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Parasites and Parasite Eggs Detected in Laboratory Mice in Bursa

Bursa'da Laboratuvar Farelerinde Tespit Edilen Parazitler ve Parazit Yumurtaları

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ABSTRACT

Objective: A mouse infected with parasites is not a suitable model for use in experiments, and therefore, it is necessary to know whether it is infected. In this study, the aim is to investigate the endo and exoparasites in BALB/c laboratory mice.
Methods: In this study, the presence of parasites in 250 mice obtained from Bursa Uludağ University Experimental Animal Centre was investigated by faecal flotation and cellophane tape methods. In addition, helminths recovered during necropsy of selected mice were examined to confirm species identification.
Results: According to the results of the research, ectoparasites found in mice were mites such as *Otodectes cynotis*, *Myobia musculi* and *Myocoptes musculinus*, and endoparasites were nematodes of the species *Syphacia obvelata*, *Syphacia muris* and *Aspiculuris tetraptera*. In addition to adults of these species, many unidentified parasite eggs were also found. An *Aspicularis tetraptera* nematode exhibiting an unusual cervical alae structure not previously described in the literature was detected.
Conclusion: Although helminths from the *Strongylidae* and *Heligmosomidae* families were not found in the necropsy, helminth eggs belonging to this family were found in the fecal flotation. This study has presented different parasites detected in laboratory mice and original images were presented for some samples with unusual morphological structures.
Keywords: *Aspicularis tetraptera*, Bursa, ectoparasites, laboratory mouse, *Syphacia*

ÖZ

Amaç: Parazitler ile enfekte bir fare, deneylerde kullanmak için uygun bir model değildir ve bu nedenle enfekte olup olmadığının bilinmesi gerekir. Bu çalışmada BALB/c laboratuvar farelerinde bulunan endo ve ekzoparazitlerin araştırılması amaçlanmıştır.
Yöntemler: Bursa Uludağ Üniversitesi Deney Hayvanları Merkezi'nden temin edilen 250 faredeki parazitlerin varlığı fekal flotasyon ve selofan bant yöntemleriyle araştırılmıştır. Ayrıca, seçilen farelerin nekropsilerinden elde edilen helmintler tür teşhisi yapılması için incelenmiştir.
Bulgular: Yapılan araştırmanın sonuçlarına göre, farelerde bulunan ektoparazitler; *Otodectes cynotis, Myobia musculi* ve *Myocoptes musculinus* gibi akarlar, endoparazitler ise *Syphacia obvelata, Syphacia muris* ve *Aspiculuris tetraptera* türündeki nematodlardı. Bu türlerin erginleri yanı sıra çok sayıda teşhis edilemeyen parazit yumurtasına da rastlanmıştır. Ayrıca literatürde tanımlanmamış farklı bir servikal alae yapısı sergileyen *Aspicularis* cinsi bir nematoda rastlanmıştır.
Sonuç: Nekropside, *Strongylidae* ve *Heligmosomidae* ailesinden helmintler bulunamamış olsa da, fekal flotasyonda bu aileye ait helmint yumurtalarına rastlanmıştır. Bu çalışma ile Bursa'da laboratuvar farelerinde saptanan farklı parazitler ortaya konmuş ve ilginç morfolojik yapılara sahip bazı örnekler konusunda orijinal görseller sunulmuştur.
Anahtar Kelimeler: *Aspicularis tetraptera*, Bursa, ektoparazitler, laboratuvar faresi, *Syphacia*

INTRODUCTION

The laboratory mouse is one of the most frequently used model organisms in experimental research due to its physiological similarity to humans. Some parasites carried by mice are known to affect the immune system (1). In addition, some parasitic infections cause observable changes in the behavior of mice (2). Coprophagic mice serve as ideal hosts for sustaining parasite life cycles; however, infected mice are unsuitable for experimental use, making infection screening essential. For these reasons, it is helpful to

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have information about the parasite load of mice before *in vivo* studies with mice.

Laboratory mice can be infested with different types of external parasites. Due to the environment they are in, infestation with mites is generally observed. *Myobia musculi*, *Myocoptes musculinus* and *Radfordia affinis* are more common among these mites, while *Demodex musculi* and *Ornithonyssus bacoti* are less common species. None of these ectoparasites are zoonotic. These mites feed on intercellular fluid and skin fragments (3).

Internal parasites that can be seen in laboratory mice are of two groups: protozoa and helminths. Cestode parasites are very rare in laboratory mice with modern conditions, while pinworms are much more common. *Hymenolepis diminuta*, *H. nana* and *Rodentolepis microstoma* are the three cestode species that can be seen in laboratory mice (3).

Syphacia obvelata is the oxyurid nematode that most commonly infects laboratory mice. Eggs are approximately 134x36 µm in size and are banana-shaped. *Syphacia muris* and *Trichuris muris* are other important species that less commonly infect laboratory mice. Simultaneous infection with both *S. obvelata* and *S. muris* is very rare. *Aspiculuris tetraptera* is another pinworm species most commonly found in laboratory mice. *Heligmosomoides polygyrus* is routinely used for parasitic immunology and the evaluation of the effects of anthelmintic compounds (3,4).

Protozoan parasites can be found in the intestines or tissues. Those found in tissues include *Klossiellla*, *Hepatozoon*, *Babesia*, *Toxoplasma* and *Plasmodium*. These are almost never found in modern laboratory mice. Only *Spironucleus muris* is occasionally found in modern laboratory mice, but *Giardia muris* and *Crytosporidium muris* are extremely rare. Non-pathogenic enteric protozoan species include *Entamoeba muris*, *Chilomastrix bettencourti* and various species of trichomonads (3).

The aim of this study is to determine the parasite species carried by some laboratory mice used in Bursa and to present original visual data on their morphology.

