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

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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. were 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.

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Keywords: Iran, Leptospira spp., Rattus norvegicus, urban environments, vector-borne pathogens, zoonotic disease

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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
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, Germany]).

![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.](https://creativecommons.org/licenses/by-nc-nd/4.0/).
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’- GCCTAGCTCCTTTGTAC -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’- GGGGACGCTCATGGTGG -3’ and gltA-R: 5’- AATGCAAAAAAGACATGAAAC -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 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’-AAGCATTACCGCTTGGTGTTG-3’, lipL32-R: 5’-GAATCTCCTTCCAGCATT-3’, probe: 5’-FAM- AAAGCCAGGAAACGGC-BHQ1-3’. The secY typing was performed using conventional PCR with specific primers including secY-F: 5’-GGGATTACAGTTATACCTGC-3’ and secY-R: 5’- GAGTTAGAGCTCAAATCAG-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/
Tehran was as follows: northern (10%, n = 2/20), southern (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.

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

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).

**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, Kamanzi et al. [18] from Nigeria, and Pangjai et al. [19] from Thailand. These studies found that the prevalence of *Bartonella* spp. in the urban rat population was 19%, 25%, 25.7%, 25%, 17%, 13.7%, 26%, and 16.9%, respectively. However, Krügel et al. [20] from Germany, Holiday 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 Raie Tabatabaei, Mohammad Reza Pourmand, and Taher Azimi: Writing – original draft; and Writing – review & editing.

Taher Azimi and Mohammad Reza Pourmand: Language editing.

Transparency declaration

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