ORIGINAL ARTICLE

Detection and distribution of zoonotic pathogens in wild Norway rats (*Rattus norvegicus*) from Tehran, Iran

T. Azimi[1], L. Azimi[2], F. Fallah[3], M. R. Pourmand[1], H. Peeri Dogaheh[4] and S. Rafiei Tabatabaei[2]

1) *Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences,* 2) *Pediatric Infection Research Center, Research Institute for Children's Health,* 3) *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran and* 4) *Department of Microbiology, School of Medicine, Ardabil University of Medical Science, Ardabil, Iran*

Abstract

This is the first study on the prevalence of vector-borne zoonotic pathogens found in *Rattus norvegicus* (*R. norvegicus*) in urban areas of Tehran, Iran. Serological tests were used to detect IgG antibodies against *Coxiella burnetii* (*C. burnetii*) and *Rickettsia* spp. using a commercial qualitative rat ELISA kit. The frequency of *Streptobacillus moniliformis* (*S. moniliformis*) and *Bartonella* spp. was determined using a conventional PCR method. Molecular detection and characterization of *Leptospira* spp. were conducted using TaqMan real-time PCR based on *lipL32* gene and *SecY* typing methods. A total of 100 *R. norvegicus* rats were collected from five regions in Tehran, Iran, and investigated to determine their zoonotic pathogens. *S. moniliformis* and *Bartonella* spp. were detected in 23 of 100 (23%) and 17 of 100 (17%) *R. norvegicus* populations, respectively. The highest prevalence of *S. moniliformis* and *Bartonella* spp. with similar frequency rates (n = 6/20; 30%) was seen among the *R. norvegicus* rats captured from the northern and southern parts of Tehran, respectively. Seroreactivity against *C. burnetii* and *Rickettsia* spp. was detected in 4% and 1% of *R. norvegicus*, respectively. *C. burnetii.* was identified only in one rat captured from the eastern part of Tehran. Results showed that *Leptospira* spp. was detected only in two rats, collected from the southern part (n = 2/20; 10%) of Tehran. The secY typing method identified two different *Leptospira* species including *L. interrogans* and *L. kirschneri.* The results showed that urban rats might play an important role in transmission of zoonotic pathogens to humans.

Keywords: Iran, *Leptospira* spp., *Rattus norvegicus*, urban environments, vector-borne pathogens, zoonotic disease
Original Submission: 26 February 2021; **Revised Submission:** 12 May 2021; **Accepted:** 7 June 2021
Editor: Michel Drancourt
Article published online: 24 June 2021

Corresponding author: M.R. Pourmand, Department of Pathobiology, School of Public Health, and Biotechnology Research Center, Tehran University of Medical Sciences, Poursina St., Tehran, Iran
E-mail: mpourmand@tums.ac.ir

Introduction

Zoonotic pathogens cause approximately 60−70% of all new and emerging human infections [1]. *Rattus norvegicus* (*R. norvegicus*) population is scattered all over urban areas and considered a hygienic threat to public health worldwide [2]. In urban environments, *R. norvegicus* has a close contact with the human population [3]. Rodent populations are highly concentrated in urban environments, have mobile nature, and are resistant to various pathogens. These features facilitate the transmission and spread of various zoonotic pathogens to humans [4,5]. *R. norvegicus* plays a major role in transmission of several zoonotic pathogens and is known to be reservoirs and vectors of a variety of emerging zoonotic pathogens including bacteria, viruses, and protozoa [6]. These rodents carry these zoonotic pathogens without exhibiting overt clinical symptoms of the illness [7]. Rattus population contaminates water and food sources and accounts for numerous human morbidity and mortality rate. In general, transmission risk of rodent-borne pathogens increases owing to many factors including (1) poor hygiene conditions, (2) the increasing

https://doi.org/10.1016/j.nmni.2021.100908

frequency of contact between human and animal reservoirs, (3) inhalation of aerosols and consumption of contaminated water and food with feces and urine from infected rodents, and (4) direct contact by bites of arthropod vectors such as rat fleas, lice, mites, and ticks [1,8]. Globally, many zoonotic pathogens including *Leptospira* spp., *Streptobacillus moniliformis* (*S. moniliformis*), *Coxiella burnetii* (*C. burnetii*), *Rickettsia* spp., and *Bartonella* spp. are thought to be endemic in rodent populations [9,10]. Although the surveillance of rodent population in urban environments is critical, a comprehensive study of zoonotic pathogens including *Leptospira* spp., *S. moniliformis*, *C. burnetii*, *Rickettsia* spp., and *Bartonella* spp. carried by *R. norvegicus* in Tehran has not been carried out. Therefore, to identify the presence and frequency of these zoonotic pathogens carried by *R. norvegicus* in urban areas, we investigated the main pathogens carried by *R. norvegicus* in Tehran, Iran, from 2018 to 2019. This is the first study on the prevalence of vector-borne zoonotic pathogens and *S. moniliformis* related to *R. norvegicus* in urban areas of Tehran, Iran.