METHODS

Preparation of Experimental Animals

The experimental animals used in this study are the BALB/c type of mice known as laboratory mice, *Mus musculus albinus*. These albino mice are 1-2 years old, weigh approximately 28-40 g, are sensitive to light and are active at night. Fifty-four BALB/c mice of both sexes were obtained from the Bursa Uludağ University Experimental Animal Breeding and Application Center. Mice infected with oxyurid nematodes were selected from 250 mice by cellophane tape and fecal flotation methods. The reason for the special selection of mice infected with oxyurid nematodes was that these mice would be treated with extracts that were thought to have anthelmintic effects. For studies on extracts (5-7), images of various endo- and ectoparasite eggs observed in the selected mice were also recorded while the mice were being cared for. The animals were kept in standard polypropylene cages at 20-24 °C, 55% relative humidity, and fed ad libitum with standard pellets and tap water (Figure 1).

All experiments were approved by the Bursa Uludağ University Experimental Animals Local Ethics Committee (decision no: 2017-10/07, date: 11.07.2017). A total of 54 mice (approximately 30 cages) were kept by washing their cages every two days and renewing their food, water and bedding. The cages were washed with water only and dried with paper towels. The waterers were washed with boiling water, salt and alcohol and rinsed thoroughly.

Detection of Parasite Eggs

Perianal Tape Method

The cellophane tape method is recommended especially for the determination of *S. obvelata* infection (8). In order to apply this method, approximately 9 cm long cellophane tape was pressed on the perianal region of the mouse 10-15 times and stuck on the microscope slide, and then parasite eggs and ectoparasites were examined (Figure 2). After the tape was cut with a pair of scissors and stuck on the slide properly, all samples were brought to the parasitology laboratory. Eggs and parasites were examined along the slide with a 5x objective. Pictures of some parasite eggs were taken with a light microscope. The preparations are kept in the parasitology laboratory where the study was conducted.

Flotation Method with Fulleborn Technique

To apply the fecal flotation method, mice were placed in separate cages and a single mouse was kept in each cage during the experiment. Fecal materials were collected from each mouse cage with forceps and fecal samples were placed in sterile plastic sample containers previously numbered according to the number of each mouse and wrapped in labeled nylon and transported from the Experimental Animal Center to the parasitology laboratory.

Fecal samples were collected in plastic cups numbered with each mouse number. Saturated salt water solution prepared and cooled beforehand was used for fecal flotation. A saturated salt water was added little by little to the stool and it was suspended with a plastic baguette. The suspension was filtered into another container with a wire strainer and salt water was added so there was a 1-2 cm gap in the container. Two coverslips were placed on it so that they would float and waited for 15-20 minutes. The coverslip was lifted parallel to the water surface with forceps and placed on the slide, taking care not to drop, and examined under a microscope at 5× and 10× magnifications.

The studies of Pritchett (4) were used for the identification of eggs, and Hedrich (3) was used for the identification of external parasites.

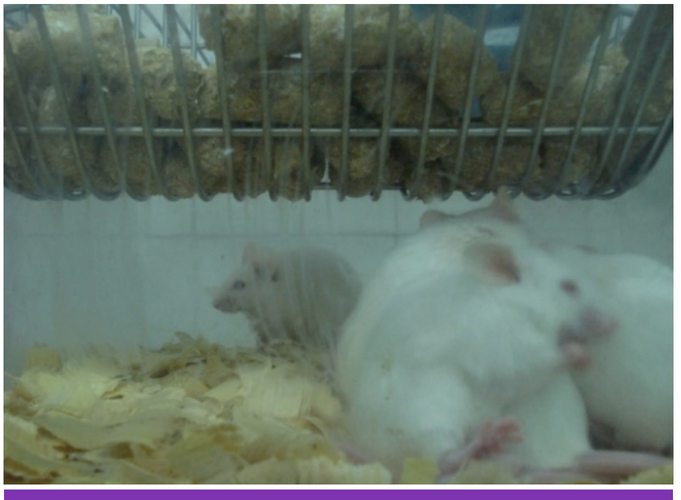

Figure 1. A group of experimental mice

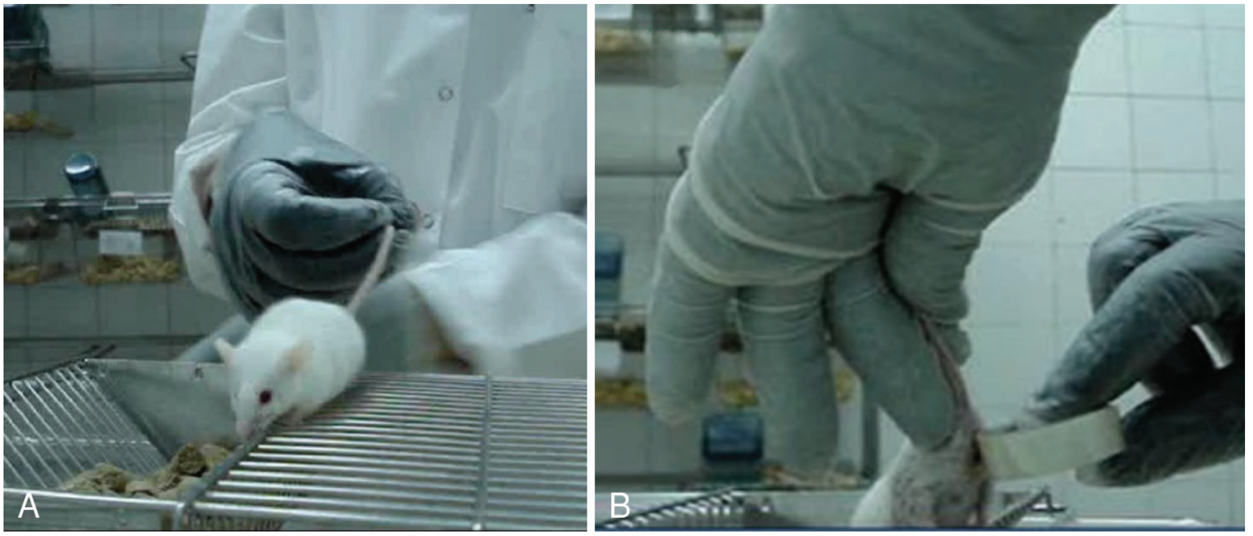

Figure 2. Application of the perianal tape method alone to laboratory mice. **A)** Each mouse is placed in its cage, **B)** The reverse-wound cellophane tape is pressed 15 times on the mouse rectum along the 9 cm tape

Detection of Helminths at Necropsy

In order to show the location of the abdominal organs removed during the necropsy phase of this study, the abdominal organs of *Mus musculus* are schematically outlined and given in Figure 3.

