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Original Article

High Parasitic Infections in the Laboratory Animals: Complications for the Research Outcomes and Zoonotic Importance

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Abstract

Background: Laboratory animals are widely used for medical research; hence, their health status is a determinant of the outcome and success of the research. We aimed to investigate the possible infection of rodents with intestinal parasites in the Hamadan University of Medical Sciences animal house.

Methods: From October to November 2022, one hundred and twenty healthy laboratory rodents including 60 Wistar rats, 30 BALB/c mice, and 30 NMRI mice were randomly collected and examined for parasitic infections. The digestive tracts were removed and examined for infection by helminths and parasitic protozoa using naked eye inspection and microscopy.

Results: 75.8% (95% CI: 68.1–83.5%) of the rodents were infected by at least one helminth or protozoa. Protozoal infections (41.7%) with *Giardia* spp. (15%), *Trichomonas* spp. (10%), *Blastocystis* spp. (6.7%), *Entamoeba* spp. (5.9%), *Cryptosporidium* spp. (3.3%), and *Eimeria* spp. (0.8%) were observed relatively higher than helminthic infections (34.2%) with *Syphacia obvelata* (10.8%), *Syphacia muris* (10%), *Aspicularis tetraptera* (7.5%), *Hymenolepis nana* (3.3%), and *Hymenolepis diminuta* (2.5%).

Conclusion: This study showed high parasitic infection without clinical signs in laboratory rodents. Therefore, monitoring these animals and improving their breeding conditions can eliminate the adverse effects of these parasitic infections in the animal research processes and improve the health of researchers and staff.

Introduction

Laboratory animals are widely used in many fields of bio-medical sciences, including medicine, biology, veterinary

medicine, and pharmacy (1). Among laboratory animals, small rodents, including mice and rats, are the most commonly used



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animals for research (2). Infection of these rodents with various pathogens, such as parasites and especially zoonoses, can be a potential risk to people who are in close contact with these animals (3). Symptoms of disease caused by these parasites in the rodents are usually mild to moderate, and sometimes, no symptoms are observed, but in rare cases, some parasites can be fatal to the animals under study. Since laboratory rodents are kept in colonies and are in direct contact with each other, the infection can spread quickly throughout the colony (4, 5).

About 150 to 200 diseases may be transmitted from laboratory animals to humans, such as rat bite fever, tuberculosis, hemorrhagic fever, salmonellosis, lymphocytic choriomeningitis, leptospirosis, as well as various endo and ectoparasites (6-8). Infection with these agents can cause tissue damage by changing immunological, pathophysiological, biochemical, and hematological processes, stimulate abnormal tissue growth, change the volume of host body fluids, or even animal death, which directly or indirectly affects the results of research (9, 10). The animal houses of academic and research institutions, such as those affiliated with universities of medical sciences; provide a conducive environment for the study and maintenance of laboratory animals, including rodents (11). However, these facilities may inadvertently serve as reservoirs for a variety of parasites, including gastrointestinal parasites, thus posing potential risks for zoonotic infections among personnel working within these facilities (12). Moreover, parasitic infection can cause the death of the animals, or interpretation of research results becomes problematic (13).

Hamadan University of Medical Sciences located in the western region of Iran, houses laboratory animals for research and educational purposes. Despite the critical role of these facilities in advancing scientific knowledge and investigations using laboratory animals, there is a paucity of data regarding the prevalence and diversity of gastrointestinal

parasites among rodents inhabiting such environments in this region. Understanding the prevalence of these parasites is essential for implementing effective control measures and minimizing the risk of zoonotic infections, and improving the animal based research results (14).

Therefore, we aimed to investigate the gastrointestinal parasites of rodents.

Materials and Methods

Rodents

From October to November 2022, 60 Wistar rats, 30 BALB/c mice, and 30 NMRI mice of different sex and ages were randomly selected from the outed rodents available in the animal house of Hamadan University of Medical Sciences, Hamadan, west of Iran. Animals that were excluded from the experimental cycle for any reason and were maintained as other animals used for research were selected for the study. The use of animals was approved by the Ethical Committee of Hamadan University of Medical Sciences (Code: IR.UMSHA.REC.1401.771).

Parasitological assay

Rodents were transferred to the Parasitology Laboratory of Hamadan University of Medical Sciences, and their gastrointestinal tract (GI) was examined. Briefly, inside a plate containing phosphate-buffered saline (PBS), all contents of intestines and their lining membrane was gently scraped, and the contents were carefully examined by eyes and also with a stereomicroscope for the presence of helminths. Acetocarmine staining was used to identify isolated cestodes, and nematodes were transferred into 70% alcohol containing 5% glycerin, clarified with lactophenol, and identified by microscopy (5). Formalin-ether concentration method was used to identify intestinal protozoa and parasitic helminth eggs. Also, modified Ziehl-Neelsen staining was used to identify *Cryptosporidium* spp.

oocysts and trichrome staining was used to identify other intestinal protozoa (15, 16). The identification of parasites was performed using morphological characterization and valid taxonomic keys (16, 17).

