Helminthic Infections of Laboratory Animals in Animal House of Shiraz University of Medical Sciences and the Potential Risks of Zoonotic Infections for Researchers

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Abstract

**Background:** Different parasitic diseases may be transferred from laboratory animals to human (zoonoses). The current study was designed to determine the helminthic infections in animal house of Shiraz University of Medical Sciences to prevent the possible transmission of zoonotic ones from laboratory animals to the staff and researchers.

**Methods:** Sixty laboratory animals including mouse, rats, Guinea pigs and rabbits were randomly selected and examined for any helminthic infections.

**Results:** 83.33% rats were infected with *Syphacia muris* and *Aspiculuris tetraptera*, 100% of Guinea pigs were infected with *Paraspidodera uncinata*, rabbits were infected with *Passalurus ambiguous* (40%), inbred BALB/C mice were infected with *Hymenolepis nana* (50%), *Aspiculuris tetraptera* (90%) and *Syphacia obvelata* (90%), outbred BALB/C mice were infected with *Hymenolepis nana* (50%), *Aspiculuris tetraptera* (90%), *Syphacia obvelata* (90%) and *C57BL/6* mice were infected with *Hymenolepis nana* (66%), *Aspiculuris tetraptera* (100%) and *Syphacia obvelata* (100%). Our study was revealed minimum and maximum infection frequency in rabbits and guinea pigs respectively.

**Conclusion:** It seems that low and unsuitable space of infected animals in mentioned research center was the main cause of distribution of infection among rats and mice in Animal House of Shiraz University of Medical Sciences.

**Keywords:** Animal house; Laboratory animals; Parasite infection, Sanitary barrier, Sanitary monitoring

Introduction

The use of living laboratory animals in experimental biomedical researches has provided knowledge to better understanding physiological and pathological processes in both man and other animals.\(^1\)\(^2\) Experimental results from research on living laboratory animals are affected by environmental and infectious conditions.\(^3\)\(^4\) About 150 to 200 diseases may be transferred from laboratory animals to human (zoonoses) including rat bite fever, tuberculosis, leptospirosis, lymphocytic choriomeningitis, salmonellosis and hemorrhagic fever as well as various parasitic worms such as *Hymenolepis nana*, *Hymenolepis diminuta*, *Syphacia muris*, *Syphacia obvelata*, *Physaloptera*, *Schistosoma*, *Trichinella*.\(^5\)

Considering the importance of healthy condition of living laboratory animals for research and researchers, periodical monitoring of these animals for evaluation and presence of parasites, viruses, bacteria, fungi and genetic disorders is very important.\(^1\)\(^6\) So,
the aim of present study was evaluation of helminthic infections of laboratory animals of Animal House at Shiraz University of Medical Sciences in Shiraz, southern Iran in order to prevent the possible transmission of zoonotic helminthic infection between laboratory animals and personnel or researchers.

Materials and Methods

Sixty adult laboratory animals were randomly chosen from both sexes in Animal House of Shiraz University of Medical Sciences in Shiraz, southern Iran, (12 Sprague dawley rats; 6 C57BL/6 mice; 10 outbred BALB/C mice; 10 inbred BALB/C mice; 12 Short Hair England guinea pigs and 10 Dutch rabbits). Their average weights were 220±20 gram, 20±4.99 gram, 20±5.50 gram, 20±5.50 gram, 700±45 gram, and 2500±300 gram respectively. The animals were housed at a temperature of 24±2°C and relative humidity of 40% to 70% with weekly floor exchange. They had free access to water and standard pelleted laboratory animal diets. A 12:12 light: dark cycle was followed in the mentioned Animal House Center.

All animals were submitted in an appropriate chamber and sacrificed humanely by placing them in a small container with ether using standard Animal ethics of Iran. Consequently, necropsy was done for all animals. The animal’s gastrointestinal (GI) tracts were completely removed after suture of beginning and terminal portions of GI tract with silk (No. 3/0 and 0 [Silk HEILING, design in Germany]) and were set in 10% formalin solution in plastic container. Samples were immediately submitted to Helminthology Laboratory of Parasitology and Mycology Department at Shiraz Medical School and the lumen contents were examined using different sieves including 80 mesh. The emulsified stool was placed on a slide and observed under the microscope. Iodine was used to stain eggs. Eggs, larvae and adult worms were diagnosed by light microscopy.\(^7\) Collected worms were transferred into the small glass bottles containing 70 degree alcohol with 5-10% of glycerine.\(^8\) The worms were cleared and stained by FAAL solution (formaldehyde, pure ethyl alcohol, azocarmine, lactophenol) dye.\(^9\) Some worms were stained by Carmine and mounted by Canada balsam.\(^10\) The different parts of worms were measured using micrometry. The worm’s identification were determined using different keys.\(^11,12\)

Results

Our results were summarized in Table 1. Out of 12 rats, 10 (83.33%) were infected to two types of parasites from nematoda, and 2 (16.6%) were free from any infection. All of the examined Guinea pigs (100%) were infected to *Paraspidodera uncinata* (Figures 1-3). Among the rabbits, frequency of infection to Passalurus ambiguus (only identified parasite) were 40% (Figure 4 and 5). There were three types of parasites in inbred BALB/C mice and outbred BALB/C mice including *Hymenolepis nana* (Figures 6-8), *Aspiculuris tetraptera* (Figure 9 and 10) and