Mice were euthanized by taking blood from the heart under sevofluron anesthesia. Each mouse was laid down with its abdomen facing up and opened with scissors from the anus to the anterior. All internal organs of the mice (esophagus, lungs, stomach, liver, kidneys, heart and intestines) except the brain, pancreas and reproductive organs were opened one by one and placed in petri dishes containing saline or 70% alcohol and examined under a stereomicroscope. Helminths in these organs were collected using a brush, dropper and needle tip and placed in labeled vials containing 70% alcohol. The date of collection, helminth species, mouse number from which they were collected and the name of the organ from which they were collected were written on each vial.

Helminths suitable for examination under the light microscope were used in glycerin water to identify species and take their pictures under a Nikon brand light microscope. The information given by Pritchett (4) was used as a key for identification. All helminths were fixed on slides with the numbers of mice they were taken from and turned into permanent preparations. In order for the glycerin to enter between the slide and the coverslip, alcoholic water was drawn from one end of the coverslip with blotting paper, while glycerin was slowly dropped from the other end to fill the coverslip and replace it with the alcoholic water. After this, the excess material on and around the coverslip was cleaned with blotting paper again, transparent nail polish was applied to the edges of the coverslip and left to dry. All preparations are kept in the Parasitology Laboratory of the Biology Department of the Bursa Uludağ University Faculty of Arts and Sciences.

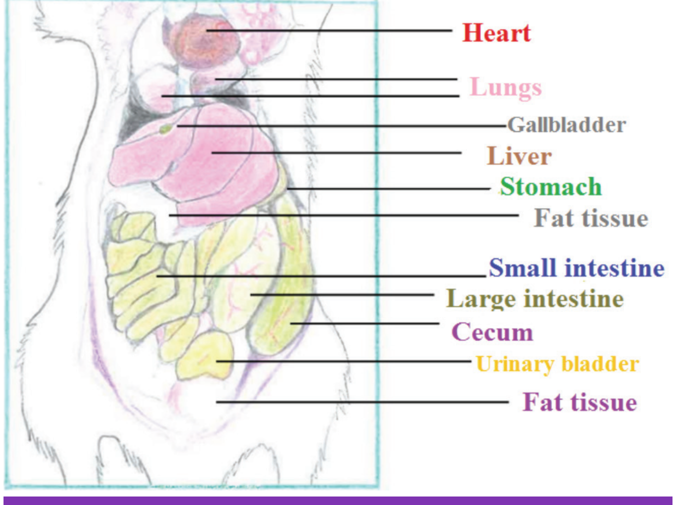

Figure 3. Abdominal organs of the mouse (original)

Statistical Analysis

The infection rate with the oxyurid nematodes among laboratory mice was calculated, and a chi-square test was conducted on the data of infected mice.

RESULTS

A total of 808 oxyurid nematodes were found in the small intestine, cecum and colon of laboratory mice. Of the 250 mice screened for infection, 54 (21.6%) were naturally infected with at least one nematode species. The infection rate in female mice was 16% (24/150), while the infection rate in male mice was higher than in females, at 30% (30/100). The significance of this data between genders was also confirmed by the chi-square test ($p<0.05$).

In fecal flotation tests, approximately 2000 parasite eggs were counted for some mice. These mice were numbered 36E, 43E, 21E, 42E and 40E. Since the letter E represents a male mouse, it was observed that all the most severely infected mice were male mice.

Helminth Eggs Observed in Fecal Flotation

In this study, the main criterion for the investigation of parasite eggs was the morphology of the eggs. As can be seen in the images below, different morphologies were encountered in the microscope images of the eggs. Although it is easy to distinguish the elliptical morphology of *Aspicularis tetraptera* eggs and the banana-shaped *Syphacia obvelata* eggs, it is quite difficult to distinguish ectoparasite eggs and some helminth eggs by looking only at their morphology. Nevertheless, when the egg sizes and shapes are considered, an egg belonging to the *Heligmosomoides polygyrus* species and another egg belonging to the *Strongylidae* family were found. Although these species are very rare in laboratory mice, these infections can still be encountered in mice. Images of these nematode eggs are shown in Figure 4.

Some parasite eggs could not be identified due to their different morphologies. Although the egg in Figure 5A resembles an *A. tetraptera* egg at first glance, genetic analyses are required for the identification of the two eggs in Figure 5.

When various parasite eggs stuck to the cellophane tape were examined, it was seen that the majority of them were *S. obvelata* eggs. Ectoparasite eggs were seen in some samples. Although they resembled *S. obvelata* eggs in shape, they were slightly larger in size and had dark granular structures inside (Figure 6).

Ectoparasite Eggs Observed in Fecal Flotation

A large number of insect eggs were encountered in fecal flotation and some of them could be identified. Various egg types in the developmental stages of insects and the condition of the embryo inside the transparent egg shell are shown in Figure 7.

Ectoparasites Observed in Fecal Flotation and Cellophane Tape

Some of the ectoparasites observed in fecal flotation and cellophane tape during the screening of mice for pinworm infection were photographed. Two different species of mite, *Myocoptes musculinus* and *Myobia musculi* were identified according to literature (3). Females can be seen carrying eggs, and are larger than males (Figure 8).

In insects, molting is inevitable at some stages of development. Figure 8G shows a *Myobia musculi* photographed while performing this process. The ectoparasite shown in Figure 8F, identified as a male *Myocoptes musculinus*, appears shrunken, likely due to osmotic effects of the saline solution. In addition to these insects, various mites were also encountered both in the fecal flotation and in the cellophane tape. A picture of the *Otodectes cynotis* mite is given in Figure 9.

