

Prevalence of Ecto and Gastrointestinal Parasites of *Rattus rattus* in Mazandaran Province, North of Iran

İran'ın Kuzeyinde Mazandaran Eyaletinde *Rattus rattus*'un Ekto ve Gastrointestinal Parazitlerinin Prevalansı

Ahmad Daryani¹, Afsaneh Amouei², Abdol Sattar Pagheh³, Mehdi Sharif¹,

Shahabeddin Sarvi¹, Mohammad Taghi Rahimi⁴, Fatemeh Rezaei⁵

¹Toxoplasmosis Research Center, Mazandaran University of Medical Sciences, Sari, Iran

²Department of Parasitology, Sari Medical School, Mazandaran University of Medical Sciences, Sari, Iran

³Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran

⁴Shahroud University of Medical Sciences Faculty of Medicine, Department of Basic Medical Sciences, Shahroud, Iran

⁵Islamic Azad University of Chalous Branch, Chalous, Iran

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ABSTRACT

Objective: Rodents act as reservoir hosts and are an important potential source for many zoonotic pathogens such as parasites, which pose a public health risk to humans. Therefore, it is necessary to investigate the prevalence of parasites among rodents.

Methods: A total of 118 *Rattus rattus* were captured in Mazandaran province, north of Iran, using snap live traps. Various samples were collected from feces and each rat was combed with a fine-tooth comb to extricate any ectoparasite. Fecal specimens were examined by direct wet mounting, formalin-ether concentration, modified acid-fast, and trichrome staining methods.

Results: The overall prevalence of gastrointestinal parasites in the examined rats was 75.4%. *Cryptosporidium* spp. (30.5%) were the most prevalent protozoan, followed by *Giardia* spp. (20.3%), *Entamoeba muris* (13.5%), *Trichomonas muris* (10.1%), and *Spironucleus muris* (3.3%). Regarding helminths' eggs, *Syphacia obvelata* (24.5%), *Hymenolepis diminuta* (10.1%), and *Trichuris muris* (9.3%) had the highest prevalence, respectively. Furthermore, 3060 ectoparasites collected from 102 rodents were infested with lice (40% *Polyplax* spp.), mites (33.3%), and flea (16.1% *Xenopsylla cheopis* and 10.6% *Xenopsylla astia*).

Conclusion: According to the results of this study, the prevalence of ecto and gastrointestinal parasites in the collected rats in the area being studied was remarkably high. Additionally, *Rattus rattus* can be considered a potential risk to human health.

Keywords: Prevalence, rodent, gastrointestinal parasites, ectoparasites, Iran

ÖZ

Amaç: Kemirgenler, halk sağlığı riski oluşturan parazitler gibi birçok zoonotik patojen için rezervuar konak ve önemli potansiyel kaynak rolü oynadığından, kemirgenlerde parazitlerin yaygınlığının araştırılması önem taşımaktadır.

Yöntemler: İran'ın kuzeyindeki Mazandaran eyaletinde, anlık canlı tuzaklar kullanılarak toplam 118 sıçan-*Rattus rattus* yakalandı. Dışkularından numuneler toplanan her bir sıçan ayrıca ektoparazitler amaçlı inceleme için ince dişli bir tarakla taranarak toplandı ve incelendi. Dışkı örnekleri ıslak yayma, formalin-eter yöntemi ile incelendi ve modifiye aside dayanıklı boyama ve trikrom ile boyandı.

Bulgular: İncelenen sıçanlarda gastrointestinal parazitlerin genel prevalansı %75,4 idi. *Cryptosporidium* spp. (%30,5) en yaygın protozoon iken, bunu *Giardia* spp. (%20,3), *Entamoeba muris* (%13,5), *Trichomonas muris* (%10,1) ve *Spironucleus muris* (%3,3) izledi. Helmint yumurtalarının prevalansı, *Syphacia obvelata* (%24,5), *Hymenolepis diminuta* (%10,1) ve *Trichuris muris* (%9,3) şeklinde idi. Enfeste 102 kemirgenden toplanan 3060 ektoparazitlerin dağılımı ise, bit (%40 *Polyplax* spp.), akar (%33,3) ve pire (%16,1 *Xenopsylla cheopis* ve %10,6 *Xenopsylla astia*) şeklinde idi.