Materials and methods

Study site and rat trapping

From May 2018 to December 2019, a total of 100 *R. norvegicus* rats were collected from five regions (north, south, west, east, and centre) of Tehran Province, Iran (Fig. 1). All rats were captured using Sherman and Tomahawk professional live traps (Tomahawk Live Trap, Hazelhurst, WI) which had been baited with alluring baits such as sunflower seeds and peanut/sesame butter during the peak of their activities in four seasons [3]. In general, 15 traps were distributed in each of the five selected regions after sundown to cover the areas. All collected rats were transferred to a special laboratory in animal houses within 48 h of their capture and were maintained for one week. In the next step, all rats were sacrificed through intramuscular injection of xylazine and ketamine (0.1 mg/kg), followed by bilateral thoracotomy. The subsequent serological and molecular tests were carried out at the Department of Pathobiology, Division of Medical Microbiology, School of Public Health, Tehran University of Medical Sciences.

Sample collection and DNA extraction

Blood samples were collected from each captured rat using a 5-ml syringe and cardiac puncture. All blood samples were centrifuged and, then, the serum was kept at −20 °C before serological analysis. Moreover, fresh fecal samples (~500 mg) were collected from each rat and washed with distilled water by centrifugation for 10 min at 13,000 rpm at 25 °C. Genomic DNA was extracted from fecal samples (approximately 250 mg) using the DNA extraction kit (AllPrep DNA minikit [QIAGEN, Hilden,

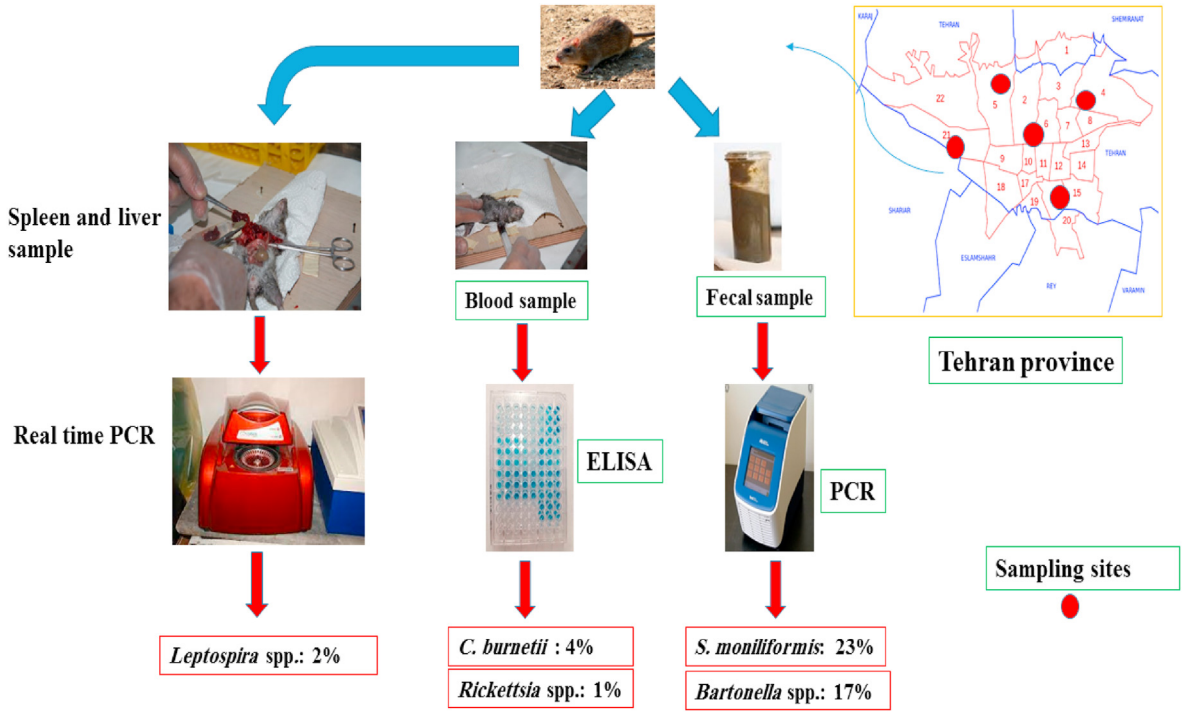

FIG. 1. A schematic map of the method was carried out and the prevalence of each surveyed zoonotic pathogens among the *Rattus* population in Tehran, Iran.

Germany]) according to the manufacturer's protocol. The extracted DNA samples were eluted in 50 µl of elution buffer stored at −20 °C before PCR analysis. Finally, the rats were dissected to reach out to their spleen and liver tissues.