Helminths Observed at Necropsy

A large number of nematodes were found in the necropsies of mice. Some of them show morphological features different from those normally expected. Figure 10 shows the interesting anterior structure of a nematode shrinking in salt water. It is difficult to determine the sex of this specimen because its posterior part has been severed by the scalpel. Still, this type of annular cervical alae structure can be found in the genus *Aspicularis*. No nematode similar to this specimen was found in the literature review. Since nematodes other than *Syphacia obvelata*, *S. muris* and *A. tetraptera* are rarely detected in laboratory mice, the specimen in Figure 10 was not expected to be an alien species belonging to a genus other than *Aspicularis* or *Syphacia*.

Normal morphological characters are observed in the *A. tetraptera* specimens shown in Figure 11. There is a developed bulbous cervical alae around the lips. The body of the male individual is thinner and smaller than the female. The appearance of the tail in male *A. tetraptera* is very characteristic, the cloacal apparatus on the tail is clearly visible, allowing for reliable sex determination.

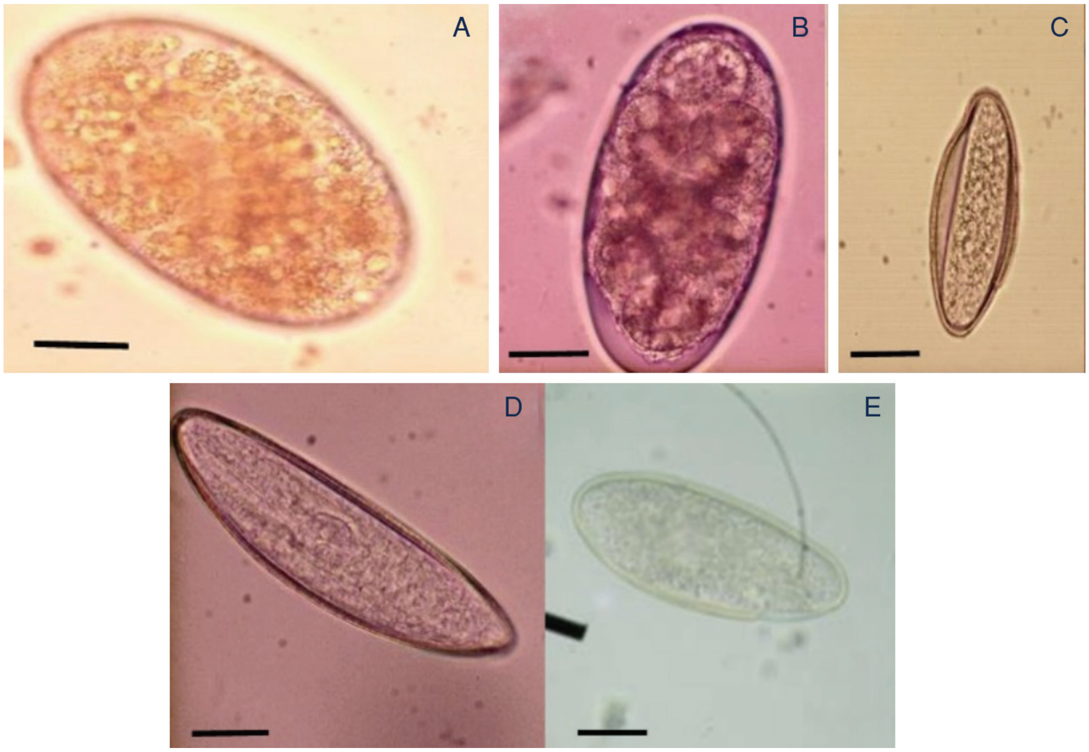

Figure 4. Helminth eggs detected in the faeces of laboratory mice (scale bar: 20 µm): **A)** *Heligmosomoides polygyrus*, **B)** *Strongylidae*, **C)** *Aspicularis tetraptera*, **D)** *Syphacia obvelata*, **E)** *Syphacia muris*

Females were carrying eggs in many of the examined specimens. The numerous eggs carried by this type of nematode under its transparent body cover could be distinguished by its characteristic elliptical appearance (Figure 11B). A veil-like cervical alae structure is characteristic in female *A. tetraptera* specimens (Figure 12B), but this structure was not observed in *S. obvelata* adults. Interestingly, an asymmetrically developed cervical alae structure was observed in the female *Syphacia* specimen (Figure 13A). In another female *Syphacia* nematode, a vulva structure that protrudes outwards was observed, unlike other females (Figure 13B). Many specimens of the genus *Syphacia* were egg-free, while one specimen was recorded during spawning (Figure 14).

In Figure 15, the body parts of female and male adults of *S. obvelata* and *A. tetraptera* are shown schematically.

DISCUSSION

Oxyurids (pinworms) are cosmopolitan nematode parasites of public health importance (9). Mild infection in animals is usually subclinical, but intense parasite infection can cause decreased activity, weight loss and sometimes intestinal lesions (10,11). In addition to the negative impact on animal health, some members of the Oxyuridae family have zoonotic potential, but do not cause significant harm (4). Among the oxyurids, only *S. obvelata* is known to be transmitted to humans (12). Male mice carry more parasites in pinworm infections than females (13), and this finding was confirmed in our study, where 30% of males and 16% of females were affected.

A. tetraptera and *Syphacia* sp. are located in the large intestine (14). In this study, the fact that in some examples *Syphacia* eggs were not found on cellophane tape and that such eggs were found in the fecal flotation of mice indicates that *S. obvelata* does not always migrate to the anus to lay eggs. In addition, a picture showing that it lays eggs in the large intestine (Figure 14) supports this.

In a previous study, laboratory rats and mice in Ankara were investigated for *Syphacia* infection and only *S. obvelata* was found in the mice, and the infection rate was found to be between 21 and 100% (15). In the current study, both *S. obvelata* and *S. muris* eggs were observed in laboratory mice in Bursa. However, *S. muris* infection was detected in only one out of the 250 mice (0.4%) examined. Among the 250 mice scanned, at least one instance of either *S. obvelata* or *A. tetraptera* infection was found in 54 of them (21.6%).

