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Is the *Rattus norvegicus* Population Playing a Role in the Transmission of Zoonotic Diseases to Children? A Pilot Study in Tehran, Iran

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Abstract

Background: Due to frequent exposure to surface water and contact with animals, children represent a group susceptible to zoonotic diseases.
Objectives: The present study aims to determine the presence and prevalence of the main zoonotic agents in *R. norvegicus* populations in Tehran, Iran.
Methods: In the present study, 100 *R. norvegicus* were captured within a time span of one year from five districts of Tehran, Iran. Fecal and blood samples were collected from rodents and serum was recovered after centrifugation. The presence of specific IgG antibodies against *Leptospira* spp. and Rabies virus was detected using a commercial qualitative rat ELISA kit. A conventional PCR assay was employed to detect the presence of *Vibrio vulnificus* in the commensal *R. norvegicus* population.
Results: In general, 80% (n = 80/100) and 20% (n = 20/100) of rats were males and females, respectively. The results of the ELSA assay showed that of the 100 *R. norvegicus* captured in Tehran, 7% (n = 7/100) and 1% (n = 1/100) were positive for *Leptospira* spp. and Rabies virus, respectively. *Leptospira* spp. revealed the highest frequency (20%; 4/20) among *R. norvegicus* collected from the eastern part of Tehran. Rabies virus was detected only from the southern (5%; 1/20) part of Tehran. Results of the PCR method showed that the percentage of the rats tested positive for *V. vulnificus* was 5%. Overall, the surveyed zoonotic microorganisms had the highest (n = 5/20; 25%) and lowest (n = 1/20; 5%) frequency rates in the eastern and northern parts of Tehran, respectively.
Conclusions: The results accentuate the necessity of implementing rodent control programs and regular disinfection as well as avoiding contact with rodent populations in urban environments.

Keywords: *Rattus norvegicus*, *Leptospira* spp., Zoonotic Diseases, Rabies, Children, Tehran, Iran

1. Background

Rattus norvegicus (*R. norvegicus*) are recognized to be reservoirs for different zoonotic diseases and are associated with important hygienic problems (1). In urban environments, *R. norvegicus* often live in proximity to human populations and are linked to significant human morbidity and mortality in developing and developed countries (2). These rodents carry different microorganisms including bacteria, viruses, and parasites and they are considered to be a concern for global public health in urban environments (3). So far, 79 different species of rodents have been identified in Iran (2). Among these rodents, *R. norvegicus* had the highest frequency and were frequently isolated in urban areas. It has been revealed that 61% of 1500 known human pathogens are common among humans and animals. Therefore, these pathogens can cause zoonotic diseases (4). In general, the rodent's population contaminates water and food and can infect the human population in different ways. However, contact with different rat secretions such as urine, saliva, and feces, consumption of contaminated water or food, inhalation of aerosols, direct contact by bites, direct contact with contaminated infected domestic animals, and infections via vectors are considered the main pathways (5-7). Based on several studies conducted in different countries, *R. norvegicus* represent a reservoir for *Leptospira* spp. Moreover, it is predicted that these rodents carry other microorganisms such as *Vibrio vulnificus* (*V. vulnificus*) and Rabies virus (3, 8, 9). Despite the existence of an effective vaccine regimen, the rabies virus continues to be a global health concern with an estimated human death rate

of 55,000 each year, worldwide (10). *V. vulnificus* can cause severe infections from gastroenteritis to 'primary sepsis' and necrotizing fasciitis and is associated with most human death cases caused by Vibrios (11). Leptospirosis is an important endemic zoonotic disease, has a global distribution, and is considered a public health concern (12). Based on the World Health Organization's (WHO's) Leptospirosis Burden Epidemiology Reference Group reports, approximately 60,000 human deaths occur each year, worldwide (13). Direct contact with rodent population or infected livestock and wild animals, contact with surface water, soil, and plants, consumption of contaminated water, direct contact with the urine of infected animals, or contact with a urine-contaminated environment are the main ways that *Leptospira* spp. can infect children (14). It has been found that due to frequent exposure to surface water and contact with animals, children represent a more susceptible group to *Leptospira* infections (13). Tehran, home to a population of 10 - 12 million people, is the most populous city in Iran and Western Asia that has a hot-summer Mediterranean climate (1). However, data about the prevalence of *Leptospira* spp., *V. vulnificus*, and Rabies virus in urban rat populations are limited and remain unexplored. The current study carried out a pilot survey of rats collected from five districts of Tehran for *Leptospira* spp., *V. vulnificus*, and Rabies virus.

