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Sensitivity of Perianal Tape Impressions to Diagnose Pinworm (*Syphacia* spp.) Infections in Rats (*Rattus norvegicus*) and Mice (*Mus musculus*)

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We determined the sensitivity of perianal tape impressions to detect *Syphacia* spp. in rats and mice. We evaluated 300 rat and 200 mouse perianal impressions over 9 wk. Pinworm-positive perianal tape impressions from animals with worm burdens at necropsy were considered as true positives. Conversely, pinworm-negative perianal tape impressions from animals with worm burdens were considered false negatives. The sensitivity of perianal tape impressions for detecting *Syphacia muris* infections in rats was 100%, and for detecting *Syphacia obvelata* in mice was 85.5%. Intermittent shedding of *Syphacia obvelata* ova is the most probable explanation for the decreased sensitivity rate we observed in mice. We urge caution in use of perianal tape impressions alone for *Syphacia* spp. screening in sentinel mice and rats.

The rat pinworm, *Syphacia muris*, and the mouse pinworm, *Syphacia obvelata*, inhabit the cecum and colon of rodents and are still found in many modern laboratory animal facilities.[3,9,11] Although usually nonpathogenic in immunocompetent rodents, pinworm infections may have untoward effects on behavior, growth, intestinal physiology, and immunology.[7,8,10,14] These effects and the demand for defined experimental animals, possible hindrances to interinstitutional collaborative research, and increased operating cost associated with treatment and environmental decontamination make effective pinworm surveillance and eradication vital for many animal facilities. Several techniques have been described to diagnose pinworm infections in laboratory rodents. Common diagnostic techniques include perianal tape impression, anal swab, fecal flotation, necropsy with microscopic examination of cecal and colonic contents, and histologic examination.[11]

Early, effective diagnosis of pinworm infections is critical to pathogen containment and eradication. The validity of different diagnostic techniques varies widely. For diagnosis of *S. obvelata* in mice, demonstration of adult worms in the intestine has been reported as the most likely to yield a correct diagnosis, followed by perianal tape impression, and demonstration of eggs in fecal smears has been described as the least likely.[1] Here we report the sensitivity of the commonly used perianal tape impression method to detect *Syphacia* spp. infections in rats and mice.

Materials and Methods

Animals. ***Rats.*** Male Sprague–Dawley SPF rats (Crl:SD; $n = 40$; age, 24 d; Charles River Laboratories, Wilmington, MA) were obtained; all rats acquired patent *S. muris* infections as a result of 3-wk continuous exposure to pinworm-contaminated bedding from an inhouse colony of parasitized rats. Colony animals were antibody-negative for sialodacryoadenitis virus, Sendai virus, lymphocytic choriomeningitis virus, rat parvoviruses, reovirus 3, Theiler murine encephalomyelitis virus, and *Mycoplasma pulmonis* (Research Animal Diagnostic Laboratory, Columbia, MO). Infection with *S. muris* infection was confirmed in individual rats by cellophane tape impressions of the anus. Anal tapes were mounted on glass slides and examined microscopically for ova under a 4× objective lens; the same person performed all microscopic examinations. Animals were included in the study only if they had an *S. muris*-positive tape test on the day the study began (day 0).

Mice. Male SPF mice (Crl:CD1; $n = 30$; age, 24 d; Charles River Laboratories) were obtained; all mice acquired patent *S. obvelata* infections as a result of 6-wk continuous exposure to pinworm-contaminated bedding from an inhouse colony of parasitized mice. Colony animals were antibody-negative for the following viral and bacterial pathogens: mouse hepatitis virus, Sendai virus, pneumonia virus of mice, reovirus 3, Theiler murine encephalomyelitis virus, *Ectromelia*, *Mycoplasma pulmonis*, mouse parvovirus, mice minute virus, mouse rotavirus, and lymphocytic choriomeningitis virus (Research Animal Diagnostic Laboratory). Infection with *S. obvelata* was confirmed in individual mice by microscopic evaluation of cellophane tape anal impressions. Animals were included in the study only if they had an *S. obvelata*-positive tape test on the day the study began (day 0).

