

Video Article

Diagnosis of Ecto- and Endoparasites in Laboratory Rats and Mice

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Abstract

Internal and external parasites remain a significant concern in laboratory rodent facilities, and many research facilities harbor some parasitized animals. Before embarking on an examination of animals for parasites, two things should be considered. One: what use will be made of the information collected, and two: which test is the most appropriate. Knowing that animals are parasitized may be something that the facility accepts, but there is often a need to treat animals and then to determine the efficacy of treatment. Parasites may be detected in animals through various techniques, including samples taken from live or euthanized animals. Historically, the tests with the greatest diagnostic sensitivity required euthanasia of the animal, although PCR has allowed high-sensitivity testing for several types of parasite. This article demonstrates procedures for the detection of endo- and ectoparasites in mice and rats. The same procedures are applicable to other rodents, although the species of parasites found will differ.

Video Link

The video component of this article can be found at <https://www.jove.com/video/2767/>

Protocol

1. Endoparasite examination (Table 1)

1. Perianal tape test (also see section 5; these are usually performed at the same time)

1. Remove a length of clear, not frosted, cellophane tape from a dispenser. Tape should be long enough to handle by one end without touching the middle (approximately 5 cm). It may be easier to dispense several lengths at one time, attaching them to the edge of a clean work surface and using as needed.
2. Lift a mouse from its cage and place on the cage lid, holding it by the tail. Perform this action in a laminar flow cabinet or biosafety cabinet if the health status of the animal requires it.
3. Restrain the mouse by the tail, lifting its hind legs from the cage. Grasp the end of the tape between thumb and forefinger, then apply the middle of the tape firmly to the mouse's perineum, including the perianal area several times. Hair should be seen to be adherent to the tape for the assay to be considered successful.
4. Place the mouse back in the cage.
5. Place a drop of mineral oil on a labeled clean glass slide, apply the tape to the slide, then another drop of mineral oil. Cover with a glass cover slip.
6. Read the microscope slide using the 10x and 40x objectives on a light microscope. The perianal tape test is best at detecting *Syphacia* eggs, although other parasite eggs are sometimes found.

2. Fecal flotation

1. Assemble flotation solution, a flat-bottomed vial (pill vial or fecal flotation device such as Ovatector), petri dish, cover slip, microscope slide, and applicator/stirring sticks. Flotation solution, such as Fecasol, should have a specific gravity of 1.20-1.30 and may be made from various sodium salts, sugar, zinc sulfate, or purchased commercially. (Table 2)
2. Collect 2-5 fecal pellets from the cage or fresh from the animal(s) into the flotation chamber. If feces are extremely dry, either due to age or species producing the fecal matter, moistening the feces with 500 µl of 0.9% saline may be beneficial.
3. Place the vial in the petri dish to protect the working surface from overflow of dissolved feces. Add a small volume of flotation medium and mash and stir thoroughly. No large pieces of material should remain. Continue to add flotation medium until a meniscus forms above the edge of the vial.
4. Place the cover slip on the meniscus and incubate at room temperature for 15 minutes. Parasite eggs and some protozoan oocysts will rise to the top and adhere to the cover slip.

5. After incubation, lift and invert the cover slip. Place the cover slip on a glass microscope slide.
6. Examine the slide using the 10x and 40x objectives under a light microscope.
7. Although low-tech and easy to perform, in general, this technique is not recommended for mice or rats. As a rule, it is more suitable for animals that produce a larger volume of feces.

3. Fecal concentration and centrifugation

1. Assemble flotation solution, centrifuge tube, cover slip, microscope slide, and applicator/stirring sticks and tube caps. Flotation solution should have a specific gravity of 1.18-1.30 and may be made from various sodium salts, sugar, zinc sulfate, or purchased commercially (Table 2)
2. Collect 2-10 fecal pellets from the cage or fresh from the animal(s) into a collection tube. If feces are extremely dry, either due to age or species producing the fecal matter, moistening the feces with 500 μ l of 0.9% saline or with the flotation solution you are using may be beneficial.
3. Mix sample in an appropriate flotation solution within a glass centrifuge tube. Mechanical agitation with a vortexer may be used to mix samples. If a vortexer is used, snap caps should be placed on the tops of the tube to prevent spillage and cross contamination. Routinely, samples are prepared in 1.18 specific gravity zinc sulfate, however other solutions may be used in addition (in a separate preparation tube) or in substitution.
4. Add additional flotation solution to each tube to form a slight positive meniscus on each tube. Apply a plastic cover slip to each tube, and ensure that full contact with tube lip is made. Place tube(s) into the centrifuge.
5. Centrifuge at approximately 616-760 RCF for 10 minutes. If cover slips are lost or broken during the centrifugation process, a new cover slip can be placed on the sample tube and the tube can be gently tipped so that the meniscus touches the new cover slip. No additional centrifugation is necessary.
6. Remove cover slip from centrifuge tube and place on a labeled, clean glass microscope slide. If multiple centrifugation solutions were used to evaluate one fecal sample, two cover slips may be placed on the same slide.
7. Stain the slide with iodine. This allows for easier identification of cysts.
8. Examine the slide using the 10x and 40x objectives on a light microscope.

