0.36

NA: not applicable.

[a] Wald t values over 1 or below −1 indicate substantial differences from the baseline. The baseline case is a rat of unknown sex sampled in autumn in the Central district.

Discussion

In this study we found low parasite and pathogen prevalences among brown rats in Helsinki compared with other studies in Europe. For example, in different European cities, prevalence in brown and/or black rat population of *H. diminuta* varied from 1.2 to 36.3% [32-37] according to estimations between 1975 and 2017, while based on work between 2010 and 2017, the prevalence of *H. spumosa* ranged from 35 to 82.5% [33-38], and that of *Nippostrongylus brasiliensis* from 6.2 to 46.0% [33-37]. In a publication from 2010, ratHEV was detected through RT-PCR in 0 to 27.2% of rats when sampling across continental Europe [39], whereas Seoul hantavirus (SEOV) prevalence reported in 2009 in wild rats across Flanders, Belgium was 15 to 33% [40]. *Leptospira* seroprevalences have been found to vary in Europe between 1.2% and 100% in urban or peri-urban environments in studies conducted between 1995 and 2016 [41-54]. Thus, rats in Helsinki seem to be in the lower bounds of the prevalence ranges for viral and bacterial pathogens and helminths.

The temporal trend in rat pathogens also appears to be declining: two of the taxa that we surveyed had been investigated previously in the Helsinki region: *Leptospira* prevalence was 43.5% in 1952–53 [55], whereas the prevalence was only 1.2% in our sample. The comparison is not straightforward, as the sampling method is not described in the previous study and *Leptospira* infections were earlier detected serologically, but the difference seems stark. Also, human *Leptospira* cases have been rare in Finland, overall, with 16 reported allochthonous cases between 2011 and 2023, of whom the last one in 2016 [56]. *Trichinella spiralis* prevalence in rats at the Helsinki Zoo was 12% in 1965 [57], and

the overall *Trichinella* prevalence in dump pits in the Helsinki area was 19% in 1994–2000 [58], whereas we found no occurrences in our sample. The general decrease of sylvatic *T. spiralis* in Finland is attributed to the absence of spillover from the domestic cycle [59]. Other *Trichinella* species prevalent in Finland do not readily infect rats [60]. Rats are currently usually not considered an important reservoir for *T. spiralis*, but rather an indicator of its presence in the environment [61].

Campylobacter jejuni prevalence in adult rats in the Helsinki City area (15%) was comparable to levels previously reported around animal-production farms in Finland (20%, n = 10 [62]), lower than on pig farms in France (40%, n = 40 [63]), yet higher than on pig and chicken farms in Sweden (3%, n = 58 [64]). In urban rats in New York city and in fish markets and restaurants in Tokyo, lower prevalences of *C. jejuni* and *C. coli* (4%, 5% and 0%, respectively) have been reported [65,66] whereas rats in the Lyon sewage system had a prevalence of 18% (n = 92 rats caught in 1982) [67]. Interestingly, the older parts of the Helsinki City have a mixed sewage system, i.e. both household waste and rainwater run in the same sewage system. This mixed system is thought to be beneficial for rats as they can easily access it from rainwater drains and then forage among household waste. This also leads to the questions whether transmission is occurring between rats and humans or rather vice versa, as this setting also potentially allows for anthroponotic infections in rats if and when the latter come into contact with human faeces. It is difficult to track how many, and which individual, rats move in the sewage system and conclusively deduce whether *Campylobacter* infections were more common in these areas (10 positives out of 128 in

mixed sewer area vs 4 of 130 in other areas; $x^2_1 = 2.81$, $p = 0.09$). Nevertheless, this calls for more detailed studies on the infection risks that humans pose to rats, and to understand the importance of this transmission route regarding zoonosis persistence and spread in the urban environment.

No previous surveys have been conducted on sero-prevalence in rats in Helsinki and our results suggest the first evidence for the occurrence of rat-associated Seoul hantavirus in Finland. This detection needs to be followed up with genetic characterisation. On a positive note, we found no signs of *Angiostrongylus* or other lung- or heart-related nematodes. The first autochthonous cases of rat heart worm *Angiostrongylus cantonensis* were recorded in *R. rattus* and *R. norvegicus* in 2021 in Valencia, Spain [68], thus calling for continued surveillance of this zoonotic parasite. *A. cantonensis* is currently spreading across the world and it has been limited to subtropical and tropical regions [69]. As the biotic and abiotic limitations of its spread are to date unknown [70], it remains an open question whether the parasite could survive in Helsinki rats.

Numerous factors influence the transmission and spread of rat-borne parasites and pathogens, all of which could explain why the prevalences in our survey appear comparably low. Indeed, the spatial heterogeneity of within-rat communities is known to be pervasive across various scales [71]. There are reasons to expect that no single explanation would cover all the different species as the studied pathogens and parasites are transmitted from individual to individual (viral pathogens, *Leptospira*, *Campylobacter, Trichinella*), via the environment (*Leptospira*, *Campylobacter*, *Nippostrongylus*, *Heterakis*, *Syphacia*) or through intermediate hosts (*Hymenolepis*, *Trichinella*). They have varying levels of host specificity and competence, with some mainly infecting brown rats, while others circulate in a wider range of local rodents and other mammals. Different transmission modes, reservoir host communities and survivability in the environment lead to differing drivers for these zoonoses.