Another study conducted in Ankara between 2011 and 2012 revealed that all 283 BALB/c mice cared for in the laboratory were infected with at least one of the helminths *A. tetraptera*, *S. obvelata* and *H. nana*. *A. tetraptera* was found in all mouse cages (16). As for the current study, although the majority of infected mice were infected with *A. tetraptera*, this rate is not 100%.

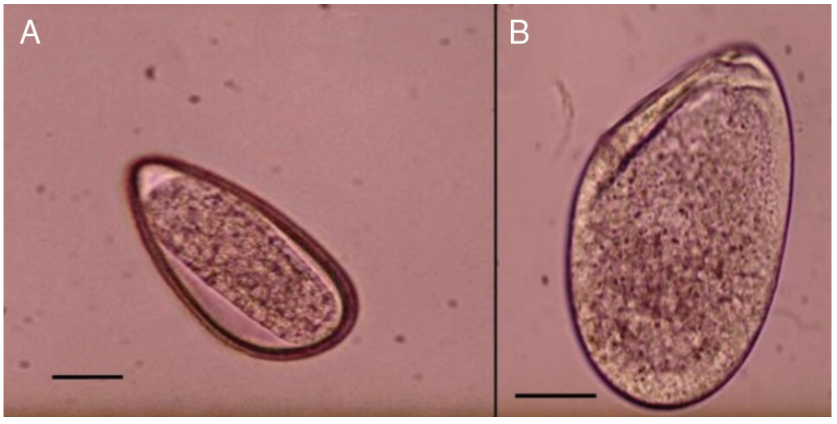

Figure 5. A,B) Two unidentified eggs detected in faecal flotation (scale bar: 20 µm)

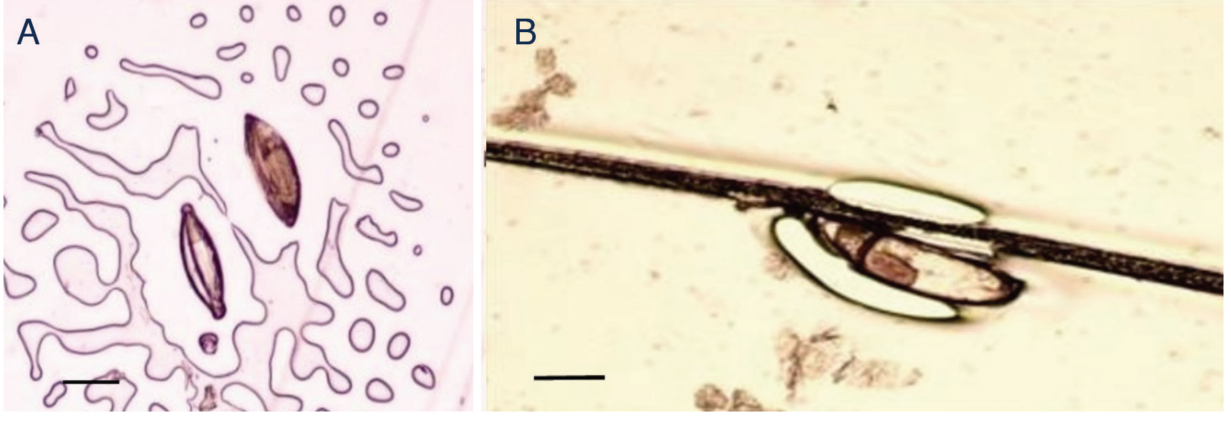

Figure 6. Parasite eggs detected on cellophane tape (scale bar: 50 µm). **A)** *Syphacia obvelata*, **B)** A mite egg stuck to a mouse hair