Conventional PCR

Molecular identification of *S. moniliformis* and *Bartonella* spp. was conducted based on fecal DNA by PCR assay. In brief, *S. moniliformis* DNA was amplified and detected using the specific primer targeting 269-bp regions of the 16S ribosomal RNA gene. The sequence of primer pairs was as follows: 16S rRNA-F: 5′-CATACTCGGAATAAGATGG -3′ and 16S rRNA-R: 5′-GCTTAGCTCCTCTTTGTAC -3′. Moreover, the whole extracted DNA was tested in the presence of *Bartonella* spp. by amplification of a 379-bp nucleotide fragment of citrate synthase (*gltA*) gene. The sequence of primer pairs was as follows: *gltA*-F: 5′-GGGGACCAGCTCATGGTGG -3′ and *gltA*-R: 5′- AATGCAAAAAGAACAGTAAACA -3′. PCR conditions were set based on a previously published study by Firth *et al.* [6]. In general, PCR amplification was performed with the final volume of 25 µl including 12.5 µl of 2 × Master Mix (Amplicon, Pishgam Biotech Company, Tehran, Iran; Cat. no. PR901638), 1 µl of 10 pmol of each forward and reverse primer, 2 µl of template DNA, and 8.5 µl of sterile distilled water. Each PCR reaction comprised 1 cycle at 95 °C for 5 min (initial denaturation), followed by 32 cycles of denaturation at 95 °C for 45 s, annealing at 54 °C to 56 °C, according to the primers for each gene, for 45 s, extension at 72 °C for 30 s, and final extension at 72 °C for 7 min. Finally, all PCR products were screened on 1.5% agarose gels after staining with DNA safe stain (SinaClon Co., Iran) and visualized by UV light. The results of electrophoresis were confirmed by sequencing using ABI 3730X capillary sequencer (Pishgam; Macrogen, Seoul, Korea).

TaqMan real-time PCR and SecY typing

DNA extraction from the spleen and liver tissue was performed using a DNA extraction kit (SinaPure DNA, Kat. No, EX6011) in line with the manufacturer's instruction, and all the extracted DNA samples were standardized at 10–20 ng/µl. For identifying *Leptospira* spp., we used the probe-based real-time PCR using a specific primer and a probe targeting the *lipL32* gene. Genomic DNA was amplified using the following primer and probe sequence: *lipL32*-F: 5′-AAGCATTACCGCTTGTGGTG-3′, *lipL32*-R: 5′-GAACTCCCATTTCAGCGATT-3′, probe: 5′-FAM-AAAGCCAGGACAAGCGC-BHQ1-3′. The secY typing was performed using conventional PCR with specific primers including *secY*-F: 5′-GCGATTCAGTTTAATCCTGC-3′ and *secY*-R: 5′-GAGTTAGAGCTCAAATCTAAG-3′. The TaqMan real-time PCR and secY typing method were applied in line with the study by Azhari *et al.* [11]. Distilled water and *L. interrogans* were used as negative and positive controls, respectively. The PCR products were sequenced commercially using ABI 3730X capillary sequencer (Pishgam, Macrogen, Seoul, Korea).

Enzyme-linked immunosorbent assay

All serum samples were tested for specific IgG antibodies against *C. burnetii* and *Rickettsia* spp. using a commercial qualitative rat ELISA kit (Shanghai Crystal Day Biotech Co., Ltd). The ELISA assay was performed following the manufacturers' protocols. The optical density (OD value) of each well was read spectrophotometrically at 450 nm (OD450) within 15 min after adding the stop solution (sulphuric acid) using a microplate reader (model 680; Bio-Rad Laboratories, Hercules, CA).

Statistical analysis

All data were included in an SPSS file, version 23.0 (SPSS Inc., Chicago, IL, USA), and the frequency of each surveyed zoonotic pathogen carried by *R. norvegicus* population was analysed using descriptive statistic tests.

Results

Prevalence of *S. moniliformis* and *Bartonella* spp. in rat feces

From May 2018 to December 2019, a total of 100 live *R. norvegicus* rats from five different regions (north, south, west, east, and centre) in Tehran Province were trapped and screened to determine their zoonotic pathogens. The frequency of the surveyed zoonotic pathogens between male and female *R. norvegicus* is shown in Table 1. Among the captured *R. norvegicus*, 22% (n = 22/100) and 78% (n = 78/100) of them were female and male, respectively. Their distribution among *R. norvegicus* in five different regions of Tehran is shown in Table 2. In general, 23% (n = 23/100) of fecal samples were positive for *S. moniliformis* spp. in five regions of Tehran. Among the *R. norvegicus* captured in Tehran, *S. moniliformis* had the highest and lowest rates of prevalence in the north (30%, n = 6/20) and west (10%, n = 2/20) regions, respectively. The prevalence of *S. moniliformis* was higher in female rats (27.2%; n = 6/

TABLE 1. The frequency of surveyed zoonotic pathogens between male and female *R. norvegicus*

Pathogens	Total positive	Positive cases among genders	
		Male	Female
S. moniliformis	23% (23/100)	21.8% (17/78)	27.2% (6/22)
Bartonella spp.	17% (17/100)	15.3% (12/78)	22.7% (5/22)
C. burnetii	4% (4/100)	3.8% (3/78)	4.5% (1/22)
Rickettsia spp.	1% (1/100)	1.3% (1/78)	0% (0/22)
Leptospira spp.	2% (2/100)	0% (0/78)	9% (2/22)

