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Prevalence of *Cryptosporidium* in Wild Brown Rat (*Rattus norvegicus*) Population at Shoushtar, Iran

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Abstract

Background: Cryptosporidiosis is a zoonotic disease with public health importance, especially for individuals with weak immune system, such as children, elderly people and immune suppressed ones. Evaluation of the prevalence of *Cryptosporidium* in wild rodents such as wild brown rats could help to establish an epidemiological map of Cryptosporidiosis. The aim of the current study was to assess the prevalence of *Cryptosporidium* in wild brown rat population at Shoushtar, Iran.

Methods: In this descriptive study, 14 trapping districts were selected in Shoushtar, Iran by simple random method. Trapping conducted between January 2012 and January 2014. Trapped brown rats were euthanized and then dissected. Then, fecal content of large intestine was collected. After that, fecal samples were passing through a mesh and centrifuged at 10000 g for 10 minutes. The supernatant removed and the pellet was washed with Phosphate-buffered saline (PBS). *Cryptosporidium* oocysts were detected by microscopy after sugar flotation and modified acid-fast staining. The prevalence was calculated using descriptive statistics via SPSS (ver. 16).

Results: A total of 42 wild brown rats were captured alive during the trapping period. From 14 trapping districts, wild brown rats were captured in 9 districts. The prevalence of *Cryptosporidium* in the current study was 7.1%. The three positive cases belong to 3 districts.

Conclusion: The present study demonstrates that wild brown rats are contaminated with *Cryptosporidium* at Shoushtar, Iran. Reply to this question that either positive samples detected in this study are related to species that have public importance or not, needed molecular studies. However, previous studies with molecular techniques are limited and have controversial results.

Keywords: *Cryptosporidium*, Rats, Iran, Prevalence, Microscopy, Zoonoses

Introduction

The ubiquitous presence of rodents in many environments and their sharing of habitats with farmed animals and contamination of drinking source water have led to many studies on the prevalence of *Cryptosporidium* in these animals (1). Wild rats may act as reservoirs for some important parasitic disease such as toxoplasmosis (2), cryptosporidiosis (1, 3) and leishmaniasis (4, 5). The mentioned diseases endanger life of many individuals with suppressed or weak immune system in the world annually (6). Wild rats are very adaptable to urban environments and they are highly adapted to coexisting with human populations (7). *Cryptosporidium* is an intestinal protozoan parasite, which has been identified as an important enteric pathogen of humans and animals (8). The disease, cryptosporidiosis, usually manifests as watery diarrhea, with symptoms ranging in severity and chronicity depending on the age and immunological status of the host (9). Cryptosporidiosis is typically a self limiting disease in individuals with normal immune system. Infective stage of *Cryptosporidium* is oocyst which shed by feces of contaminated organisms to environments. The disease transmitted by fecal-oral route. Environmental pollution with human and animal fecal material is postulated as a potential pathway for infections with *Cryptosporidium*, thus amplifying environmental contamination (1). Studies show different rates of infection with some species of *Cryptosporidium* in wild rats at Iran and other parts of the world (8-14). Previous studies show that species of *Cryptosporidium* such as *Cryptosporidium hominis*, *Cryptosporidium parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, *Cryptosporidium canis*, *Cryptosporidium muris*, *Cryptosporidium suis*, *Cryptosporidium andersoni*, *Cryptosporidium cuniculus*, *Cryptosporidium ubiquitum*, *Cryptosporidium fayeri*, *Cryptosporidium scrofarum*, and *Cryptosporidium viatorum* can infect humans which two of them i.e. *Cryptosporidium hominis* and *Cryptosporidium parvum* are responsible for about

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90% of human infections (15). Assessment of the prevalence of *Cryptosporidium* spp. in wild rats is important, because some species of *Cryptosporidium* are zoonoses. The present study was carried out to evaluate the prevalence of *Cryptosporidium* spp. in wild brown rat population at Shoushtar, Iran.

Methods

Study site

Shoushtar is situated in Khuzestan province of Iran (Figure 1). Geographical coordinates of Shoushtar are 32° 2' 39" N, 48° 51' 27" E. Shoushtar has hot summers and temperate winters. The Karun River flows through the middle of the city. Shoushtar has a very poor condition in municipal infrastructures. The city is a tourist destination because of its water mills.

Figure 1. Geographic Location (red dot) of Shoushtar in Iran (16).

Trapping

Fourteen trapping districts were selected by simple random method in Shoushtar (Figure 2). Trapping conducted between January 2012 and January 2014. Trapping carried out by live trap at 10 places for each selected trapping districts (shown in Figure 2 by white circles with number). The numbers of trapping nights were 30 for each district. Traps set under manholes for sewerage network access and other suitable places in commercial and residential areas of each district (8) (Figure 3).

Figure 2. Location of Trapping Districts on the Aerial Map (17) of Shoushtar in Iran: This map shows 14 trapping districts (white circles with number) of the current study, which selected by random number table.

Figure 3. Some Trapping Sites (Pictures 1, 2, 3, and 4) of This Study and Wild Brown Rat (Picture 5) Captured in Shoushtar.

Sample preparation

After transfer to laboratory, trapped rats were euthanized. Then, rats were dissected and content of their large intestine were collected and placed in 15 mL tube containing 2.5% potassium dichromate (11, 18). The samples were kept in 4 ^{0}C until further examination. For examination, samples were passing through a 200 µm mesh and centrifuged at 10000 g for 10 minutes. After that, the supernatant removed and the pellet was washed by PBS (9). The pellets were undergoing sugar flotation for preparation of smear. The smear stained with modified acid-fast staining method (11, 13, and 18).