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>Genus/species of parasite</th>
<th>No. of animal</th>
<th>No. of infected animal</th>
<th>Infection frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td><em>Syphacia muris</em></td>
<td>12</td>
<td>10</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td><em>Aspiculuris tetraptera</em></td>
<td>12</td>
<td>10</td>
<td>83.3</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td><em>Paraspidodera uncinata</em></td>
<td>12</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Passalurus ambiguus</td>
<td>10</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>BALB/C mice</td>
<td><em>Hymenolepis nana</em></td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>(Inbred)</td>
<td><em>Aspiculuris tetraptera</em></td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td><em>Syphacia obvelata</em></td>
<td>10</td>
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<td>90</td>
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<tr>
<td>BALB/C mice</td>
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<td></td>
<td><em>Syphacia obvelata</em></td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>C57BL/6 mice</td>
<td><em>Aspiculuris tetraptera</em></td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td><em>Syphacia obvelata</em></td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
</tbody>
</table>
Fig. 1: *Paraspido nella uncinata* anterior portion

Fig. 2: *Paraspido nella uncinata* posterior portion of male

Fig. 3: *Paraspido nella uncinata* egg

Fig. 4: *Passalurus ambiguous* anterior portion

Fig. 5: *Passalurus ambiguous* egg

Fig. 6: *Hymenolepis nana* Scolex,
Syphacia obvelata (Figure 11) that their types and frequencies were shown in the Table 1. Similarly, C57BL/6 mice were infected with different distributions. In Rats, there were two types of parasites including Syphacia muris (Figure 12) and Aspiculuris tetraperta. Our study revealed minimum and maximum infection frequency in rabbits and guinea pigs respectively. However, there were more kinds of parasite species in mice. Hymenolepis nana was detected from different mice.

Discussion

Application of laboratory animals free from pathogens should be considered into mind and is a priority especially in research departments. Parasitic infections in laboratory animals, even in the absence of
clinical signs, may act as an important variable during experimental assays as well as a potential of infecting personnel and researchers. In conventional animal facilities, rodent colonies are frequently infected with helminths or become infected in the laboratories where they are maintained in the course of experiments. In the present study, sixty laboratory animals were studied from viewpoint of infection to intestinal worms. Presence, type and frequency of parasites (Aspiculuris tetraptera, Hymenolepis nana and Syphacia obvelata) were similar among the studied mice. The most frequent infection (100%) was seen in C57BL/6 mice. Also, Hymenolepis nana had maximum frequency in this species (66%). However, genomic similarity indexes from various isolates of Hymenolepis nana has showed that no differences were obtained between H. nana from rat or mouse. This is an important finding as this parasite is capable of infecting other animals as well as human. Aspiculuris tetraptera was detected in rats as well as mice and Syphacia obvelata and S. muris were observed in mice and rats. Syphacia obvelata was also detected more in C57BL/6 mice. High frequency of Syphacia obvelata and Spyhacia muris showes spread of these helminthes in colonies which had kept under conventional conditions. The age of the mice to be utilized is a parameter to be considered in the evaluation of pre-existing worm burdens in these experimental animals. Despite our study (evaluation of adult mice), other studies showed, infections with S. obvelata generally occur in young mice, since adults seem to be more resistant. Thus, 4-5 weeks mice should be investigated. Others reinforce the acquired resistance to infection between the 4th and 9th weeks after birth, whereas infections with A. tetraptera affect older mice. Taff’s reported the age related resistance in mice, associated with an increase of mucus production and to the natural aging physiological process; nevertheless, no specific immune response was detected. The high frequency of S. obvelata in comparison with A. tetraptera worm burdens presently observed is not in accordance with Rosas that reports higher frequencies of the latter species. Nevertheless, this high frequency of S. obvelata in rodent colonies is justified considering the nematode lifecycle, that is shorter in this species, thus inducing the infection in a larger number of mice in short periods. Gonçalves et al. observed associations of S. obvelata and A. tetraptera in outbred and inbred mice. This parameter is to be analyzed with basis on the population dynamics of pinworms in mice, as previously reported.

Hymenolepis nana, a commonly found parasite in mice and colonies kept under conventional conditions was detected in the animal house. It is important to remark that this parasite has a zoonotic potential and is characteristic of autoinfection and direct cycle contribute to maintain the high prevalence of animal infection in the colonies. Therefore, infected animals are not recommended for research use due to the risk of transmission among technicians and researchers who handle the colonies. Besides, this parasite may affect experimental results of investigation involving gastrointestinal, hematological, immunological and nutritional issues. Parasitic prevalence was partially less in rabbits compared to other animals and 100% of infection was seen in Guinea pigs. Infected animals are not indicated for experimental design due to a possible negative influence on experimental results. Although most of these infections are subclinical, they are relevant as they are able to affect the animal physiology, leading to changes in immunological, histological, nutritional, biochemical, and hematological parameters, besides affecting susceptibility to other infectious agents. After evaluation of rats and mice cages, it was noticed that the number of animals in the cages were not at a standard level. This condition was prevalent among rats but with a lower severity. Lawlor reviewed data related to cage size and concluded that to avoid harm to the rat (such as foot lesions and premature death), large rats (over 900 g) require 1800 cm² of floor space when housed singly, or 1000 cm² per rat (as well as 30 cm of cage height) when housed in a group. Also, according to Van de Weerd et al. study, a group of eight mice was
kept in 840 cm². The rabbits were kept singly in discrete cages leading to a lower infection distribution. The results of the current study indicate the need of massive investment on laboratory animal science and technology (physical environment, equipment, human resources qualification implementation of strict sanitary barriers and sanitary monitoring) in the animal houses for enhancement of quality of living laboratory animals for biomedical research and decrease of infection transmission to human as well as other laboratory animals. Furthermore, quarantine programs are also needed for new animals or biological materials.

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**Conflict of interest:** None declared.

## References

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