TABLE 2. The prevalence of surveyed zoonotic pathogens among *R. norvegicus* in five districts of Tehran, Iran

Zoonotic parasites	Sample type	Methods	No. of positive samples/no. tested in five districts of Tehran					No. of positive samples/no. tested
			North	South	West	East	Centre	Total
S. moniliformis	Fecal	PCR	6/20 (30%)	5/20 (25%)	2/20 (10%)	5/20 (25%)	5/20 (25%)	23/100 (23%)
Bartonella spp.	Fecal	PCR	2/20 (10%)	6/20 (30%)	3/20 (15%)	2/20 (10%)	4/20 (20%)	17/100 (17%)
C. burnetii	Serum	ELISA	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/20 (5%)	0/20 (0%)	1/100 (1%)
Rickettsia spp.	Serum	ELISA	2/20 (10%)	2/20 (10%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	4/100 (4%)
Leptospira spp.	Spleen and liver	TaqMan real-time PCR	0/20 (0%)	2/20 (10%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	2/100 (2%)

22) than that in male rats (21.8%; n = 17/78). Molecular analysis of *Bartonella* spp. in fecal samples helped detect 17 of 100 (17%) samples. The prevalence of *Bartonella* spp. in five regions of Tehran was as follows: northern (10%, n = 2/20), southern (30%, n = 6/20), eastern (10%, n = 2/20), western (15%, n = 3/20), and central (20%, n = 4/20). *Bartonella* spp. had the highest frequency in the southern part of Tehran. The prevalence of *Bartonella* spp. was higher among female rats (22.7%; n = 5/22) than that among male rats (15.3%; n = 12/78).

Molecular prevalence of *Leptospira* based on *lipL32* and secY genes

Among the 100 *R. norvegicus* rats captured, their spleen and liver tissues were isolated and their genomic DNA was extracted. In total, the extracted DNA demonstrated that only 2% (n = 2/100) was positive for the *lipL32* gene. *Leptospira* spp. was detected only in two rats, collected from the southern part (n = 2/20; 10%) of Tehran. The secY typing method identified two different *Leptospira* species including *L. interrogans* and *L. kirschneri*.

Detection of *Rickettsia* spp. and *C. burnetii* in serum samples

To detect *Rickettsia* spp. and *C. burnetii* in the serum samples of the trapped rats, the presence of specific IgG antibodies was surveyed by an ELISA kit. In general, based on the results of the ELISA assay of the 100 *R. norvegicus* rats captured in Tehran, 4% (n = 4/100) of them were positive for *Rickettsia* spp., originating from northern (10%, n = 2/20) and southern (10%; n = 2/20) parts of Tehran, Iran. *Rickettsia* spp. was not isolated from *R. norvegicus* captured from the central, western, and eastern regions of Tehran. The prevalence of *Rickettsia* spp. was higher in female rats (4.5%; n = 1/22) than that in male rats (3.8%; n = 3/78). On the other hand, the results of the serological assay showed that, of the 100 *R. norvegicus* rats trapped in Tehran, 1% (n = 1/100) of them were positive for *C. burnetii*. *C. burnetii* was identified only in one rat (male rat) captured from the eastern part of Tehran. However, this vector-borne pathogen was not detected in the northern, southern, western, and central parts of Tehran.

Coinfection between the surveyed pathogens

In the present study, coinfection was seen between some surveyed zoonotic pathogens. Our results showed that 7 animals were infected simultaneously with *S. moniliformis* and *Bartonella* spp. Moreover, one animal simultaneously was infected with *Bartonella* spp. and *Leptospira* spp. We could not find the coinfection between *Bartonella* spp. and *Rickettsia* spp., between *Bartonella* spp. and *C. burnetii*, or between *S. moniliformis* and *C. burnetii*. However, coinfection between *S. moniliformis and Rickettsia* spp. was detected in one animal.

Discussion

Given that a high percentage of emerging and reemerging vector-borne diseases originates in wild animals, conducting a survey of reservoirs and frequency of zoonotic pathogens has public health importance [4]. Moreover, gaining a better knowledge of pathogenic agent ecology and its epidemiology is critical to the implementation of control measures. This study evaluates the presence and frequency of five zoonotic pathogens in *R. norvegicus* originating from five different regions of Tehran, Iran. To our knowledge, the present study is the first report of *S. moniliformis*, *C. burnetii, Rickettsia* spp., and *Bartonella* spp. in urban rats in Tehran, Iran. The results of our study revealed that *S. moniliformis* was the main zoonotic pathogen that had the highest frequency (23%; n = 23/100) among other surveyed pathogens isolated from the *R. norvegicus* population of Tehran. Several studies have assessed the prevalence of *S. moniliformis* in the urban rat population worldwide. In 2014, Firth *et al*. [6] investigated the frequency of zoonotic pathogens carried by commensal *R. norvegicus* in New York City. Results of their study revealed that 17% (n = 23/133) of Norway rats were positive for *S. moniliformis*. On the other hand, in 2008, Kimura *et al*. [12] assessed the prevalence of *Streptobacillus* spp. in feral rats by using a PCR assay. Results of their research illustrated that the frequency of *S. moniliformis* between *R. norvegicus* and *R. rattus* was 92% and 58%, respectively. They showed that an extremely high proportion of urban rats