Sonuç: Çalışma alanında toplanan sıçanlarda ekto ve gastrointestinal parazit prevalansı oldukça yüksektir. Ayrıca *Rattus rattus*, insan sağlığı için potansiyel risk olarak değerlendirilebilir.

Anahtar Kelimeler: Prevalans, kemirgen, gastrointestinal parazitler, ektoparazitler, İran



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Address for Correspondence/Yazar Adresi: Fatemeh Rezaei, Islamic Azad University of Chalous Branch, Chalous, Iran
Phone/Tel: +01133543248 E-mail/E-Posta: rezaei1363@gmail.com ORCID ID: orcid.org/0000-0002-0635-127X

INTRODUCTION

Rodents are known to be reservoir hosts for at least 60 zoonotic diseases and play an important role in disease transmission, especially where these animals are in close association with human settlements (1). Rodents comprise nearly two-thirds of the world's mammal species identified and are prosperous in biological adapting to various situations (2,3). They live in various geographical areas, including sewage channels, slaughterhouses, waste disposal sites, farms, and food storage places. These animals are mostly small in size and reproduce very quickly. Over thousands of years, the presence of rodents, particularly murine, in the human environment has been considered a pest in the agricultural and urban environments causing economic losses. They act as reservoir hosts and an important potential source for many zoonotic pathogens that pose a health risk for humans and induce significant socioeconomic problems (4,5). Rats can transmit infective agent through bite, urine, feces, and ectoparasites (6,7). Parasitic zoonoses, such as *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma gondii*, *Entamoeba histolytica*, *Syphacia obvelata*, and *Hymenolepis diminuta* infect enormous numbers of humans and are responsible for huge morbidity and mortality around the world. Murine ectoparasites, such as fleas, lice, and ticks can usually live in close association with different types of rodents (8,9). Some of the ectoparasites are vectors of important pathogenic microorganisms, and some

others directly cause itching, ulcerated skin, hair loss, skin abrasion, and asthma (6). They can also be serious infectious agents for several parasitic zoonoses, plague, leptospirosis, rat-bite fever, Omsk hemorrhagic fever, and murine typhus (10).

Although rats are widely distributed in Mazandaran province, north of Iran, there is no data on the prevalence of ecto and gastrointestinal parasites in these animals (11). Concerning the public health risks of rats, for the first time, this study aimed to evaluate the prevalence of ecto and gastrointestinal parasites in rats in Sari, Iran.

METHODS

Ethical Approval

The study protocol was approved by the Institutional Research Ethics Committee of the Mazandaran University of Medical Sciences, Iran (approval number: 875).

Geographical Information on the Study Area

Sari is the provincial capital of Mazandaran, located in the north of Iran, between the slopes of the Alborz Mountains and the southern coast of the Caspian Sea (Figure 1). Its coordinates are: 36°33'48"N 53°03'36"E, and its population is 453,782 people. Sari has a hot-summer Mediterranean climate. Winters are cool and rainy, whilst summers are hot and humid. This city has a



Figure 1. The location of Mazandaran province (northeastern of Iran)

particular geographical condition, including many plains, prairies, and forests. There are broad agricultural areas, barns, and crop fields. These conditions provide proper climatic conditions and habitats for the breeding rodents.

Sample Collection

Using live traps, a total of 118 *Rattus rattus* were captured from different sites in Sari from January 2020 to February 2021. The rats were transferred to the Parasitology Laboratory of Mazandaran University of Medical Sciences. For each captured rat, sex, morphometric measurements, trapping location, and weight were recorded. Also, the animals were classified into juveniles (≤ 2 months old) and adults (≥ 2 months old) according to their body weight (borderline value: 200 gr).

Rodent Identification

Keys and illustrations developed by Harrison & Quah (1962) and Medway (1983) were used to recognize rodent species by morphological measurements and physical appearances.