2. Objectives

The present study aims to determine the presence and frequency of these agents in the *R. norvegicus* population living in an urban environment.

3. Methods

3.1. Ethics Approval

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

3.2. Study Sites and Sample Collection

During one year from May 2018 to May 2019, 100 *R. norvegicus* were captured from five districts of Tehran, Iran, including 20 from the northern district, 20 from the southern district, 20 from the western district, 20 from the eastern district, and 20 from the central district. All the trapping locations were selected in urban areas in alleys behind residential dwellings. Within each trapping location, Sherman live traps and alluring baits were set and sampling was done through a convenient sampling method. The sampling strategy will be designed to trap similar numbers of rats in five districts. The sampling will be performed after sundown in every five districts and processed during the next morning. All rats were transported to a guaranteed special laboratory in animal houses within 48 h after being captured and fecal samples were collected and transferred to top-screwed small bottles containing formol-ether solution and labeled. All rats were euthanized by the intramuscular injection of ketamine and xylazine (0.1 mg/kg) followed by bilateral thoracotomy. In the next step, blood samples were collected by cardiac puncture using a 5ml syringe, and serum was recovered after centrifugation. All fecal and serum samples were stored at -80°C until molecular and serological analysis.

3.3. Enzyme-linked Immunosorbent Assay

Briefly, all serum samples were screened for specific antibodies against *Leptospira* spp. and Rabies virus by Enzyme-Linked Immunosorbent assay (ELISA). The ELISA method was performed using a commercial qualitative rat ELISA kit (Shanghai Crystal Day Biotech Co., Ltd) at the central laboratories of the Department of Microbiology, Shahid Beheshti University of Medical Sciences. The optical density (OD value) of each well was read with a spectrophotometer at 450/620 nm within 15 min of stopping (15 min after adding the sulfuric acid). All of the ELISA steps and the process of determining the cut-off amount were performed according to the manufacturer's instructions.

3.4. DNA Extraction and Polymerase Chain Reaction

Genomic DNA was extracted from fecal samples using the DNA extraction kit (AllPrep DNA minikit (Qiagen, Inc.), as recommended by the manufacturer. All extracted DNA samples were eluted in 50 μl of elution buffer and stored at -80°C before Polymerase chain reaction (PCR) analysis. In the next step, PCR was performed to determine the prevalence of *V. vulnificus* using specific primer pairs including F: 5'-TTCCAACTTCAAACCGAACTATGA-3' and R: 5'-ATTCCAGTCGATGCGAATACGTTG -3. Briefly, 25 μL PCR mixture consists of 12 μL of 2$\times$ Master Mix (Amplicon, Pishgam, Tehran, Iran; Cat. no. PR901638) including 0.5 μL of 10 mM of each deoxynucleoside triphosphate (dNTPs), 1 $\times$ PCR buffer, 3 mmol/L $MgCl_2$, 1 unit of Taq DNA polymerase, 0.5 μM of each primer (10 mM), 3 μL of template DNA, and 9 μL of sterile distilled water. PCR reactions were performed on a thermal cycler (Eppendorf, Mastercycler Gradient; Eppendorf, Hamburg, Germany). PCR was conducted under the following condition: one cycle at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 45 s, 72°C for 45 s, and the final extension step at 72°C for 10 minutes following the last cycle. All PCR products were electrophoresed on a 1.5% agarose

gel, visualized by DNA safe stain (SinaClon Co., Iran), and photographed under UV light (Life Technologies). The positive PCR products representing the studied gene were confirmed by sequencing analysis (Macrogen Korea). The results of sequencing were studied by the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

3.5. Statistical Analysis

All data were included in an SPSS file, and the prevalence of surveyed microorganisms was analyzed by the statistical package SPSS V.23.0 (SPSS Inc., Chicago, IL, USA) using descriptive statistic tests.