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Table 1. Details of Trapping and Prevalence of *Cryptosporidium* in Wild Brown Rats at Shoushtar

Trapping districts *	GPS coordinate (Latitude/Longitude)	Number of trapping nights	Number of trapped brown rats	Number of positive cases	Prevalence (%) in 42 examined brown rats
1	32° 3'59.64"N/48°49'18.36"E	30	0	0	0
2	32° 3'41.22"N/ 48°48'55.29"E	30	5	1	2.38
3	32° 3'40.60"N/ 48°49'36.68"E	30	7	1	2.38
4	32° 3'38.54"N/ 48°50'37.69"E	30	0	0	0
5	32° 3'37.89"N/48°51'21.81"E	30	8	0	0
6	32° 3'31.95"N/ 48°52'20.87"E	30	2	0	0
7	32° 3'21.95"N/ 48°49'56.18"E	30	6	0	0
8	32° 3'0.90"N/ 48°51'40.71"E	30	0	0	0
9	32° 2'44.55"N/ 48°50'54.32"E	30	2	0	0
10	32° 2'40.19"N/ 48°51'57.74"E	30	0	0	0
11	32° 2'26.62"N/48°50'34.28"E	30	4	1	2.38
12	32° 2'23.11"N/ 48°51'17.29"E	30	3	0	0
13	32° 2'9.10"N/48°50'52.69"E	30	0	0	0
14	32° 1'49.73"N/ 48°50'51.76"E	30	5	0	0
Total		420	42	3	≈7.1

* According to Figure 2

Parasite detection and statistical analysis

The prepared smear examined using the oil-immersion objective. Oocysts were identified on the basis of morphology and color (18). The prevalence was calculated using descriptive statistics via SPSS (ver. 16). All institutional, national and international guidelines for the care and use of animals were followed.

Results

A total of 42 wild brown rats were captured alive during the trapping period (Figure 3). From 14 districts that trapping were carried out, wild brown rats were captured in 9 districts (Table 1). In the current study, district 5 (Figure 2 and Table 1) with eight captured rats has the highest abundance of wild brown rats among all trapping districts. The prevalence of *Cryptosporidium* (Figure 4) in the present study was 7.1% (Table 1). The three positive cases belong to 3 districts (districts 2, 3 and 11) at Shoushatr, Iran (Figure 2 and Table 1).

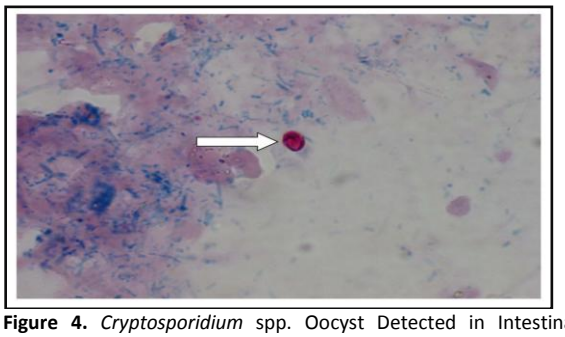

Figure 4. *Cryptosporidium* spp. Oocyst Detected in Intestinal Contents of Wild Brown Rats at Shoushtar.

Discussion

In immune-competent individuals, infections with *Cryptosporidium* are usually cleared within about 2 weeks with subsequent immunity to re-infection; however, immuno-compromised persons, have been reported to remain infected for months or longer which may lead individual to death (12). Neither this study, nor other previous studies does not provide any proved evidence that show wild brown rats play an important role in epidemiology of *Cryptosporidium* in human yet and available studies only rely on possibilities. Most epidemiological studies focused on some species with public health importance such as *C. parvum* species complex, *C. meleagridis*, *C. viatorum*, *C. felis*, *C. suis*, *C. ubiquitum*, *C. scrofarum*, *C. muris*, and *C. andersoni* (19) that were not detected in wild brown rat except for some limited molecular studies that vary in results. In addition, the results of molecular studies on wild rats also vary widely between studies and a common result was not found. Finding of the present study may be helpful because some species that have a public health importance reported in previous studies from wild rats (8, 10). Difference in prevalence of *Cryptosporidium* among trapping districts of the present study and with other studies may be due to differences in rodent density, differences in density of the human population in studied areas and time of sampling (9). Brown rat has not been captured in 5 out of 14 districts examined in this study, which might have partially resulted from seasonal variation, living conditions and food availability. The prevalence of *Cryptosporidium* reported in this study was lower

than the study of Bahrami et al. (10), and Kimura et al. (8) but kimura et al. (8) doesn't used acid fast staining method. Furthermore, prevalence of *Cryptosporidium* reported in the current study was higher than the study of Lv et al. (11). Regard to this fact that the Shoushtar does not have any effective sewage treatment system prior to release sewage to Karun River, there is a possibility that contamination could reach into the municipal water system of downstream cities. Nevertheless, the current methods which used in municipal water treatment plants and sewage treatment facilities in Iran, does not eliminate oocysts of *Cryptosporidium* due to its high resistance to typical disinfectants and little size (less than or equal to 6 µm). The advantage of this study was the detection method which is a gold standard. Limitation of the current study was absence of molecular diagnostic methods for detection of *Cryptosporidium* species.

Conclusion

Although the species of *Cryptosporidium* were not determined in this study, detection of *Cryptosporidium* in wild brown rat could help to establish the epidemiological map of this parasite in Shoushtar, Iran. The potential of the *Cryptosporidium* oocysts identified in the present study to cause disease in humans is unknown (9). Examination of a larger number of wild brown rats with molecular techniques is required as this will allow more accurate evaluation of the role that these rodents play in epidemiology of *Cryptosporidium*.

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Conflicts of interest

The author declares that there are no conflicts of interest related to the current article.

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