4. Direct examination of intestines for helminths and protozoa

1. Place the euthanized mouse or rat carcass in dorsal recumbency on a clean dissection board or similar work surface.
2. Using forceps, lift the abdominal wall at the genital area. Using scissors, carefully incise the ventral abdominal wall from the genital area to the base of the ribcage removing both skin and muscle and exposing the intestines. Remove the intestines, beginning at the duodenum (the segment of the intestine beginning at the exit of the stomach) and continuing to the descending colon (the segment of the intestine which ends at the anus and usually contains formed feces).
3. Place intestines in a 100 ml Petri dish. Collect a portion of cecum and duodenum from the euthanized animal and place on a dissecting board. Incise each intestinal segment lengthwise to expose the mucosa.
4. Using scissors, cut the remaining intestines into small sections. Add enough tap water to the dish to barely submerge the collected tissue.
5. Incubate the sample mixture at 35-40°C in a laboratory oven or incubator for a minimum of 10 minutes. This will liberate and expose luminal helminths. While the sample is incubating, carry out the following steps.
6. Place two drops of 0.9% saline side by side on a single labeled slide, to allow for preparation of samples from two intestinal segments (duodenum and cecum).
7. Heat sterilize and cool an inoculating loop or equivalent.
8. Scrape the mucosa of the duodenum and place the scrapings on the left hand side of the slide (side closest to the frosted edge).
9. Scrape the mucosa of the cecum and place the scrapings from on the right hand side of the slide.
10. Top the scrapings with a cover slip. Examine prepared slide using the 40x objective under a phase contrast microscope; increase magnification as needed for identification. If parasites are detected, identify based on morphology.
11. The dish contents will be ready for examination by this point. Examine the dish contents under a dissecting microscope. Use a probe or applicator stick as necessary to move contents within the dish to complete a thorough examination. If pinworms are present they will appear as small, white, hair-like worms. If tapeworms are present, they will appear as segmented, flat worms (larger than the thread-like pinworms).
12. If helminths are detected or suspected, collect the specimen using a small pair of forceps.
13. Mount the specimen on a labeled, clean glass slide in a drop of paraffin or mineral oil and place a cover slip on top of the specimen. Examine the slide using the 10x and 40x objectives on a light microscope.

2. Ectoparasite examination (Table 3)

5. Fur pluck examination for ectoparasites (tape test)

1. Remove a length of clear, not frosted, cellophane tape from a dispenser. Tape should be long enough to handle by one end without touching the middle (approximately 5 cm). It may be easier to dispense several lengths at one time, attaching them to the edge of a clean work surface and using as needed.
2. Lift a mouse or rat from its cage and place on the cage lid, holding it by the tail. Perform this action in a laminar flow cabinet or biosafety cabinet if the health status of the animal requires it.
3. Restrain the mouse or rat. Grasp the fur with hemostats and gently pluck fur from the mouse's scapular area, ventral cervical region, axillary area, inguinal area, and dorsal rump. Place the fur on the tape. Hair should be seen to be adherent to the tape for the assay to be considered successful. Place the mouse or rat back in its cage.
4. Place a drop of mineral oil on a labeled clean glass slide; apply the tape, then another drop of mineral oil. Cover with a glass cover slip.
5. Read the microscope slide using the 10x and 40x objectives under a light microscope.
6. This examination is best for detecting fur mites such as *Radfordia*, *Myobia*, and *Myocoptes*.

6. Skin scrape

1. Assemble the following materials: animal to be tested, mineral oil, microscope slide, cover slip, scalpel, and scissors. If this test is to be performed on live animals, they should be anesthetized before beginning.
2. Sample the dorsum near the base of the tail and the temporal region of the head. Alternatively, skin lesions and/or other sites may be scraped.
3. Deeply scrape the skin with the scalpel blade in the opposite direction of hair growth, to erode the epidermis. Trimming the hair coat prior to scraping may improve screening sensitivity by reducing visual obstruction (excess hair) on the slide.
4. Place a drop of oil on the slide. Apply the sample to the oil drop by wiping the blade (with sample attached) on the surface of the slide. Add additional oil to the slide if necessary, and top with a cover slip.
5. Read the microscope slide using the 10x and 40x objectives under a light microscope.
6. The skin scrape is generally used to detect *Demodex* (and dermatophytic fungi).