harboured *S. moniliformis* [12]. *S. moniliformis* is a causative agent of two main zoonotic diseases including rat-bite fever and Haverhill fever [13]. *S. moniliformis* is carried asymptomatically by 50–100% of wild rats, and these rodents shed the *S. moniliformis* with saliva and urine in urban environments [14]. The mortality rate from rat-bite fever is reported to vary from 13% among untreated cases to 53% in patients with endocarditis [13]. *Bartonella* spp. carried by the Rattus population cause several illnesses in humans, but little information is available about their distribution in urban areas. The finding of our research revealed that *Bartonella* spp. had the highest prevalence (30%; n = 6/20) in the *R. norvegicus* population trapped from the southern part of Tehran. The total prevalence of *Bartonella* spp. was 17% (n = 17/100). Our results are comparable with those of Costa *et al.* [9] from Brazil, Himsworth *et al.* [10] from Canada, Rothenburger *et al.* [15] from Canada, Firth *et al.* [6] from the USA, Klangthong *et al.* [16] from Thailand, Tay *et al.* [17] from Malaysia, Kamani *et al.* [18] from Nigeria, and Pangjai *et al.* [19] from Thailand. These studies found that the prevalence of *Bartonella* spp. in the urban rats was 19%, 25%, 25.7%, 25%, 17%, 13.7%, 26%, and 16.9%, respectively. However, Krügel *et al.* [20] from Germany, Halliday *et al.* [21] from the United Kingdom, Müller *et al.* [22] from West Indies, and Su *et al.* [23] from China revealed that the frequency of *Bartonella* spp. in urban rat population was 37.4%, 13–60%, 36.3%, and 9.6%, respectively. *Bartonella* spp. is of zoonotic potential and can cause various illnesses including cat-scratch disease, Oroya fever, and trench fever. Among *Bartonella* spp., several species such as *B. quintana*, *B. bacilliformis*, and *B. henselae* are associated with emerging and reemerging human illnesses [24]. Humans and several different animals such as rodents, felids, lagomorphs, and canids are considered as natural hosts to *Bartonella* spp. [25]. The transmission of *Bartonella* spp. between animal and human populations was mediated by arthropod vectors [26]. The total frequency of *Rickettsia* spp. among *R. norvegicus* population was 4% (n = 4/100). The results of our study are in agreement with those of two different studies conducted by Kim *et al.* [27,28] from Korea. In two different studies, Kim *et al.* [27,28] revealed that the frequency of *Rickettsia* spp. in rodent population was 3.2% and 3.8%, respectively. However, our obtained frequency was higher than what has been found in several other studies around the world. Many studies conducted in different countries revealed that the prevalence rate of *Rickettsia* spp. in the rodent population was 0.36% [10] and 0.8% [29], respectively. On the other hand, our obtained frequencies were lower than what have been found in several other studies conducted by Chareonviriyaphap *et al.* [30] from Thailand, Bennett *et al.* [31] from the USA, Siritantikorn *et al.* [32] from Thailand, and Ibrahim *et al.*[33] from Indonesia. They demonstrated that the prevalence of *Rickettsia* spp. in the rodent population was 23.7%, 23%, 25%, and 38%, respectively. *Rickettsia* spp. is an obligate intracellular bacterium that is distributed among different arthropod vectors such as fleas, ticks, mites, and lice [34]. This bacterium is a causative agent of rickettsioses, an emerging arthropod-borne zoonotic disease that has worldwide distribution [35]. This zoonotic disease is endemic in tropical regions, especially in Southeast Asia [36]. In general, four different groups of *Rickettsia* spp. are well known that have a major role in human and animal infection. These four groups are as follows: (1) the epidemic typhus group rickettsia transmitted by fleas, (2) the spotted fever group rickettsia transmitted by ticks, (3) the *R. canadensis* group, and (4) the *R. bellii* group [37]. Rodents represent a natural reservoir of tick-borne or flea-borne rickettsiae. Transmission of *Rickettsia* among rodents or from rodent population to humans was mediated by an ectoparasite vector such as rat flea [34].

The frequency of *C. burnetii* among the *R. norvegicus* population was 1%. This result is in agreement with that of published studies from different countries. The prevalence of *C. burnetii* among rodents in several studies performed by Runge *et al.* [38] from Germany, Kamani *et al.* [7] from Nigeria, and Rozental *et al.* [39] from Brazil was 1.3%, 2.1%, and 4.6%, respectively. However, our finding is in contrast with those of previous studies from Zambia [40], Spain (three studies) [41–43], the United Kingdom [44], and the Czech Republic [45], which reported that the prevalence of *C. burnetii* among rodents was 45%, 12.4%, 9.3%, 8%, 15.6–19.1%, and 12%, respectively. *C. burnetii* is an obligate intracellular bacterium that usually affects a wide range of hosts including ruminants, marine mammals, reptiles, ticks, and birds [46]. However, domestic mammals are the main reservoir of *C. burnetii*, which is a causative agent of Q fever disease with global distribution. However, this disease was not reported in New Zealand and Antarctica [47,48]. Inhalation of aerosolized bacteria after environmental contamination, delivery, or abortion, as well as direct contact with infected animals, mainly mediates the transmission of *C. burnetii* to humans [49]. Moreover, *C. burnetii* was isolated from more than 14 soft tick species and 40 hard tick species; therefore, arthropods could transmit this bacterium to humans and animals [48].

