Serosurvey and molecular detection of the main zoonotic parasites carried by commensal Rattus norvegicus populations in Tehran, Iran

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Abstract

Background: Rattus norvegicus are reservoirs of various zoonotic parasites that have become a global public health concern. Considering the distribution of Rattus norvegicus throughout Tehran, this study aims to assess the frequency of zoonotic parasites carried by commensal rodents in Tehran, Iran.

Methods: The study considered five regions (North, South, West, East, and center) of Tehran as case studies. The serological method was used for detecting antibodies against Trichomonas vaginalis , Babesia spp, and Cryptosporidium spp using a commercial qualitative rat ELISA kit. The frequency of Toxoplasma gondii was surveyed by the conventional PCR method. Furthermore, nested PCR was used to detect Giardia spp and Leishmania spp in commensal Rattus norvegicus in Tehran.

Results: Approximately, 76% of 100 Rattus norvegicus tested were infected with at least one zoonotic parasite, which demonstrates the significant frequency of parasites within the study areas. Seroreactivity against Trichomonas vaginalis , Babesia spp, and Cryptosporidium spp was detected in 5%, 0%, and 1% of Rattus norvegicus tested, respectively. Toxoplasma gondii DNA was detected in 32 out of 100 (32%) Rattus norvegicus , and Leishmania spp and Giardia spp DNA were found in 18 out of 100 (18%) and 76 out of 100 (76%) Rattus norvegicus investigated, respectively.

Conclusion: The findings indicate a wide geographical dissemination of Giardia spp, Toxoplasma gondii , and Leishmania spp DNA in Rattus norvegicus within five districts of Tehran. In contrast, other parasites such as Cryptosporidium spp infection rarely occurred in Rattus populations. No evidence for the circulation of Babesia spp was found in this study.

Introduction

Zoonotic parasites cause a significantly high rate of human infectious diseases [1]. It is predicted that sixty-one percent of pathogens, which are recognized to infect individuals, can cause zoonotic diseases [2]. Zoonotic parasites are transmitted between animals and persons with or without vectors; however, eating foods contaminated by rodent feces or urine and inhaling the germ in feces of rodents are considered the most important pathways for parasite transmission [3, 4, 5]. Rattus norvegicus globally live and feed in close proximity to human populations and are known to carry various pathogens including bacteria, viruses, and parasites [6]. In urban areas, Rattus norvegicus act as a reservoir of zoonotic pathogens, especially zoonotic parasites, and hold a connection to various important hygienic problems; they are also responsible for human morbidity and mortality, worldwide [7]. Many of these zoonotic parasites including Leishmania spp, Giardia spp, Toxoplasma gondii, Trichomonas vaginalis, and Cryptosporidium spp are assumed to be endemic in Rattus norvegicus populations around the world [8, 9, 10, 11]. Currently, approximately seventy-nine species of rodents have been recognized in Iran; among these previously identified rodents, Rattus norvegicus have shown greater frequency in the urban area and occupied widespread habitats in cities [12]. Although it has been proven that these rats have a potential role in the transmission of a large number of zoonotic parasites, the prevalence and diversity of

parasites in urban Rattus norvegicus populations remain unknown and, also, the data concerning zoonotic parasites of Rattus norvegicus are quite insufficient. So far, a comprehensive parasitological assessment of Rattus norvegicus populations in the case of Tehran, Iran has not been conducted. Therefore, the present study performs a comprehensive survey of Rattus norvegicus collected in five districts of Tehran for zoonotic parasites. These are the first informative data on zoonotic parasites related to Rattus norvegicus in urban areas of Tehran, Iran.

Materials And Methods

Site selection and sample collection

The study was conducted in five regions (North, South, West, East, and center) of Tehran. All the trapping locations were selected in urban areas in alleys behind the residential dwellings. The sampling strategy was designed to trap a similar number of rats between October 2018 and *June* 2019. The rodent samplings were carried out using Sherman live traps and suitable baits through the convenient sampling method. The aggregated places of rats were found around dumping garbage sites along the water open canal and gardens. Due to the physical and chemical intervention of Tehran Municipality aimed at controlling rats, the catching of rodents is problematic; therefore, a prebaiting procedure is preferable for improving the efficiency of traps. The trapping was carried out after sundown in each selected region and processed during midnight or the next morning. The traps were distributed in order to cover the present situation. The collected rodents were transferred to a guaranteened special laboratory in animal houses and were euthanized by the intramuscular injection of Ketamine and Xylazine (0.1 mg /kg) followed by bilateral thoracotomy. Finally, faecal samples were collected and blood was obtained by cardiac puncture using a 5mL syringe; then, serum was recovered after centrifugation and stored at −80°C until serological analysis. The subsequent parasitological examination was performed at the Department of Microbiology of Shahid Beheshti University of Medical Sciences.