7. Direct examination of pelage

1. Place the euthanized mouse or rat on the stage of a dissecting microscope.
2. Examine the hairs of the pelt at approximately 10X using an applicator stick or similar instrument to part the hair and observe the base of the hair shaft.
3. Examine the cranial region, between the eyes and the pinnae, between the pinnae, between the scapulae, under the jaw, and the inguinal and axillary areas. Alternatively, the whole carcass may be examined.
4. Collect any ectoparasites or suspicious material seen using a small pair of forceps. Ectoparasites may often look like dandruff or a yellow waxy buildup at the base of a hair shaft or directly on the skin.
5. Mount the specimen on a clean glass slide in a drop of paraffin or mineral oil and place a cover slip on top of the specimen. Examine the slide using the 10x and 40x objectives under a light microscope.

3. Representative Results:

See attached files identifying the following parasites: (Note: these procedures will detect any egg, helminth, or cyst present in the feces or on the skin and fur; only a few of these are listed below)

Endoparasites:

<i>Syphacia muris</i> (egg, worm)	<i>Chilomastix bettencourti</i>
<i>Syphacia obvelata</i> (egg, worm)	<i>Hexamastix muris</i>
<i>Aspicularis tetraptera</i> (egg, worm)	<i>Retortamonas</i> sp.
<i>Rodentolepis nana</i> (egg, worm)	<i>Giardia</i> spp.
<i>Tritrichomonas muris</i>	<i>Spiroucleus muris</i>
<i>Entamoeba muris</i>	

Ectoparasites:

<i>Myocoptes musculinis</i>	<i>Radfordia affinis</i>
<i>Myocoptes musculinis</i>	<i>Radfordia ensifera</i>
<i>Myobia musculi</i>	

	Tape test	Fecal flotation	FCC	Direct exam ¹	PCR
Protozoa	--	+	++	+++	+++/NA ²
Metazoa					
Pinworm	+/- ³	+/- ⁴	+ ⁴	+++	+++
Tapeworm⁵	--	+	++	+++	NA
Other roundworms⁵	--	+	+++	+++	NA

1. This method requires euthanasia of the animal.

2. There are not PCR detection methods currently available for every protozoan.

3. This method is most appropriate to detect *Syphacia* spp.

4. This method will be more likely to detect *Aspicularis*, and less likely to detect *Syphacia*.

5. Tapeworms and roundworms other than pinworms are very rare in modern laboratory mice and rats.

Table 1. Class of endoparasite and appropriate detection method. Some methods will require euthanasia of the animal. NA indicates method is not currently available for these parasites.+ indicates suitability of the method for detection of the parasite in question, and – indicates that the method is not recommended for that parasite.

Solution	Specific gravity	Ingredients per 1L H ₂ O
Sodium chloride	1.20	311 g sodium chloride
Sodium nitrate	1.20	338 g sodium nitrate
Sodium nitrate	1.30	616 g sodium nitrate
Sugar	1.20	1170 g sucrose ¹
Sheather's sugar	1.27-1.30	1563 g sucrose ¹
Zinc sulfate	1.18	493 g zinc sulfate

¹. These solutions require refrigeration or the addition of 9 ml phenol as a preservative.

Table 2. Fecal flotation solutions (from Smith *et al.*)

	Fur pluck (tape test)	Skin scrape ¹	Direct exam ¹	PCR
Lice	--	--	++	NA
Mites	+	+	+++	+++/NA ³
Fleas ⁴	--	--	+	NA
Ticks ⁴	--	--	++	NA

¹. This method requires anesthesia if it is to be performed on a live animal.

². This method requires euthanasia of the animal.

³. There are not PCR detection methods currently available for every species of mite.

⁴. Fleas and ticks are extremely rare in modern laboratory animal facilities.

Table 3. Class of ectoparasite and appropriate detection method. Some methods will require euthanasia of the animal, and other methods will require anesthesia to perform them in live animals. NA indicates method is not currently available for these parasites. NA indicates method is not currently available for these parasites.+ indicates suitability of the method for detection of the parasite in question, and – indicates that the method is not recommended for that parasite.

Discussion

When working in a laboratory, safety should always be a concern. Remember to wear appropriate protective equipment when working with animals and to clean your workstation with disinfectant before and after. These methods are primarily designed to find any parasites of laboratory rodents in the locations examined, i.e., they can detect exotic or exceedingly rare parasites as well as the more common pinworms and fur mites. Although they are equally applicable to other species, wild rodents may have additional parasites in locations such as liver, subcutis and brain, not evaluated by the above methods.

Disclosures

The authors are all employees of Charles River, a supplier of diagnostic tests and reagents, including the tests described above.

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