In conclusion, the results showed that urban rats were the main reservoirs that played a significant role in transmission of vector-borne zoonotic pathogens and *S. moniliformis* to humans. Therefore, taking several effective measures such as monitoring of pathogens in urban environments, regular disinfection of urban environments, development of suitable surveillance plans, implementation of effective rat control programs, and inter-

vention strategies to prevent the spillover and transmission of zoonotic agents from rat population to humans is critical in urban environments. Moreover, these findings highlight the urgent need for further studies on other pathogens in urban rats and other domestic and wild animals in Tehran, Iran.

Ethics approval

The present study was approved by the Ethics Committee of School of Public Health and Allied Medical Sciences, Tehran University of Medical Sciences, with reference number IR.TUMS.SPH.REC.1398.035.

Author contributions

Taher Azimi, Mohammad Reza Pourmand: Conceptualization; Data curation; Formal analysis; and Writing – original draft.

Taher Azimi, Fatemeh Fallah, Leila Azimi, and Mohammad Reza Pourmand: Conceptualization; Methodology; Project administration; and Writing – original draft.

Sedigheh Rafiei Tabatabaei, Mohammad Reza Pourmand, and Taher Azimi: Writing – original draft; and Writing – review & editing.

Taher Azimi and Mohammad Reza Pourmand: Language editing.

Transparency declaration

The authors declare that they have no competing interests. This research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sectors.

Acknowledgements

This article was extracted from the Ph.D. thesis written by Mr. Taher Azimi in the Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran (Registration No. 240/316). This study was supported by the research committee of Tehran University of Medical Sciences, Tehran, Iran (Grant No. 42072).

References

[1] Azimi T, Azimi L, Fallah F, Pourmand MR, Ostadtaghizadeh A, Abai MR, et al. Detection and characterization of *Enterobacteriaceae* family members carried by commensal *Rattus norvegicus* from Tehran, Iran. Arch Microbiol 2020;1:1–14.

[2] Desvars-Larrive A, Pascal M, Gasqui P, Cosson JF, Benoit E, Lattard V, et al. Population genetics, community of parasites, and resistance to rodenticides in an urban brown rat (*Rattus norvegicus*) population. PloS One 2017;12(9):e0184015.

[3] Azimi T, Pourmand MR, Fallah F, Karimi A, Mansour-Ghanaie R, Hoseini-Alfatemi SM, et al. Serosurvey and molecular detection of the main zoonotic parasites carried by commensal *Rattus norvegicus* population in Tehran, Iran. Trop Med Health 2020;48(1):1–7.

[4] Zhao W, Zhou H, Huang Y, Xu L, Rao L, Wang S, et al. *Cryptosporidium* spp. in wild rats (*Rattus* spp.) from the Hainan Province, China: molecular detection, species/genotype identification and implications for public health. Int J Parasitol– Parasites Wildlife 2019;9:317–21.

[5] Azimi T, Fallah F, Pourmand MR, Karimi A, Armin S, Rahbar M, et al. Is the *Rattus norvegicus* population playing a role in the transmission of zoonotic diseases to children? A pilot study in Tehran, Iran. Arch Pediatr Infect Dis 2021 (In Press). e113203.

[6] Firth C, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, et al. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. MBio 2014;5(5). e01933-14.

[7] Kamani J, Baneth G, Gutiérrez R, Nachum-Biala Y, Mumcuoglu KY, Harrus S. *Coxiella burnetii* and *Rickettsia conorii*: two zoonotic pathogens in peridomestic rodents and their ectoparasites in Nigeria. Ticks Tick-Borne Dis 2018;9(1):86–92.

[8] Bird BH, Mazet JA. Detection of emerging zoonotic pathogens: an integrated one health approach. Ann Rev Anim Biosci 2018;2:121–39.

[9] Costa F, Porter FH, Rodrigues G, Farias H, de Faria MT, Wunder EA, et al. Infections by *Leptospira interrogans*, Seoul virus, and *Bartonella* spp. among Norway rats (*Rattus norvegicus*) from the urban slum environment in Brazil. Vector-Borne Zoonotic Dis 2014;14(1):33–40.

[10] Himsworth CG, Bai Y, Kosoy MY, Wood H, DiBernardo A, Lindsay R, et al. An investigation of *Bartonella* spp., *Rickettsia typhi*, and Seoul hantavirus in rats (*Rattus* spp.) from an inner-city neighborhood of Vancouver, Canada: is pathogen presence a reflection of global and local rat population structure? Vector-Borne Zoonotic Dis 2015;15(1):21–6.