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were screened for antibodies against *Trichomonas vaginalis*, *Babesia* spp, and *Cryptosporidium spp* using commercial qualitative rat ELISA kit (Shanghai Crystal day Biotech Co., Ltd) according to the manufacturer's instructions. The optical density (OD value) of each well was measured immediately using a microplate reader set at 450 nm (OD450) within 15 minutes after adding the stop solution (sulfuric acid).

DNA extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from fecal samples using the DNA extraction kit (AllPrep DNA minikit (Qiagen, Inc.) according to the manufacturer's guidelines, and each DNA sample was eluted in 200 µl of elution buffer preserved at -80oC until the future use. PCR was conducted for the detection of *Toxoplasma gondii* using specific primer pairs. The sequence of primers used for PCR reaction is shown in Table 1. PCR was conducted at a final volume of 25 µL including 0.5 µl of 10 mM of each

deoxynucleoside triphosphate (dNTPs), 3 µl of 10x PCR buffer without MgCl2, 2.5 mmol/L MgCl2, 1 unit of Taq polymerase (Cinnagene, Iran), 0.5 µM of each primer (10mM), 3 µl of template DNA, and 7.5 µL of sterile distilled water. Amplification reactions were performed under the following condition: one cycle of 95 °C for 4 min, followed by 36 cycles of 94 °C for 45 s, annealing at 56°C for 45 s, and preserving 72 °C for 1 minute with the final extension at 72°C for 10 minutes following the last cycle. PCR products were screened on a 1%–1.5% agarose gel, visualized by DNA safe stain (SinaClon Co., Iran), and photographed under UV light. Moreover, PCR amplified products were confirmed by sequencing analysis (Macrogen Korea), and the obtained sequence results were examined by the NCBI BLAST program (Primer blast).

Nested PCR

Nested PCR was used for the detection of *Giardia* spp and *Leishmania* spp using specific primer pairs. Briefly, *Leishmania* DNA was amplified and detected using the first-round primer pairs including 5'-CTGGATCATTTTCCGATG-3' and 5'-TGATACCACTTATCGCACTT-3' and the second-round primers including 5'-CATTTTCCGATGATTACACC-3' and 5'-CGTTCTTCAACGAAATAGG-3'. The PCR conditions for the first step were based on a previously published study by Poonam et al. [13]. On the other hand, *Giardia* spp DNA was amplified using the first round and second round primers, as shown in Table 2. The PCR conditions for the first step were based on a previously published study by Adnan et al[14]. In summary, PCR was conducted at a final volume of 50 µl including 10 mM Tris-HCl (pH 8.3) and 50 mM KCl, a 200 µM concentration of each dNTP, 1.5 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase (Invitrogen), 50 ng of each primer, 5 µl DNA, and 1× PCR buffer (Invitrogen). For the second round, the method provided by Gannavaram et al. was used [15]. Briefly, the second round was performed at the total volume of 50 µl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 mM of each dNTP, 1.5 mM MgCl2, 2 mM of each primer, and 1.5 U Platinum Taq DNA Polymerase (Invitrogen). Moreover, we used 1 µl of the diluted (1:10) products from the first-round reaction as a template. Amplification reactions were performed under the following condition: initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 1 minute, annealing at 50-54 °C for 1 minute, and preserving 72 °C for 90 s with the final extension at 72°C for 3 minutes. PCR products were screened on a 1% agarose gel, visualized by DNA safe stain (SinaClon Co., Iran), and photographed under UV light; they were confirmed by sequencing analysis (Macrogen Korea). The sequencing results were examined by the NCBI BLAST program (Primer blast).