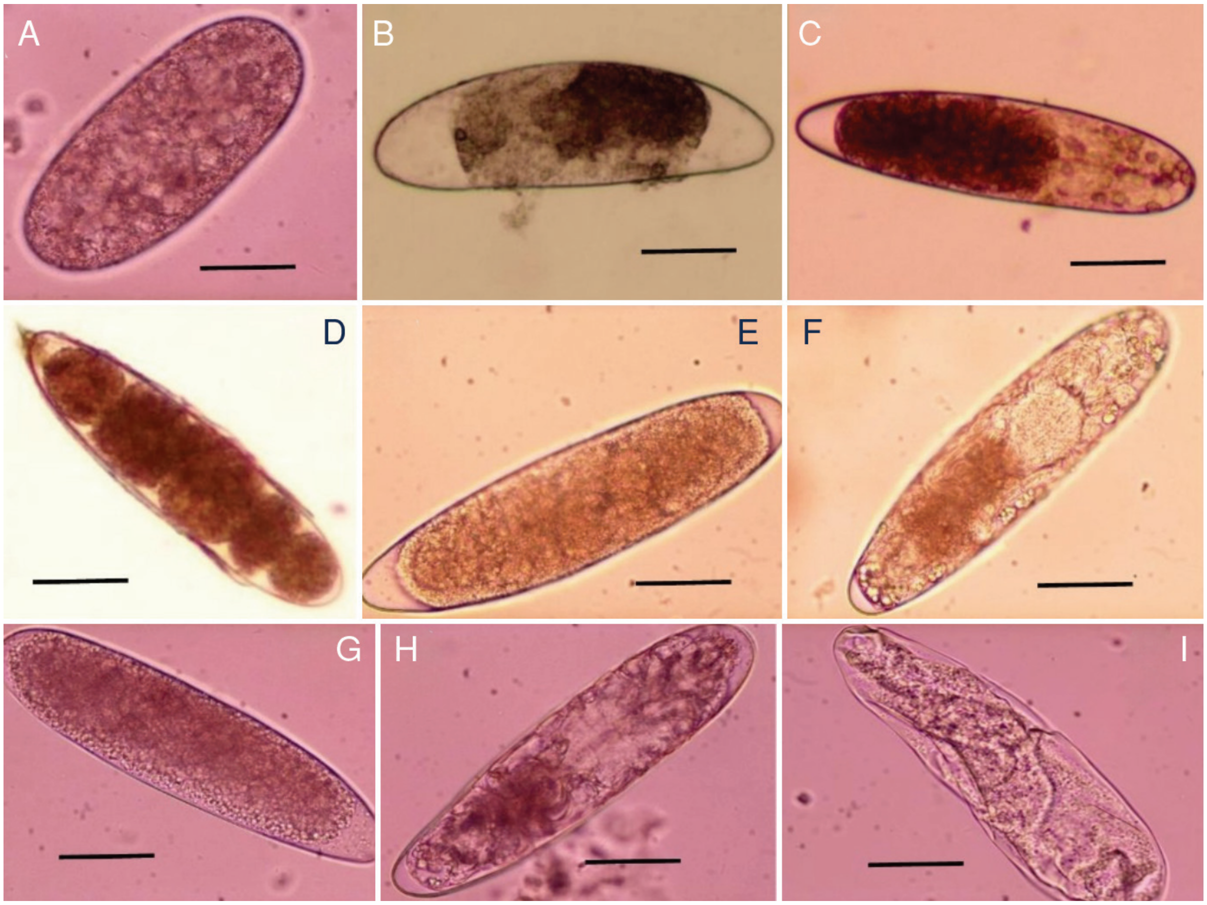

Figure 7. Various ectoparasite eggs seen in fecal flotation (scale bar: 25 µm). **A)** *Otodectes* (mite) egg, **B-H)** Various stages of mite eggs, probably of the genus *Chirodiscoides*, **I)** An abandoned eggshell of a mite

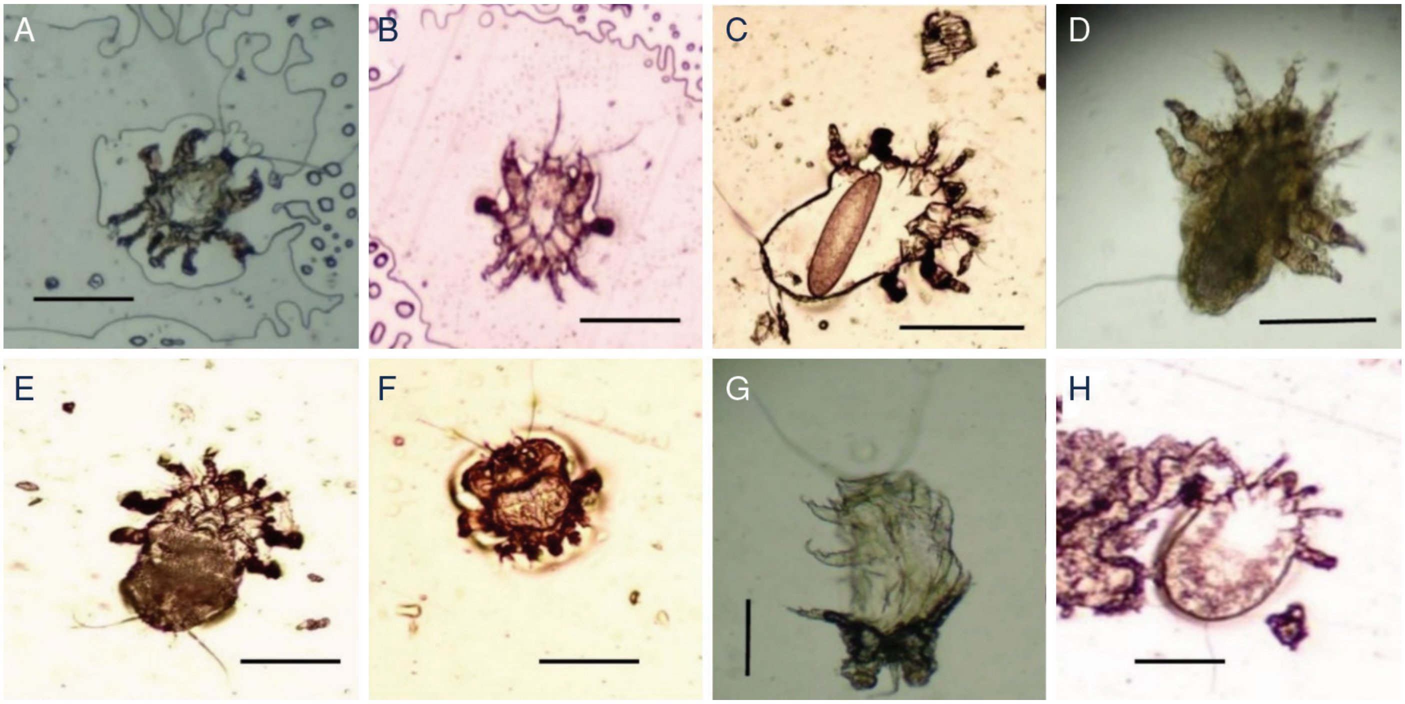

Figure 8. Some ectoparasites observed in mice (scale bar: 100 µm). **A)** and **B)** A *Myocoptes musculinus (M. musculinus)* male seen on cellophane tape, **C)** The remains of a *M. musculinus* with eggs developed inside, **D)** and **E)** *M. musculinus* female seen in fecal flotation, **F)** Shrunken *M. musculinus* male seen in fecal flotation, **G)** *Myobia musculi* shedding its coat, and **H)** An unidentified ectoparasite on cellophane tape

Myocoptes musculinus infestation in laboratory mice was observed in mice brought from abroad to Akdeniz University's laboratory (17). The agents were detected in all mice from the same herd (100%) that showed clinical signs, and were treated with the addition of ivermectin to their drinking water. In our study, no clinical signs were observed in the mice; this mite was detected during the cellophane tape or flotation technique.

According to the literature review, it was concluded that *Mus musculus* can be the host of at least 109 different species of parasites. Among the ectoparasites carried by the house mouse, *Xenopsylla cheopis*, *Nosopsyllus* spp. and *Rhipicephalus* spp. are vectors for important zoonotic diseases. Among the endoparasites, twelve species of helminths and two species of protozoa are species that can also cause disease in humans (18). However, trematodes are not found in laboratory mice because trematodes require intermediate hosts for their development, which cannot survive in laboratory animal breeding areas.