[11] Azhari NN, Ramli SN, Joseph N, Philip N, Mustapha NF, Ishak SN, et al. Molecular characterization of pathogenic *Leptospira* sp. in small mammals captured from the human leptospirosis suspected areas of Selangor state, Malaysia. Acta Tropica 2018;188:68–77.

[12] Kimura M, Tanikawa T, Suzuki M, Koizumi N, Kamiyama T, Imaoka K, et al. Detection of *Streptobacillus* spp. in feral rats by specific polymerase chain reaction. Microbiol Immunol 2008;52(1):9–15.

[13] Dendle C, Woolley IJ, Korman TM. Rat-bite fever septic arthritis: illustrative case and literature review. Eur J Clin Microbiol Infect Dis 2006;25(12):791–7.

[14] Eisenberg T, Ewers C, Rau J, Akimkin V, Nicklas W. Approved and novel strategies in diagnostics of rat bite fever and other *Streptobacillus* infections in humans and animals. Virulence 2016;7(6):630–48.

[15] Rothenburger JL, Himsworth CG, Nemeth NM, Pearl DL, Jardine CM. Beyond abundance: how microenvironmental features and weather influence *Bartonella tribocorum* infection in wild Norway rats (*Rattus norvegicus*). Zoonoses Publ Health 2018;65(3):339–51.

[16] Klangthong K, Promsthaporn S, Leepitakrat S, Schuster AL, McCardle PW, Kosoy M, et al. The distribution and diversity of *Bartonella* species in rodents and their ectoparasites across Thailand. PloS One 2015;10(10):e0140856.

[17] Tay ST, Mokhtar AS, Zain SN, Low KC. Isolation and molecular identification of *bartonellae* from wild rats (*Rattus* Species) in Malaysia. Am J Trop Med Hygiene 2014;90(6):1039–42.

[18] Kamani J, Morick D, Mumcuoglu KY, Harrus S. Prevalence and diversity of Bartonella species in commensal rodents and ectoparasites from Nigeria, West Africa. PLoS Negl Trop Dis 2013;7(5):e2246.