Sample Examination

Rats were dissected following the protocol previously described (12). Fecal samples from gastrointestinal tracts were examined by direct wet mounting with saline and iodine. A saline wet smear was made by mixing almost 2 mg of stool with a drop of saline on a glass microscope slide. Likewise, the iodine wet mount was ready by adding nearly 2 mg of stool to a drop of Lugol's iodine on a glass microscope slide, and a cover slip was inserted on the stool suspension. Primarily, these wet smears were studied using a low-power (10 \times) objective and then by a high-power (40 \times) objective of a light microscope. In addition, for more accurate diagnosis of protozoa cysts, trophozoites, and worm eggs, a piece of fecal samples used for performing the formalin-ether concentration technique (FECT) and trichrome staining (13).

Cryptosporidium Oocyst Detection

All stool samples from the intestines were studied for the parasite oocysts, including *Cryptosporidium* spp. which were identified by microscopy after sugar flotation and modified acid-fast staining (14).

Examination for Ectoparasites

Each rodent was combed with a fine-tooth comb to extricate any ectoparasite into a tray. Fine forceps were used to pick up ticks

and mites from rodents' skin when it was hard to extricate them by combing. Bags, where rodents hold, were overturned to the tray to gather extricated ectoparasites. The tray contents were scrutinized with a hand lens, and ectoparasites were collected using the moistened applicator stick and placed in a collection tube comprising 70% alcohol. A distinct container was used for each rodent (2). Ectoparasites were identified by valid entomological keys (15,16).

Statistical Analysis

Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences) version 20. Univariate Fisher's Exact test and chi-square analysis was used to determine the association between the prevalence of parasites and host factors (age, gender, and area). A p-value < 0.05 was considered significant.

RESULTS

In the current study, we included 118 rats (average weight: 250 gr). It is noteworthy that 60.2% and 39.8% of rats were trapped in urban and rural areas, respectively. The rats (67 male vs 51 female) were categorized according to their age into two groups' juveniles (27 or 22.88%) and adults (91 or 77.12%). The prevalence of parasitic infection was higher among males (83.5%) than females (64.7%). However, there was no significant difference between prevalence of parasitic infection and gender ($p < 0/05$). Out of 118 samples, 89 (75.4%) were positive for intestinal parasites. The prevalence rates for intestinal protozoa and helminths were 80.5% and 44.02%, respectively. Tables 1, 2 show the prevalence of gastrointestinal and ectoparasites in collected rats based on sex, age, and habitat. Also, Table 3 shows the prevalence of isolated gastrointestinal parasites in rats.

Out of 118 samples, 86.4% were positive for ectoparasites, and 69.4% both ectoparasite infestation and endo-parasite infection. A total of 3060 ectoparasites collected from 102 rodents. They were infested with lice (40% *Polyplax* spp.), mite (33.3%), and flea (16.1% *Xenopsylla cheopis* and 10.6% *Xenopsylla astia*). Table 4 indicates the prevalence of ectoparasite infestation among the examined rats in Sari. Moreover, mixed infections were present in some rats.

Table 1. Prevalence of gastrointestinal parasites of rats based on age, sex and habitats

Demographic factors	Total sample	Positive cases	Positive percent	Confidence interval	p-value
Age					
Juvenile	27	20	74.04	(95% CI: 53 to 88)	0.85
Adult	91	69	75.8	(95% CI: 65 to 84)	
Sex					
Male	67	56	83.5	(95% CI: 72 to 91)	0.018
Female	51	33	64.7	(95% CI: 50 to 77)	
Habitats					
Rural	47	29	61.7	(95% CI: 46 to 75)	0.24
Urban	71	51	71.8	(95% CI: 59 to 81)	

CI: Confidence interval

Table 2. Prevalence of ecto-parasites in rats of different age, sex and habitats

Demographic factors	Total sample	Positive cases	Positive percent	Confidence interval	p-value
Age					
Juvenile	27	21	77.7	(95% CI: 57 to 91)	0.12
Adult	91	81	89.01	(95% CI: 80 to 94)	
Sex					
Male	67	58	86.5	(95% CI: 72 to 91)	0.96
Female	51	44	86.2	(95% CI: 76 to 93)	
Habitats					
Rural	47	44	93.6	(95% CI: 82 to 98)	0.063
Urban	71	58	81.6	(95% CI: 70 to 89)	