Husbandry. All studies were conducted in 2 separate, identical cubicles in 1 room at the University of Tennessee Health Science Center (Memphis, TN). Animals were singly housed in static, polysulfone, microisolation caging (Alternative Design Manufacturing and Supply, Siloam Springs, AR) on autoclaved, contact, hardwood bedding (Northeastern Products, Warrensburg, NY) and maintained on a 12:12-h light:dark cycle at 22.8

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± 0.6 °C. All animals were provided acidified water (pH 2.5 to 3) and irradiated, pelleted, rodent chow (Rodent Diet 7912, Harlan, Indianapolis, IN) ad libitum. Caging, food, and water bottles were changed weekly under strict, aseptic microisolation technique within a biological safety cabinet (NU-425-600, NuAire, Plymouth, MN). All cages and implements were washed in a mechanical washer with final rinse at 180 °F and were autoclaved at 250 °F for 15 min prior to entry into the room. A temperature-recording label (Temp-Tape 180, Pharmacal Research Laboratories, Naugatuck, CT) was used daily to ensure final rinse temperature. Steam chemical integrators (SteriGage LR, 3M, St Paul, MN) were used with each autoclave cycle; quality control was assessed further monthly by using a biologic indicator (Verify, Steris, Mentor, OH). Animal use followed a protocol approved by the facility's Institutional Animal Care and Use Committee. Animals were housed, cared for, and used in compliance with the *Guide for the Care and Use of Laboratory Animals*[6] in an AAALAC International-accredited program.

Sensitivity evaluation. The findings we present here were collected in tandem with a study designed to evaluate the efficacy and safety of topical selamectin to eradicate pinworm infections in rats and mice.[5] In the parallel study, selamectin, at the administered dosages, was 100% ineffective in eliminating *Syphacia* spp. infections in rats and mice. As part of the parallel study, pinworm-positive rats were assigned randomly to 1 of 4 treatment groups [positive control (no treatment), selamectin (0.6 mg/kg), selamectin (6.0 mg/kg), or fenbendazole-medicated chow (150 ppm) containing 10 animals each. Likewise, pinworm-positive mice were randomized into 1 of 3 treatment groups [positive control (no treatment), selamectin (6.0 mg/kg), or fenbendazole-medicated chow (150 ppm). Weekly, all rodents were weighed and tested for pinworm ova by perianal cellophane tape impression. Nine weeks after the start of the study, all animals were euthanized by inhalational isoflurane overdose and necropsy examination was performed to determine pinworm infection. Using a method similar to 1 described previously[2,5] gastrointestinal content examinations were performed in all animals by longitudinally opening the cecum and colon from the ileocecal junction to the rectum and washing that portion of the gastrointestinal tract with 200 mL sterile water. The fecal mixture was passed through a 5-in. culinary mesh strainer. The resulting filtrate was passed through a 3-in. culinary mesh strainer. The remaining filtrate was passed through a 100-µm nylon screen (Miami Aquaculture, Miami, FL). The screen was rinsed with 10 mL sterile water. A 2-mL aliquot of the wash solution was placed in a RODAC plate (Becton Dickinson Labware, Lincoln Park, NJ) and stained with iodine to facilitate worm counting; worm counts then were multiplied by the dilution factor of 5. Pinworm-positive perianal tape impressions from animals with burdens at necropsy were considered true positives. Conversely, pinworm-negative perianal tape impressions from animals with worm burdens were considered false negatives. The sensitivity of perianal tape impressions to determine pinworm infection was calculated by using the following formula:

$$\text{Sensitivity} = [\text{True positives}/(\text{True positives} + \text{False negatives})] \times 100\%.$$

Results

Rats. At necropsy, worms were recovered from all rats in the following groups (n = 10 per group): selamectin (0.6 mg/kg), selamectin (6.0 mg/kg), and positive control. All 300 perianal tape impressions taken from these 30 rats were positive for *S. muris*. Therefore the sensitivity of perianal tape impressions for detecting *S. muris* infections in rats was calculated to be 100%. Worms were not recovered at necropsy of the fenbendazole-treated rats; as a result, tape impressions from these animals were not used in sensitivity calculations. At necropsy, the worm burden (mean ± SEM) recovered from pinworm positive rats was 25 ± 7 worms per rat.

Mice. At necropsy, worms were recovered from all mice in the selamectin and positive control groups (n = 10 per group). During the study, we evaluated 200 perianal tape impressions from these 20 mice. Of the tape impressions evaluated, 171 *S. obvelata* true-positive tapes and 29 false-negative tapes were identified. The 29 false-negative tape impressions were obtained from 12 different mice. The sensitivity of perianal tape impressions for detecting *S. obvelata* in mice was calculated to be 85.5%. Worms were not recovered at necropsy of fenbendazole treated mice; as a result, tape impressions from these mice were not included in sensitivity calculations. The worm burden recovered from pinworm-positive mice was 32.5 ± 8.4 worms per mouse.