Statistical analysis

The data was formatted in an SPSS file, and the frequency of each surveyed parasite was analyzed by the statistical package SPSS v.23.0 (SPSS Inc., Chicago, IL, USA) using descriptive statistic tests.

Results

Detection of *Trichomonas vaginalis, Babesia* spp, and *Cryptosporidium spp*

A total of 100 live *Rattus norvegicus* (20 rats from each district of Tehran) were captured and surveyed in order to determine their zoonotic parasites. To evaluate the seroprevalence of *Trichomonas vaginalis*,

Babesia spp, and *Cryptosporidium spp* in the trapped rats, the presence of rat IgG antibodies was examined by ELISA kit. In total, results of serological assay revealed that of the 100 rats captured in Tehran, 5% (n = 5/100) and 1% (n = 1/100) were positive for *Trichomonas vaginalis* and *Cryptosporidium spp, respectively.* Among five different districts, *Trichomonas vaginalis* had the highest frequency (15%, n = 3/20) among *Rattus norvegicus* collected from the western part of Tehran. However, this parasite was not detected in the northern and central parts of Tehran. On the other hand, *Cryptosporidium spp was detected only in one* rat, collected from the central part of Tehran. *Babesia* spp was not detected in the any serum samples of all 100 animals examined.

Detection of *Toxoplasma gondii, Giardia* spp, and *Leishmania* spp

In this study, PCR method was used to screen the presence of *Toxoplasma gondii* in fecal samples, collected from *Rattus norvegicus*. Moreover, Nested PCR was used for detecting *Giardia* spp and *Leishmania* spp using specific primer pairs. The number of *Rattus norvegicus* and sample types positive for zoonotic parasites in five districts of Tehran are shown in Table 3. Results showed that the percentage of positive animals in the five regions of Tehran for *Toxoplasma gondii* was 32%. Among *Rattus norvegicus* trapped in Tehran, *Toxoplasma gondii* had the highest and lowest frequency in the north (70%, n = 14/20) and west (5%, n = 1/20) districts, respectively. *Leishmania* spp ELISA analysis of serum samples resulted in the detection of 18 out of 100 (18%) seropositive samples, originating from northern (15%, n = 3/20), southern (15%, n = 3/20), eastern (15%, n = 3/20), western (15%, n = 3/20), and central (30%, n = 6/20) parts of Tehran. *Giardia spp has the highest frequency among surveyed parasites.* In general, according to the results of Nested PCR assay, of the 100 rats captured in Tehran, 76% (n = 76/100) were positive for *Giardia spp,* originating from eastern (95%, n = 19/20), central (80%, n = 16/20), southern (75%, n = 15/20), western (65%, n = 13/20), and northern (65%, n = 13/20) parts of Tehran.

Discussion

In general, in the urban area, rodents such as Rattus norvegicus exist in large populations and represent a significant reservoir of a range of human pathogens including bacteria, viruses, and parasites [6, 16]. In comparison to other mammalian species, Rattus norvegicus live and feed in closer proximity to humans. These rodents harbor and disseminate zoonotic parasites through their ectoparasites or via biological materials; therefore, they play an active and main role in the transmission of various zoonotic diseases [17, 18]. Tehran as the capital of Iran is a large city in the north of the country that features a continental-influenced Hot-summer Mediterranean climate. Home to a population of about 10–12 million in the city and 15 million over the larger metropolitan area of Greater Tehran, Tehran is the most populous city in Iran and Western Asia and has the second largest metropolitan area in the Middle East [19, 20, 21]. However, the prevalence and diversity of parasites in Rattus norvegicus populations in Tehran remain unknown, and a comprehensive parasitological assessment of Rattus norvegicus populations has not been conducted so far. The result of our study revealed that Giardia spp was the main parasite that was frequently (76%; n = 76/100) isolated from the Rattus population of Tehran. In addition, the frequency of Giardia spp was very high in the eastern (95%, n = 19/20) part of Tehran. This result was in contrast to