4. Results

4.1. Prevalence of Zoonotic Pathogen

During the one year from May 2018 to May 2019, 100 *R. norvegicus* were captured from five districts (North, South, West, East, and center) of Tehran, Iran. In general, 80% (n = 80/100) and 20% (n = 20/100) of rats were males and females, respectively. The prevalence and distribution of surveyed microorganisms among *R. norvegicus* in five districts of Tehran are shown in Table 1.

Moreover, the frequency of surveyed pathogens among male and female *R. norvegicus* is shown in Table 2. Results showed that 13% (n = 13/100) of all captured rats were positive from which different surveyed microorganisms were isolated. The presence of specific rat IgG antibodies against *Leptospira* spp. and Rabies virus was detected using an ELISA kit. In total, results of the ELSA assay showed that of the 100 *R. norvegicus* captured in Tehran, 7% (n = 7/100) and 1% (n = 1/100) were positive for *Leptospira* spp. and Rabies virus, respectively. Among the five districts, the frequency of *Leptospira* spp. is as follows: North (0%; 0/20), south (0%; 0/20), west (5%; 1/20), east (20%; 4/20), and center (10%; 2/20). *Leptospira* spp. revealed the highest frequency among *R. norvegicus* collected from the eastern part of Tehran. Rabies virus was only detected from the Southern (5%; 1/20) part of Tehran. PCR assay was applied to survey the presence of *V. vulnificus* in fecal samples collected from *R. norvegicus*. Results revealed that the percentage of the rats tested positive for *V. vulnificus* in the five districts of Tehran was 5%. The frequency of *V. vulnificus* among five districts was as follows: North (5%; 1/20), south (10%; 2/20), west (5%; 1/20), and east (5%; 1/20). *V. vulnificus* was not detected in the central parts of Tehran. Overall, the surveyed zoonotic microorganisms had the highest (n = 5/20; 25%) and lowest (n = 1/20; 5%) frequency rates among *R. norvegicus* collected from the eastern and northern parts of Tehran, respectively.

5. Discussion

In urban areas, rodents exist in large populations and live with and near human populations (2). *R. norvegicus* is recognized as the reservoir for different human zoonotic pathogens and has a significant role in zoonotic disease ecology, worldwide (1). In the current study, *R. norvegicus* were captured from five districts in Tehran to obtain data about the presence and frequency of *Leptospira* spp. *V. vulnificus* and Rabies virus. The results showed that *Leptospira* spp. were the main zoonotic pathogens that were (7%; n = 7/100) isolated from the *R. norvegicus* population in Tehran. Besides, the highest prevalence (20%; n = 4/20) of *Leptospira* spp. was identified in the eastern part of Tehran. Several studies have surveyed the presence and prevalence of *Leptospira* spp. in Rattus in cities around the world. Azhari et al. (15) surveyed the prevalence and molecular characterization of pathogenic *Leptospira* spp. in the Rattus population captured from Selangor state, Malaysia. The finding of their study revealed an overall *Leptospira* detection rate of 14.3% among the 266 Rattus captured. Results of another study conducted by Firth et al. (3) in the USA revealed that the prevalence of *Leptospira* interrogans among *R. norvegicus* was 12%. Maas et al. (8) in 2018 reported that the prevalence of *Leptospira* spp. in *R. norvegicus* in four regions in the Netherlands was 33% - 57%. Costa et al. (16) in Brazil and Runge et al. (17) in Germany found that the frequency of *Leptospira* spp. in Norway rats was 83% and 25%, respectively. In Iran, Mosallanejad et al. (18) surveyed the frequency of *Leptospira* infection among *Rattus rattuss* of Ahvaz District, southwest of Iran. The results of their study revealed that from a total of 120 *Rattus rattuss*, 3.3% were serologically positive to *Leptospira* infection. In general, rodent population and domestic animals such as pigs, dogs, and cattle are considered as the reservoirs for and carriers of *Leptospira* spp. (12). *Leptospira* spp. can infect humans in several ways including direct contact with urine or feces of infected animals as well as contact with contaminated soil and water (19). Children spend more time playing games in contaminated surface water and animal. They are considered as a group susceptible to leptospirosis and run an increased risk of contracting this zoonotic disease (13, 14, 20, 21). *Leptospira* spp. can cause febrile illnesses and lead to high mortality and morbidity in children. It has been revealed that fever, myalgia, gastroenteritis-like symptoms, and conjunctival suffusion are common clinical features of leptospirosis among children (22). The difference in the prevalence rate of *Leptospira* spp. among conducted studies results from several factors such as (1) variation in samples type; (2) difference in diagnostic methods; (3) different sanitary conditions of the urban environment in countries; (4) awareness levels of peoples about the rat-