Discussion

Effective diagnosis of adventitial infections in laboratory animals is crucial for managing rodent colonies to promote valid research. With regard to diagnosis of pinworms, perianal tape impression has been described as the most widely used for detecting eggs of *Syphacia* spp.[4] Among 28 institutions surveyed, 21 used the tape test with at least 1 other method for detection of pinworm infections.[3] Despite widespread use of the perianal tape test, to our knowledge, ours is the first report to describe the sensitivity of perianal tape impressions to diagnose pinworm infections in rats and mice. Sensitivity measures the proportion of true positives that are identified as such. By definition, a sensitive diagnostic assay produces a low percentage of false-negative results or, conversely, a high percentage of true-positive results from tests of exposed animals.[12] In the present study, we determined that the sensitivity of perianal impressions for detecting *S. muris* infections in rats was 100%. The sensitivity of this modality for detecting *Syphacia obvelata* in mice was determined to be 85.5%.

False-negative perianal tape impressions may result from several factors, including operator training and performance, worm burden, and worm sex. Other factors reported to affect tape test efficacy include time of testing[13] and age of host.[1] In the present study, all tape impressions were performed by 1 veterinarian and 1 veterinary technician, each of whom had more than 5 y experience in laboratory animal science. Therefore, training deficiencies and performance error likely were not variables that decreased test performance in the current study. At necropsy, the worm burden (mean ± SEM) recovered from pinworm-positive rats was 25 ± 7 worms per animal. In 8 rats (positive control, n = 3; 0.6 mg/kg selamectin, n = 2; and 6.0 mg/kg selamectin, n = 3), the calculated worm burden was 5 worms per animal. Each of these 8 rats had positive tape tests during each week of the study. The worm burden recovered from pinworm-positive mice was 32.5 ± 8.4 worms per mouse. Of the 3 mice that were tape test-negative at necropsy, 2 had worm burdens of 20 (positive control, n = 1; selamectin group, n = 1); the remaining mouse had a worm burden of 5 (positive control n = 1). From these observations, we conclude worm burden was not a likely contributing factor to the false-negative results we recorded. The sex of recovered worms was not determined, but because all rodents with patent pinworm infections at necropsy had multiple positive perianal tape impressions

throughout the study period, we excluded the possibility of male-only infections.

Periodicity of egg production has been described to occur in *S. muris*. Mature female *S. muris* reportedly migrate from the intestines and cecum at approximately noon, and more ova are found on the perianal skin during the afternoon than during the rest of a 24-h period.[13] No periodicity of ova production with *S. obvelata* has been observed. During our study, the time of tape test performance was variable and therefore might represent an experimental variable. Age of host at the time of examination has been cited to affect the efficacy of anal tape impressions in diagnosing *S. obvelata* infections in mice.[1] During our study, 100% of mice with patent *S. obvelata* infections at necropsy had positive perianal tape impressions at 8 wk of age. However, by 9 wk of age, the positive detection rate diminished to 50%; by 10 wk of age the positive detection rate returned to 100%. From these data, we conclude that *S. obvelata* ova are shed intermittently.

Intermittent shedding of *S. obvelata* ova is the most probable explanation for the sensitivity rate we recorded. The intermittent shedding of *S. obvelata* we observed is most likely a natural phenomenon and unrelated to selamectin treatment. This hypothesis is supported by the observation that of the 12 mice that exhibited intermittent shedding, 10 were positive controls and received no drug treatment. Moreover, we ruled out the possibility that truly negative animals were reinfected because the time until subsequent positive tape tests in each animal was less than the 11- to 15-d prepatent period for *S. obvelata*. We did not observe intermittent shedding of *S. muris* ova in rats. Continuous shedding of ova would explain the 100% sensitivity of the perianal tape impression to detect *S. muris* infections in rats. Further work is needed to corroborate our findings on the shedding patterns of *S. obvelata* and *S. muris.*

Notwithstanding the results reported here, we urge caution in use of perianal tape impressions alone for *Syphacia* spp. screening in sentinel mice and rats. The efficiency of pinworm detection through use of indirect contact sentinels can be affected by a number of factors including: use of microisolation caging, quantity of dirty bedding transferred, frequency of bedding transfer, number of cages sampled, and time elapsed between bedding transfer and examination of sentinels.[3] To maximize diagnostic effectiveness, colony health surveillance for endoparasites should be multimodal and based on the institution's predetermined acceptable risk level.

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