those of published studies of Carolina and Tiwari in Brazil and India, respectively. These studies found that the frequency of Giardia spp in the Rattus norvegicus population was 17.1% and 42.9%, respectively [22, 23]. On the other hand, the result of our study revealed that Toxoplasma gondii had the highest frequency (70%; n = 14/20) among Ratti captured from the northern part of Tehran. The total frequency of Toxoplasma gondii was 32%. These results were similar to those of published studies conducted by Dellarupe and Chao Yan. They revealed that the frequency of Toxoplasma gondii in the Rattus norvegicus population in Argentina and China was 32.8% and 23.9%, respectively [24, 25]. However, Pellizzaro et al. in Brazil [8], Saki et al. from Ahvaz province of Iran [10], Gennari from Brazil [9], and Cheng Yin from China [26] showed that the frequency of Toxoplasma gondii in Rattus population was 4.6%, 6%, 8.6%, and 3.2%, respectively. Generally, the high frequency of Giardia spp and Toxoplasma gondii in the Rattus population in Tehran is an important concern. Accordingly, humans and animals have been infected mainly by the ingestion of oocysts in the environment or consumption of foods containing cysts of Toxoplasma gondii [11]; therefore, sanitary control is extremely important to observe in Tehran. Moreover, these data will help veterinarians and physicians to better plan diagnostic and preventative measures. The frequency of Leishmania positive Rattus population was 18% lower than what has been found in other studies. For example, Motazedian et al. (2010) detected Leishmania major (52%) in the Rattus population in Iran [27]; Marcelino et al. (2011) detected Leishmania (36.25%) in the Rattus population in Brazil [28]; Dohlen et al. (2018) detected Leishmania (23.3%) in the Rattus population in the USA[29]; Tsakmakidis et al. (2017) detected Leishmania (70%) in the Rattus norvegicus population in Greece [30]. On the other hand, Echchakery et al. (2017) detected Leishmania (11.11%) in the Rattus population in Morocco [31]. Leishmaniasis is a vector-borne infectious disease and is considered to be a major public health problem in the urban environment. The diagnosis of natural hosts of Leishmania spp in urban areas is critical and will facilitate a better understanding of the epidemiology of the Leishmaniasis [32].

In conclusion, this finding indicates that the Rattus norvegicus population is a significant reservoir of Giardia spp, Toxoplasma gondii, and Leishmania spp infection for humans in Tehran. It is important to raise public attention to and awareness of the transmission risk of illness to people through the Rattus population. Information about zoonotic parasites carried by the Rattus norvegicus population in Tehran province is critical to developing suitable surveillance plans and intervention strategies.

Declarations

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Ethics approval and consent to participate

The present study was approved by the Ethics Committee of National Institutes for Medical Research Development (NIMAD) with reference number IR.NIMAD.REC. 1396.323. All authors of this research paper

have directly participated in the planning, execution, or analysis of this study.

Consent for publication

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data. They played an active role in drafting the article or revising it critically to achieve important intellectual content, gave the final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Competing interests: The authors declare that they have no competing interests.

Data Availability Statement: All data generated or analysed during this study are included in this published article.

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Tables

Table 1. Primers used for the detection of the *Toxoplasma gondii*

Primers	(´Primer sequence (5´-> 3	Amplicon size
Forward	GTAGCGTGCTTGTTGGCGAC	Variable
Reverse	ACAAGACATAGAGTGCCCC	

Table 2. Primers used for the detection of the *Giardia lamblia* using nested PCR method.

First round	G7 F	5-AAGCCCGACGACCTCACCCGCAGTGC-3
	G759 R	5-GAGGCCGCCCTGGATCTTCGAGACGAC-3
Second round	BG1 F	5-GAACGAGATCGAGGTCCG-3
	BG2 R	5-CTCGACGAGTTCGTGTT-3

Table 3: Numbers of *Rattus norvegicus* and sample types positive for zoonotic parasites identified by ELISA, PCR, and Nested PCR methods in five districts of Tehran.

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	Center	Total
Leishmania spp	Fecal	Nested PCR	3/20	3/20	3/20	3/20	6/20	18/100
Giardia lamblia	Fecal	Nested PCR	13/20	15/20	13/20	19/20	16//20	76/100
Trichomonas vaginalis	Serum	ELISA	0/20	1/20	3/20	1/20	0/20	5/100
Babesia spp	Serum	ELISA	0/20	0/20	0/20	0/20	0/20	0/100
Cryptosporidium spp	Serum	ELISA	0/20	0/20	0/20	0/20	1/20	1/100
Toxoplasma gondii	Fecal	PCR	14/20	6/20	1/20	5/20	6/20	32/100

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