Table 1. Numbers of *Rattus norvegicus* and Sample Types Positive for Surveyed Zoonotic Microorganisms Identified by ELISA and PCR Methods in five Districts of Tehran

Zoonotic Parasites	Sample Type	Methods	No. of Positive Samples / No. of Tested in Five Districts of Tehran					
			North	South	West	East	Center	Total
***Leptospira* spp.**	Serum	ELISA	0/20 (0)	0/20 (0)	1/20 (5)	4/20 (20)	2/20 (10)	7/100 (7)
V. vulnificus	Fecal	PCR	1/20 (5)	2/20 (10)	1/20 (5)	1/20 (5)	0//20 (0)	5/100 (5)
Rabies virus	Serum	ELISA	0/20 (0)	1/20 (5)	0/20 (0)	0/20 (0)	0/20 (0)	1/100 (1)
Total	-	-	1/20 (5)	3/20 (15)	2/20 (10)	5/20 (25)	2/20 (10)	13/100 (13)

Table 2. The Frequency of Surveyed Microorganisms Among Male and Female *R. norvegicus*

Parasites	Total Positive, %	Positive Cases Among Genders, %	
		Male	Female
***Leptospira* spp.**	7	100	0
V. vulnificus	5	100	0
Rabies virus	1	100	0

borne disease; (5) climate variations and different rainfall patterns among countries; and (6) education status and poverty (22, 23).

In the present study, *V. vulnificus* had the highest frequency (10%; n = 2/20) in the *R. norvegicus* population captured from the southern part of Tehran. The total frequency of *V. vulnificus* was 5% (n = 5/100). To the best of our knowledge, the current study is the first research to have reported the prevalence of *V. vulnificus* in the *R. norvegicus* population, worldwide. In 2014, Firth et al. in the USA investigated the frequency of *V. vulnificus* in Norway rats. However, the results of their research revealed that none of the samples tested was positive for *V. vulnificus* (3). *V. vulnificus* can cause a potentially fatal disease among children, especially among those with a weakened immune system. Therefore, rapid diagnosis, immediate management, and better treatment of disease are critical (11, 24, 25). The frequency of the Rabies virus among the *R. norvegicus* population was 1%. In general, we found two different studies conducted by Kantakamalakul et al. (26) in Thailand and Patabendige and Wimalaratne (27) in Colombo, who surveyed the prevalence of Rabies virus among local rodents and domestic rats. Results of both studies revealed that the rabies antigen was not detected in any rodent population.

The results of the present study revealed that the Rattus population carried the zoonotic organisms in the urban environment. Children are vulnerable to infectious diseases and can be infected with different zoonotic pathogens in several ways. The limitation of this study lies in the small number of rodents in each geographical region in Tehran. This limitation is associated with the difficulty of capturing such rodents.

5.1. Conclusions

The finding of the current study emphasizes a number of important steps: (1) implementation of better rodent control programs in urban environments; (2) the need to prevent children from playing outside and in contaminated soil or water; (3) disinfection of urban areas; (4) the necessity of avoiding contact with Rattus and other rodent population in urban environments for children, especially immunocompromised patients; and (5) suitable maintenance of sanitary conditions and adoption of better waste disposal measures. Since this study is a pilot survey, the results of the present study point to the need for conducting further studies on a larger scale in Tehran, Iran.

Footnotes

Authors' Contribution: Taher Azimi and Mohammad Reza Pourmand did conceptualization, data curation, formal analysis, and writing-original draft. Fatemeh Fallah, Abdollah Karimi, and Lela Azimi did conceptualization, methodology, project administration, and writing-original draft. Mohammad Rahbar and Shahnaz Armin did data curation, formal analysis, writing-original draft, and writing-review and editing. Taher Azimi and Leila Azimi did language editing.

Conflict of Interests: The authors declare that they have no competing interests.

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