Laboratory mice can only be infected with a limited number of parasites. Among these parasites, *A. tetraptera*, *S. obvelata*, *S. muris*, *Strobilocercus fasciolaris*, *H. nana* and *H. diminuta* species of helminths have been identified by various researchers (14). In the present study, two parasite eggs were considered to belong to *Heligmosomoides polygyrus* and *Strongylidae* because they were morphologically similar in appearance. *H. polygyrus* is a nematode species widely used for research purposes and is not found in mice raised in modern laboratories (3). Therefore, in order to reach a definitive conclusion, it is necessary to develop the eggs or compare the sequence similarity by genetic analysis. In addition to these eggs, a large number of *A. tetraptera* and *S. obvelata* eggs were found, and the eggs of these oxyurid species can be easily distinguished by their specific appearance, and adults of these species were also seen at necropsy.

S. obvelata is zoonotic, and the first report of human infection occurred in a child from the Philippines, when eggs and two mature females of the parasite were identified (19). In the same year, oxyurid eggs were reported in 429 cases among 140,000 soldiers examined in Texas, Oklahoma, New Mexico, and Arizona in the United States (20). In 2009, 25 of 200 patients (12.5%) screened for infection had unexplained abdominal pain and eosinophilia, which were reported to be associated with *Syphacia* spp. infection (21). There are also records of *S. obvelata* eggs in mummified human bodies from Nubia dating from 700-300 BC (22).

According to the study on the morphology of *S. obvelata*, the body of females was measured as 2.9-4.6 (3.5±0.1) mm in length and 0.12-0.23 (0.15±0.001) mm in width based on 10 mature specimens (23). In the present study, the length of the

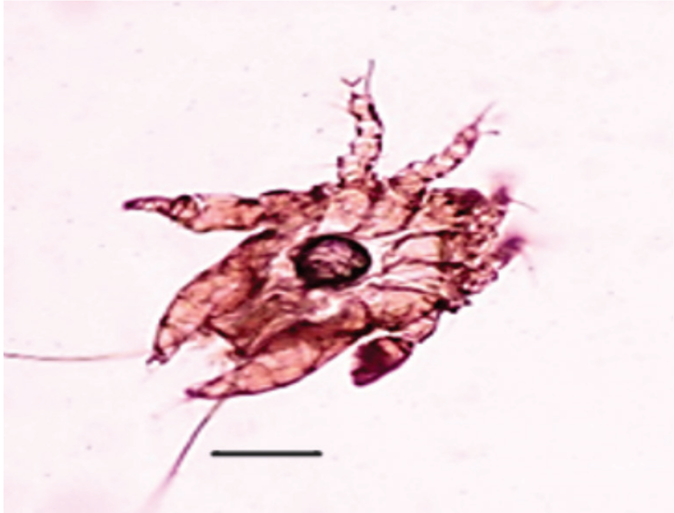

Figure 9. *Otodectes cynotis* (ear mite), female (scale bar: 100 µm)

Figure 10. An *Aspicularis* with a different cervical alae structure. The posterior part was severed by a scalpel blow while opening the intestine of a mouse (scale bar: 0.5 mm)

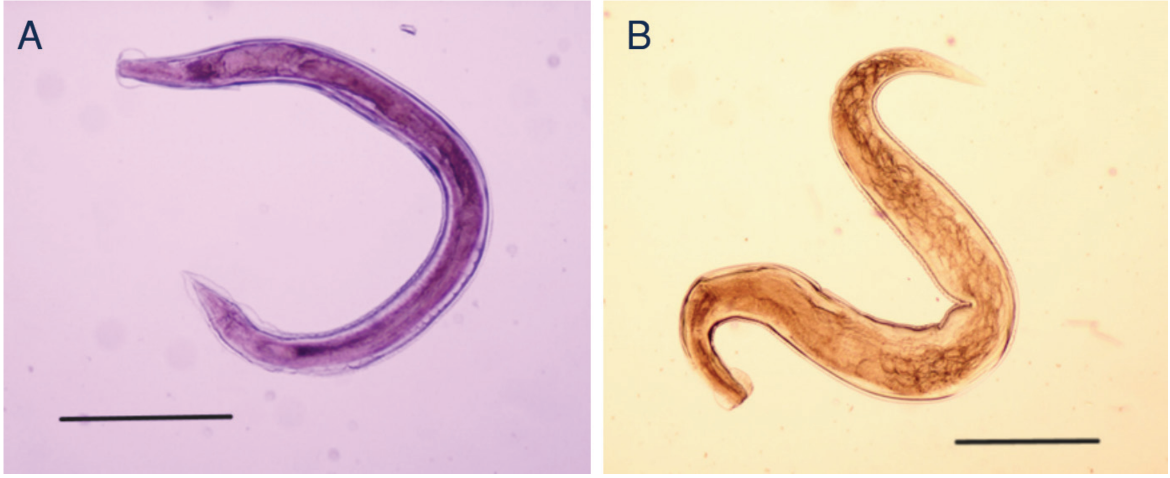

Figure 11. Two examples of the *Aspicularis* genus. **A)** Male *Aspicularis tetraptera*, **B)** Female *Aspicularis tetraptera* (scale bar: 0.7 mm)

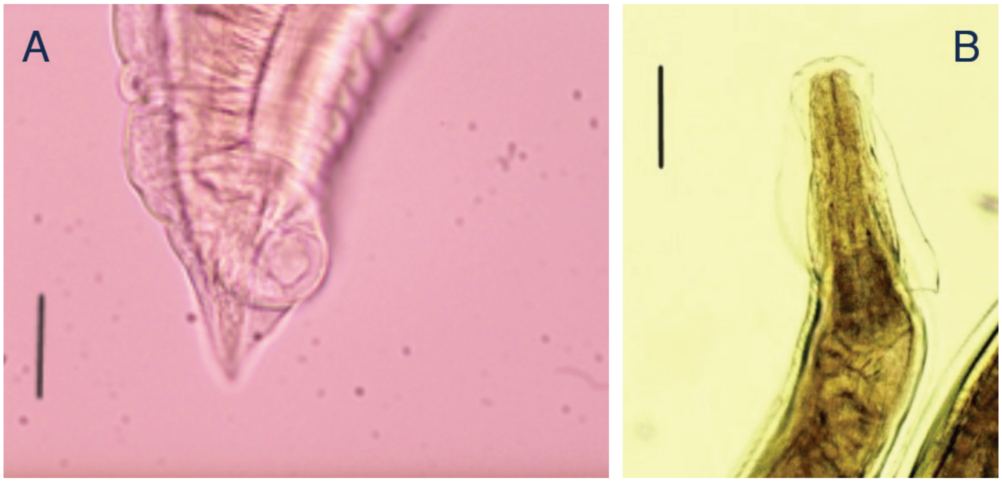

Figure 12. Some morphological observations in *Aspiculuris tetraptera (A. tetraptera)*. **A)** Male *A. tetraptera* tail (scale bar: 0.1 mm), **B)** Veil-like cervical alae of a female *A. tetraptera* (scale bar: 0.2 mm)