Statistical evaluation

SPSS statistical software (Ver. 20) (IBM Corp., Armonk, NY, USA) and the chi-square test were used to analyze statistical associations between the data findings. The probability of $P < 0.05$ was significant with a confidence interval of 95%.

Results

A total of 120 rodents (86 males, 34 females), including 30 BALB/c mice, 30

NMRI mice, and 60 Wistar rats, were randomly collected for this study. In general, 75.8% (95% CI: 68.1–83.5%) of rodents were infected with at least one helminth or protozoa (Fig. 1). Helminth infection was found in 34.2% of rodents, whereas 41.7% of rodents were infected with protozoan parasites. Five species of helminths, including three species of nematodes and two species of cestodes, were identified. *Syphacia obvelata* and *S. muris* had the highest helminth infection (10.8% and 10%, respectively), followed by *Aspicularis tetraaptera* (7.5%), *Hymenolepis nana* (3.3%), and *H. diminuta* (2.5%). The highest helminth infection was recorded in BALB/c mice (56.7%), followed by NMRI mice (46.67%) and Wistar rats (16.7%) (Table 1).

Table 1: Helminth species of laboratory rodents in the animal house of Hamadan University of Medical Sciences, west of Iran

Helminth species	BALB/c mice (n=30)		Positive	NMRI mice (n=30)		Positive	Wistar rat (n=60)		Positive	Total n (%; 95% CI)
	Male	Female		Male	Female		Male	Female		
<i>Syphacia obvelata</i>	2	4	6	4	3	7	0	0	0	13 (10.8; 5.3–16.4)
<i>Syphacia muris</i>	0	3	3	1	1	2	4	3	7	12 (10; 2.8–12.2)
<i>Aspicularis tetraaptera</i>	2	3	5	2	2	4	0	0	0	9 (7.5; 4.6–15.4)
<i>Hymenolepis nana</i>	1	2	3	0	1	1	0	0	0	4 (3.3; 0.1–6.5)
<i>Hymenolepis diminuta</i>	0	0	0	0	0	0	2	1	3	3 (2.5; 0–5.3)
Total -	5	12	17	7	7	14	6	4	10	41 (34.2; 25.7–42.7)

In addition to helminth infection, protozoan infection was found in 41.67% of rodents. Six genera of intestinal protozoa were identified, and the highest infection rate was observed in Wistar rats (45%), followed by BALB/c mice (43.3%) and NMRI mice (33.3%). *Giardia* spp. infection was the most common (15%), followed by *Tritrichomonas* spp. (10%),

Blastocystis spp. (6.7%), *Cryptosporidium* spp. (3.3%), and *Eimeria* spp. 0.8% (Table 2). There was no significant relationship between the sex and breed type of rodents with individual helminth and protozoa infection ($P > 0.05$). All these findings were reported for the first time from laboratory rodents in Hamadan province, western Iran.

Table 2: Protozoa species of laboratory rodents in the animal house of Hamadan University of Medical Sciences, west of Iran

Protozoaspecies	BALB/c mice (n=30)		Positive	NMRI mice (n=30)		Positive	Wistar rat (n=60)		Positive	Total n (%; 95% CI)
	Male	Female		Male	Female		Male	Female		
<i>Giardia</i> spp.	3	3	6	3	1	4	1	7	8	18 (15;8.6–21.4)
<i>Tritrichomonas</i> spp.	1	2	3	2	2	4	3	2	5	12 (10; 4.6–15.4)
<i>Entamoeba</i> spp.	0	0	0	0	0	0	1	6	7	7 (5.8; 1.6–10.0)
<i>Blastocystis</i> spp.	0	2	2	1	1	2	1	3	4	8 (6.7;2.2–11.1)
<i>Cryptosporidium</i> spp.	1	1	2	0	0	0	0	2	2	4 (3.3; 0.1–6.5)
<i>Eimeria</i> sp.	0	0	0	0	0	0	0	1	1	1 (0.8; 0–2.5)
Total	5	8	13	6	4	10	6	21	27	50 (41.7; 32.8–50.5)