CI: Confidence interval

Table 3. Prevalence of gastrointestinal parasites among examined rats in Sari, North of Iran

Parasite (no. sample: 118)	Positive sample (no.)	Prevalence (%)
Intestinal protozoa		
<i>Cryptosporidium</i> spp.	36	30.5%
<i>Giardia</i> spp.	24	20.3%
<i>Entamoeba muris</i>	16	13.5%
<i>Trichomonas muris</i>	12	10.6%
<i>Spiroucleus muris</i>	4	3.3%
Intestinal helminth		
<i>Syphacia oblevata</i>	29	24.5%
<i>Hymenolepis diminuta</i>	12	10.1%
<i>Trichuris muris</i>	11	9.3%
Total	89*	75.4%

* 89 rats were infected with at least one parasite

Table 4. Prevalence of ectoparasites infestation among examined rats in Sari, North of Iran

Ectoparasites (no. samples: 118)	Positive rates (no.)*	Prevalence of ectoparasites (%)	No. isolated ectoparasites from all samples
Lice <i>Polyplax</i> spp.	50	40%	1224
Flea <i>Xenopsylla cheopis</i> <i>Xenopsylla astia</i>	34	16.1% 10.6%	492 324
Mite spp.	42	33.3%	1020

* Mix infections were present in some rats

DISCUSSION

Our study demonstrated a high prevalence of intestinal parasite infection (75.4%) and ectoparasite infestation (86.4%) in the examined samples. Intestinal parasites prevalence in the present study was significantly higher than the results from Iranshahr and Nikshahr districts in Sistan and Baluchistan province, southeastern Iran (47%) (17). However, it was similar to the reports from Ardabil province located in northwestern Iran (74%) (4), Belgrade area in Serbia (68.5%) (18), and Nile rat in rural and urban regions of Sudan (70%) (19).

Our data clearly demonstrate that there was no significant difference between prevalence of ecto and

gastrointestinal parasites and gender. However, it was higher among male than female rats; this might be attributed to the fact that infected males have greater territories than uninfected males, which could increase their exposure to infection (20). In addition, the higher prevalence of infection among male rats may be explained by the fact that larger bodies of males are easier targets for parasites (18).

According to our results, the prevalence of ecto and gastrointestinal parasites in adults' rats was slightly higher than juveniles' rats. An acceptable justification may be that adults have more exposure time than juveniles due to their age. In this study, *Cryptosporidium* spp. was the most prevalent protozoan (30.5%) observed in the

examined rodents. These findings were in agreement with the results of the previous study, which reported *Cryptosporidium* species in rats from Poland (68.1%), the Philippines (28.5%), and Australia (8.2%) (21-23). This protozoan was also found in the wild, laboratory, and pet rodents in China (11.5%) and Japan (27%) (14,24). Rodents are naturally infected with zoonotic *Cryptosporidium* spp. These animals have been considered potential reservoirs of Cryptosporidiosis in humans and farm animals because they are frequently found in agricultural areas and have opportunities to contact other animals and humans (25).

In the current study, the prevalence of *Giardia* spp. among rats was considerable (20.3%). In other studies, the prevalence of *Giardia* spp. was reported as 27% in Iran (11), 38.4% in southwestern Poland (21), 65.9% in Pennsylvania, the united states (26), and 48.3% in the Mazury Lake District region of Poland (27). Giardiasis is an intestinal infection with the protozoan flagellate parasite *Giardia* spp., which causes major public and veterinary health concerns. Giardiasis has variable clinical symptoms ranging from asymptomatic to acute or chronic diarrhea, dehydration, nausea, vomiting, abdominal cramps, disaccharide intolerance, weight loss, and malabsorption (28).