Figure 13. Two female individuals of the genus *Syphacia*. **A)** Asymmetrically developed cervical alae (scale bar: 0.5 mm), **B)** Vulva clearly protruded (scale bar: 0.4 mm)

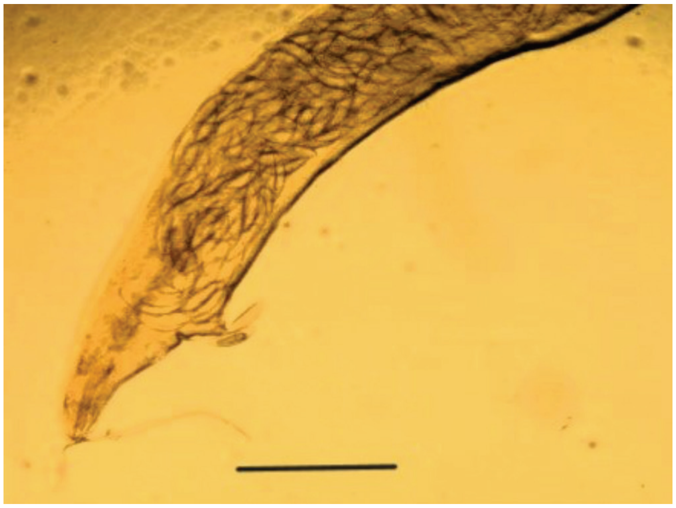

Figure 14. An egg-laying *Syphacia obvelata* (scale bar: 0.5 mm)

helminths was not measured; hundreds of pinworms of various sizes, including newly hatched larvae, were collected. All collected specimens were first identified under a stereomicroscope and then under a light microscope, and these specimens are preserved in the parasitology laboratory.

A. tetraptera is the only species of the genus *Aspicularis* and has been found to infect mice extensively in this study, as in many other studies (14-16). *A. tetraptera* females are characterized by having a veil-like cervical alae, which was seen under the microscope in almost all females in this study, but in one specimen the cervical alae were quite different, the picture of which is shown in the results (Figure 10). For the specimen with the severed posterior shown in Figure 10, since a severed posterior image filled with *Aspicularis* eggs was also taken, this species is possibly a different species of *Aspicularis* with its very different circular cervical alae. A similar difference was also seen in an adult of *Syphacia* (Figure 13A). Although a definitive conclusion cannot be reached regarding species identification since polymerase chain reaction analysis of these two samples could not be performed,

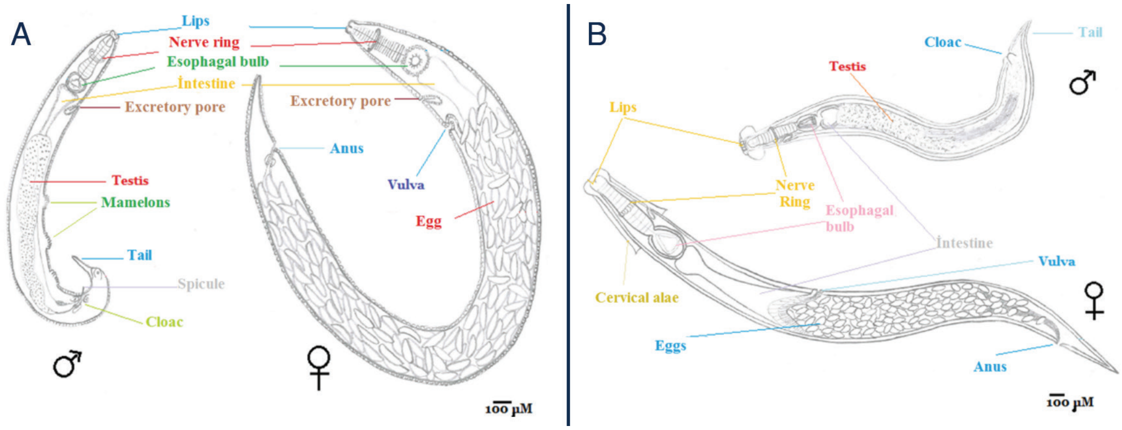

Figure 15. Schematic representation of oxyure nematodes. **A)** *Syphacia obvelata*, **B)** *Aspicularis tetraptera* female and male individuals (original)

if oxyurids similar to these two samples are encountered, their genetic analysis may be useful for the discovery of new species.

CONCLUSION

With this study, some examples of the most common parasites found in laboratory mice, showing different morphologies, are presented with pictures and new visual data provided to researchers working in this field. Even if clinical signs aren't present, different types of parasites can be observed in laboratory animals. Scientists conducting studies in mice are advised to first treat potential parasites in the herd before beginning experimental studies.

*Ethics

Ethics Committee Approval: All experiments were approved by the Bursa Uludağ Experimental Animals Local Ethics Committee (decision no: 2017-10/07, date: 11.07.2017).

Informed Consent: This study is an animal experiment, so patient consent is not required.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: D.K., Concept: D.K., A.O.G., Design: O.G., D.K., A.O.G., Data Collection or Processing: D.K., Analysis or Interpretation: O.G., D.K., A.O.G., Literature Search: O.G., D.K., Writing: O.G., D.K., A.O.G.

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