Observed communities always result from biogeographic events where rat population connectivity and individuals' movements shape pathogen spread, and limited rat movement has oftentimes been suggested as a reason for variation in pathogen communities across the scales [72]. Spatial discontinuities and bottlenecks in rat populations can also be caused, for example, by pest management operations [73] or adverse environmental events, such as cold winters or dry summers [74]. Indeed, Helsinki is a northern city and has comparably cold winters in comparison to many European cities, which can cause rat population bottlenecks. In contrast, Helsinki has no coordinated rat control plans, and private companies work on a site-by-site basis [75]. Interestingly, the effect of pest management is poorly understood. While lethal rat control is performed to reduce pathogen and parasite

circulation, there is evidence only to suggest the contrary [76]. Anecdotally, the sites where the most rat individuals were collected in this study also had higher parasite and pathogen richness, but this needs to be studied more carefully. An assessment of rat population size, its seasonal variation and the effect of pest management operations is under way in the Helsinki Urban Rat Project.

The future trends of rat pathogen and parasite prevalences are difficult to assess. Larger-scale green areas have diminished in Helsinki [77], whereas the effect of small-scale greenery, such as the inner courtyards of buildings, on rat populations is poorly known. While current population densities are unknown, high rat population densities have historically been linked, for example, to waste dumps and landfills which have all been closed in the city of Helsinki [78]. Climate change could increase mean temperature and rainfall, both of which could likely affect rat population sizes and the transmission of parasites and pathogens.

The reliability regarding the observation that the prevalence of rat-borne pathogens is low is supported by the lack of diagnosed rat-associated human cases of these zoonoses. Indeed, we have found only two described cases in the literature of rat-borne infections in humans in Finland in recent decades, both outside of Helsinki [13]. While the lack of known cases could be due to actual low numbers of zoonotic infections, our results show that potentially zoonotic pathogens and parasites are present in urban rats in Helsinki. Thus, there is a possibility of undiagnosed rat-borne infections. For example, Seoul hantavirus infections can present in a similar manner as Puumala hantavirus infections for which the annual incidence is ca 31 cases/100,000 person years (mostly based on clinical symptoms) [79]. To assess the actual risks caused by these rat-borne pathogens and parasites, better detection of human cases is needed.

Biased sampling of rats across the city is another possible explanation for the untypical prevalences. Due to the expectation of highly heterogenous rat-associated pathogen and parasite communities, representative sampling is difficult on a city-wide scale and as rats are difficult to catch at any scale [80]. As pest management companies are contacted commonly only when rats are encountered on urban sites, we would expect that our samples are from sites with higher rat population densities than in the city overall. Thus, the rats included in this study are likely to overrepresent a situation with numerous rats or larger rat colonies in contrast to sites that have fewer rats. The samples were biased towards the winter months (November to April), as rat carcasses were better preserved in traps at this time. Our preliminary data suggest that also rat population densities might represent at their annual lowest during the winter. Nevertheless, a previous study has shown that carcass collection by pest management company broadly corresponds to random sampling [15].

Due to different methods, we expect that the detection analysis of different pathogens and parasites have, for example, varying reliabilities and rates of false negatives. For instance, helminths are very reliably detected as only carcasses with no intestinal decomposition were used. In contrast, the quantity of faecal matter varied in the intestinal samples, and this could not be standardised, likely affecting *Campylobacter* spp. detection. Also freezing the carcasses before analysis potentially reduced the number of live *Campylobacter* cells and thus culture-positive sample numbers. For viruses, we mostly used antibody identifications which are quite reliable even in older carcasses. We would expect sample quality to be more compromised when it comes to PCR or genome sequencing methods, especially in relation to RNA viruses, such as ratHEV.

Even though Helsinki may have lower overall prevalence of certain rat-borne pathogens and parasites than other previously surveyed cities in Europe, it is important not to interpret our results as an indication of the local rat-borne zoonotic risk. As mentioned, rat-borne pathogen communities vary substantially, and even in situations of true low overall prevalence across the city, local prevalence at individual sites can be very high. Similarly, the probability of rat-borne microbes being transmitted to humans also depends on several other risk factors other than rat population-level prevalences, such as exposure [81]. It should also be noted that we do not know whether these pathogens and parasites could cause infections in humans and thus assessing the risk to humans is difficult. Further work is under way to especially identify viral species and genotype *Campylobacter* spp. with whole genome sequencing.

Conclusion

Here, we present a survey of potentially zoonotic parasites and pathogens in urban rats in Helsinki, Finland, during 2018 to 2023. While several pathogens and parasites encountered in other cities in Europe were found, we also noted an apparent absence of rat lung and heartworms and *Trichinella* nematodes. In general, parasite and pathogen prevalences appeared low compared with other European cities. Our survey suggests that low or non-existent diagnosis levels of rat-borne pathogen and parasite infections in humans may partly be due to a limited transmission in rats.

Ethical statement

We did not handle live rats. Rats were killed following national best practices in pest management in line with the Finnish Animal Welfare Act and based on the principle of integrated pest management. Rats are an unprotected species according to the Finnish Hunting Act, thus requiring no specific permits. Rodenticides for rat control are only available for professional, registered pest management personnel.

Use of artificial intelligence tools

None declared.

Data availability

The raw data can be found at Figshare with doi: 10.6084/m9.figshare.24866958

Conflict of interest

None declared.

Authors' contributions

Concept and design: TA, TS and OH. Sample collection and processing, and morphological analyses and interpretation of data: TA and ReK. Molecular analyses and interpretation of data: HA, NS, PH, AO, RaK, OH and TS. Supervision: TA, AO, RaK and TS. Drafting of the manuscript: TA. Critical revision of the manuscript: TA, HA, NS, ReK, PH, AO, OH, RaK and TS.

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