Spironucleus muris is another protozoan parasite observed in 3.3% of the examined samples. Although *S. muris* has been isolated from a few infected individuals, it is usually considered non-pathogenic for humans. However, in our study, its prevalence was lower than in the previous study conducted on laboratory mice in Iran (64.86%) (11). In addition, examining the gastrointestinal content of rodents showed infection with two nematoda and one cestode, but the infection was distinguished only by the presence of the eggs. The prevalence of *Syphacia obvelata*, a murine pinworm species, was 25.1% among rats examined in this study. *S. obvelata* can cause disease in humans (17). This parasite was reported among rats in South Korea (21.7%) (29) and in laboratory mice from Iran (48.6%) (11). In this study, *Hymenolepis diminuta*, also known as rat tapeworm, was detected in 10.1% of samples. Other researchers also reported this parasite among rats in southeastern Iran (23.4%) (17), Dashte-Mogan, Ardabil province, Iran (38.8%) (4), Belgrade, Serbia (30.5%) (18), and Baltimore, the United States (34.4%) (30). Man acquires the infection via ingestion of infected intermediate host (rat's flea). Although its mild infection is usually asymptomatic, severe infection may cause headache, dizziness, pruritis, diarrhea or occasionally cachexia in humans. In this study, the prevalence of *Trichuris muris* was 9.3%. The whipworm *Trichuris muris* (family: Trichuridae) is a gastrointestinal nematode parasite of house mice and rats (18). This parasite has also been reported among rats in different parts of the world (4,18,30).

Ectoparasites play an essential role in the spread of disease to humans and animals. For instance, fleas are important vectors to plague and murine typhus in many parts of the world, ticks are important due to their role in CCHF (Crimean-Congo hemorrhagic fever), theileriosis, babesiosis, anaplasmosis, and ehrlichiosis transmission, and lice are important for epidemic typhus or exanthematic typhus transmission. In the present study, 3060 ectoparasites collected from 102 rodents were infested with lice (40% *Polyplox* spp.), mite (33.3%), and flea (16.1% *Xenopsylla cheopis* and 10.6% *Xenopsylla astia*). In a study in the Baluchistan area, southeast of Iran, 67 individual rodents from four species of gerbil and jird (*Tatera indica* (55.2%), *Meriones hurrianae* (37.3%), *Gerbillus nanus* (4.5%), and *Meriones libycus* (3%) were captured

from 2008 to 2009. Out of 1,276 ectoparasites, 299 were related to mites, 127 to fleas, 972 to lice, and 24 to ticks (6). In another study which presented ectoparasites for four species of rodents *Rattus rattus palelae*, *R. argentiventer*, *R. exulans*, and *Mus musculus castaneus* in Sulawesi Utara, Indonesia, *Xenopsylla cheopis* flea was the most common in *R. rattus* (31).

Data from some studies have showed that the overall prevalence of flea infestation was higher than that of lice or mite. For instance, in a study in Bandar Abbas, southern Iran, ectoparasites were collected from 77 rodents (including *R. rattus*, *R. norvegicus*, *Mus musculus*, and hamster) and the rate of fleas isolated from rats was 87% (32). In a similar study conducted by El Kady et al. (33) in Egypt, among 135 captured rats (including *R. norvegicus*, *R. rattus frugivorous*, *R. rattus alexandrines* and *Mus musculus*), fleas were observed more prevalent than other ectoparasites.

CONCLUSION

According to the findings of this study, monitoring rodent populations and their ectoparasite infestation is essential for preparedness and early warning preparation for possible control of zoonotic arthropod-borne diseases. In our study, the prevalence of gastrointestinal and ectoparasites was remarkably high in the collected rats in the studied area. In addition, *R. rattus* can be considered a potential risk to human health due to collected gastrointestinal and ectoparasites. Therefore, controlling these animals in the study area is of particular importance. The results of the present study can provide information to the authorities for the prevention and control of rodent-borne diseases in the region. Further studies are required due to the considerable unexplored area of this province to increase knowledge on endo-parasites and ectoparasites in rats and potential zoonosis and veterinary diseases.

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* Ethics

Ethics Committee Approval: The study protocol was approved by the Institutional Research Ethics Committee of the Mazandaran University of Medical Sciences, Iran (ethics code: 875).

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* Authorship Contributions

Concept: A.D., F.R., Design: F.R., Data Collection or Processing: F.R., A.A., A.S.P., S.S., Analysis or Interpretation: M.S., M.T.R., Literature Search: F.R., M.T.R., A.S.P., Writing: F.R., A.D., S.S.

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