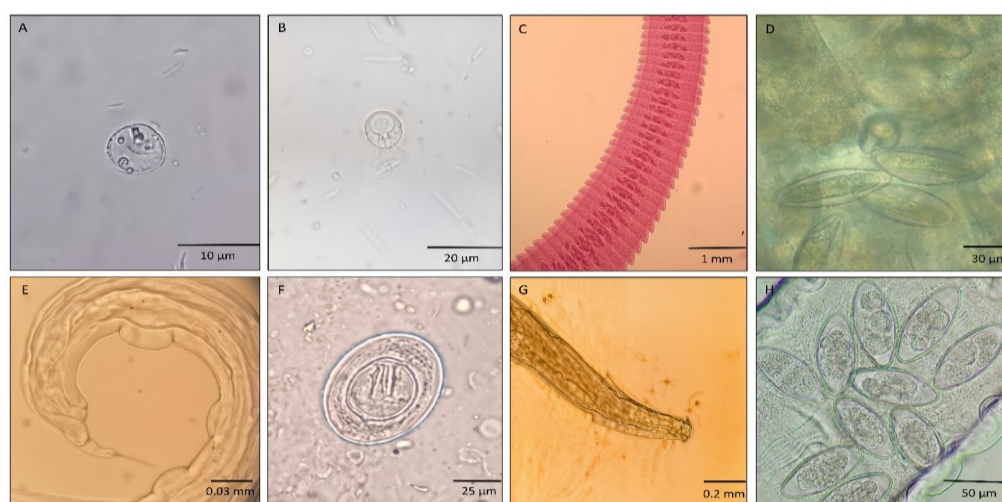


Fig. 1: Parasitic infections in laboratory rodents of the Hamadan University of Medical Sciences animal house. A: Cyst of *Blastocystis* spp., B: Trophozoite of *Giardia* spp., C: *Hymenolepis nana* mature proglottids, D: *Syphacia obvelata* eggs in the female uterus, E: Posterior end of *S. obvelata* male, F: Egg of *Hymenolepis nana*, G: Anterior end of *Aspiculuris tetraptera*, H: *A. tetraptera* eggs in the female uterus

Discussion

This study is the first report of infection of animal house rodents at the Hamadan University of Medical Sciences in west of Iran. In this study, 75.8% of the rodents were infected with at least one protozoan or

helminth species. These results are consistent with those of other studies conducted in different breeds of laboratory rodents in Iran (10, 18–22). *Syphacia* species, *Hymenolepis nana* and *Aspiculuris tetraptera* among helminths, and *Giardia muris* and *Cryptosporidium* spp. among protozoa are the most common parasites in

laboratory rodents, and only *A. tetraoptera* has no zoonotic significance (23). In a similar study conducted in the center of Iran, rats were infected with *H. diminuta* (36.1%), *E. muris* (6.3%), and *Cryptosporidium* spp. (1.3%), and mice were infected with *E. muris* (6.6%) (24). In another study in South Iran, mice were found to be infected with *H. nana* (50%), *A. tetraoptera* (90%), and *S. obvelata* (90%), and rats were infected with *S. muris* and *A. tetraoptera* (83.3%) (25). Heavy infection in these rodents can be caused by autoinfection, and there is no need for an intermediate host (26). In many studies, crowding has been mentioned as one of the most important risk factors in the occurrence of parasitic infection in animal houses (27). In addition, the unwanted entry of wild rodents into animal houses, especially the access of these animals to food storage areas, and failure to comply the hygiene regulations by animal house staff can be the other reasons for creating parasitic infections in laboratory animals (11, 28).

S. obvelata was the most common infection in this study (10.8%). Some studies have confirmed the zoonotic potential of this nematode (29, 30). The first human infection with *S. obvelata* was reported in 1919 in a child from the Philippines (31). In addition to the zoonotic importance of this parasite, hyperinfection in laboratory rodents can cause digestive disorders and, in severe cases, even death, which will affect on the results of the experiments performed on them (32).

H. nana was another helminth found in this study (3.3%). This cestode has been reported in laboratory rodents in Iran repeatedly (33). The release of surface antigens during the differentiation and growth of this cestode and other helminths produces multiple antibodies in rodents, which can cause immunological stimulation effects and ultimately disrupt the study types of laboratory rodents. In general, the prevention of zoonotic parasites in humans is directly related to their control in non-domestic animals and subsequently in

domestic animals, including rodents in research centers (1, 34, 35).

The highest protozoan infection in this study was *Giardia* spp. (15.8%), whereas it was 21.3% in laboratory rodents of three animal breeding centers in North Iran (21). *Giardia muris* infects a wide host range globally, including laboratory rodents, non-human primates, and humans; however, clinical signs or pathological lesions accompanying the organism are very low, although heavy infection in immunodeficient hosts can be dangerous (36). More studies are needed to determine the prevalence of parasitic infection in rodents kept in the animal houses of research and university centers in other parts of Iran to adopt control and prevention programs.

Although this study provides insights, some limitations should be mentioned. First, the study's scope was limited to a specific geographic location and animal facility, which may not fully represent the broader spectrum of gastrointestinal parasites found in rodents across different regions and facilities. Second, although commonly used, the detection methods employed, such as parasitological examination, may have limitations in sensitivity and specificity, potentially leading to underestimation or misidentification of certain parasite species. Additionally, the cross-sectional design of the study provides a snapshot of parasite prevalence at a single point in time without capturing potential seasonal variations or long-term trends. Future research employing more sensitive diagnostic techniques, like molecular methods, longitudinal studies, and broader geographic sampling, would contribute to a more comprehensive understanding of gastrointestinal parasites in laboratory rodents in Iran.

Conclusion

The results showed the high prevalence of parasitic infection in rodents of the animal house of Hamadan University of Medical Sciences. According to zoonotic potential of some identified parasites, the control and prevention of parasitic infection of rodents can be a factor in maintaining the health and safety of researchers and animal house personnel, as well as increasing the quality, validity, and accuracy of the study results conducted on rodents.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Ethics approval

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences (Code:IR.UMSHA.REC.1401.771).

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