[19] Pangjai D, Maruyama S, Boonmar S, Kabeya H, Sato S, Nimsuphan B, et al. Prevalence of zoonotic *Bartonella* species among rodents and shrews in Thailand. Comp Immunol Microbiol Infect Dis 2014;37(2):109–14.
[20] Krügel M, Pfeffer M, Król N, Imholt C, Baert K, Ulrich RG, et al. Rats as potential reservoirs for neglected zoonotic *Bartonella* species in Flanders, Belgium. Parasites Vectors 2020;13:1–12.
[21] Halliday JE, Knobel DL, Agwanda B, Bai Y, Breiman RF, Cleaveland S, et al. Prevalence and diversity of small mammal-associated *Bartonella* species in rural and urban Kenya. PLoS Negl Trop Dis 2015;9(3):e0003608.
[22] Müller A, Gutiérrez R, Seguel M, Monti G, Otth C, Bittencourt P, et al. Molecular survey of *Bartonella* spp. in rodents and fleas from Chile. Acta Tropica 2020;212:105672.
[23] Su Q, Chen Y, Wang B, Huang C, Han S, Yuan G, et al. Epidemiology and genetic diversity of zoonotic pathogens in urban rats (*Rattus* spp.) from a subtropical city, Guangzhou, southern China. Zoonoses Publ Health 2020;67(5):534–45.
[24] Cheslock MA, Embers ME. Human bartonellosis: an underappreciated public health problem? Trop Med Infect Dis 2019;4(2):69.
[25] Kosoy M, Goodrich I. Comparative ecology of *Bartonella* and *Brucella* infections in wild carnivores. Front Veter Sci 2019;5:322.
[26] Álvarez-Fernández A, Breitschwerdt EB, Solano-Gallego L. *Bartonella* infections in cats and dogs including zoonotic aspects. Parasites Vectors 2018;11(1):1–21.
[27] Kim HC, Yang YC, Chong ST, Ko SJ, Lee SE, Klein TA, et al. Detection of *Rickettsia typhi* and seasonal prevalence of fleas collected from small mammals in the Republic of Korea. J Wildlife Dis 2010;46(1):165–72.
[28] Kim HC, Klein TA, Chong ST, Collier BW, Yi SC, Song KJ, et al. Seroepidemiological survey of rodents collected at a US military installation, Yongsan Garrison, Seoul, Republic of Korea. Military Med 2007;172(7):759–64.
[29] Heuser E, Fischer S, Ryll R, Mayer-Scholl A, Hoffmann D, Spahr C, et al. Survey for zoonotic pathogens in Norway rat populations from Europe. Pest Manag Sci 2017;73(2):341–8.
[30] Chareonviriyaphap T, Leepitakrat W, Lerdthusnee K, Chao CC, Ching WM. Dual exposure of *Rickettsia typhi* and *Orientia tsutsugamushi* in the field-collected *Rattus* rodents from Thailand. J Vector Ecol 2014;39(1):182–9.
[31] Bennett SG, Comer JA, Smith HM, Webb JP, et al. Serologic evidence of a *Rickettsia akari*-like infection among wild-caught rodents in Orange County and humans in Los Angeles County, California. J Vector Ecol 2007;32(2):198–201.
[32] Siritantikorn S, Sangkasuwan V, Eamsila C, Singchai C, Kantakamalakul W, Puthavathana P. Seroprevalence of rickettsial infection in commensal rodents and shrews trapped in the Bangkok Metropolitan Area. J Med Assoc Thailand= Chotmaihet Thangphaet 2003;86(6):516–21.
[33] Ibrahim IN, Okabayashi T, Lestari EW, Yanase T, Muramatsu Y, Ueno H, et al. Serosurvey of wild rodents for rickettsioses (spotted fever, murine typhus and Q fever) in Java Island, Indonesia. Eur J Epidemiol 1999;15(1):89–93.
[34] Dehhaghi M, Kazemi Shariat Panahi H, Holmes EC, Hudson BJ, Schloeffel R, Guillemin GJ. Human tick-borne diseases in Australia. Front Cell Infect Microbiol 2019;9:3.
[35] Botelho-Nevers E, Raoult D. Host, pathogen and treatment-related prognostic factors in rickettsioses. Eur J Clin Microbiol Infect Dis 2011;30(10):1139–50.
[36] Blanton LS. The rickettsioses: a practical update. Infect Dis Clin 2019;33(1):213–29.
[37] Bermúdez CSE, Troyo A. A review of the genus *Rickettsia* in Central America. Res Rep Trop Med 2018;9:103.
[38] Runge M, von Keyserlingk M, Braune S, Becker D, Plenge-Bönig A, Freise JF, et al. Distribution of rodenticide resistance and zoonotic pathogens in Norway rats in Lower Saxony and Hamburg, Germany. Pest Manag Sci 2013;69(3):403–8.
[39] Rozental T, Ferreira MS, Guterres A, Mares-Guia MA, Teixeira BR, Gonçalves J, et al. Zoonotic pathogens in atlantic forest wild rodents in Brazil: *Bartonella* and *Coxiella* infections. Acta Tropica 2017;168:64–73.
[40] Chitanga S, Simulundu E, Simuunza MC, Changula K, Qiu Y, Kajihara M, et al. First molecular detection and genetic characterization of *Coxiella burnetii* in Zambian dogs and rodents. Parasites Vectors 2018;11(1):1–4.
[41] Foronda P, Plata-Luis J, del Castillo-Figueruelo B, Fernández-Álvarez Á, Martín-Alonso A, Valladares B, et al. Serological survey of antibodies to *Toxoplasma gondii* and *Coxiella burnetii* in rodents in north-western African islands (Canary Islands and Cape Verde). Onderstepoort J Veter Res 2015;82(1):1–4.
[42] Izquierdo-Rodríguez E, Fernández-Álvarez Á, Martín-Carrilo N, Feliu C, Marchand B, Quilichini Y, Foronda P, et al. Rodents as reservoirs of the zoonotic pathogens *Coxiella burnetii* and *Toxoplasma gondii* in Corsica (France). Vector-Borne Zoonotic Diseases 2019;19(12):879–83.
[43] Bolaños-Rivero M, Carranza-Rodríguez C, Rodríguez NF, Gutiérrez C, Pérez-Arellano JL, et al. Detection of *Coxiella burnetii* DNA in peridomestic and wild animals and ticks in an endemic region (Canary Islands, Spain). Vector-Borne Zoonotic Diseases 2017;17(9):630–4.
[44] Meredith AL, Cleaveland SC, Denwood MJ, Brown JK, Shaw DJ, et al. *Coxiella burnetii* (Q-Fever) seroprevalence in prey and predators in the United Kingdom: evaluation of infection in wild rodents, foxes and domestic cats using a modified ELISA. Transboundary Emerg Dis 2015;62(6):639–49.
[45] Bártová E, Kučerová HL, Žákovská A, Budíková M, Nejezchlebová H, et al. *Coxiella burnetii* and *Francisella tularensis* in wild small mammals from the Czech Republic. Ticks Tick-Borne Dis 2020;11(2):101350.
[46] Salifu SP, Bukari AR, Frangoulidis D, Wheelhouse N. Current perspectives on the transmission of Q fever: highlighting the need for a systematic molecular approach for a neglected disease in Africa. Acta Tropica 2019;193:99–105.
[47] Abnave P, Muracciole X, Ghigo E. *Coxiella burnetii* lipopolysaccharide: what do we know? Int J Mol Sci 2017;18(12):2509.
[48] Eldin C, Melenotte C, Mediannikov O, Ghigo E, Million M, Edouard S, et al. From Q fever to *Coxiella burnetii* infection: a paradigm change. Clinical Microbiology Reviews 2017;30(1):115–90.
[49] Boarbi S, Fretin D, Mori M. *Coxiella burnetii*, agent de la fièvre Q. Canadian J Microbiol 2016;62(2):102–22.