Detection of three pinworm species in laboratory mice and rats through an antemortem fecal PCR assay

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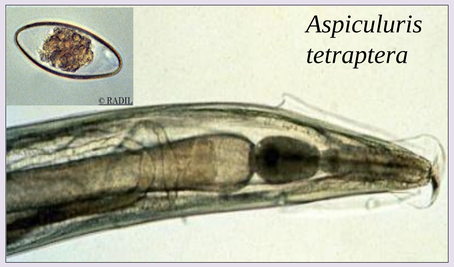
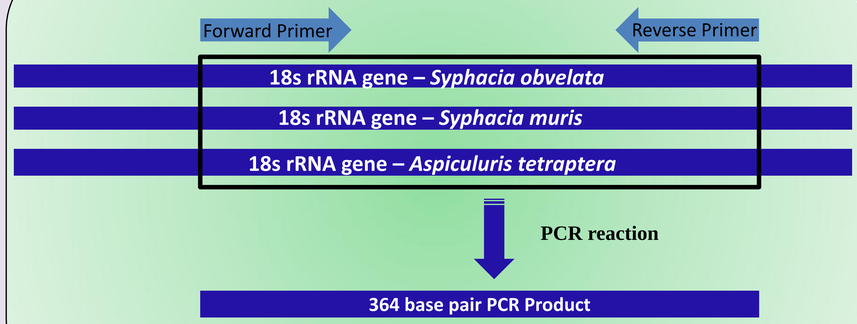


Abstract

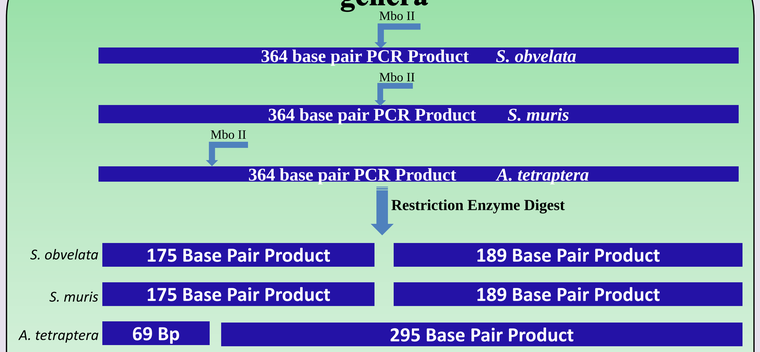
Syphacia muris, *Syphacia obvelata*, and *Aspiculuris tetraptera* are three pinworm species that are prevalent in laboratory mice and rats. It has been well documented that pinworm infections can have confounding influences on the results of immunological studies; thus, there is a need to identify infected animals so that they are not used in immunological research. The current methods for detection lack sensitivity, leading to false negative test results and animal facilities with recurrent pinworm infestations. The current 'gold standard' test is direct examination of cecal and colon contents for adult worms, but to perform this test the animal must be sacrificed. **The objective of this study was to develop a sensitive and specific antemortem PCR assay for the detection of *S. muris, S. obvelata*, and *A. tetraptera* in mice and rat fecal pellets.** A unique region of the 18S rRNA gene was identified that was common to *S. muris*, *S. obvelata*, and *A. tetraptera* and primers were designed to amplify a 364 bp product. The analytical sensitivity of this pinworm PCR assay was determined to be 10 template copies per PCR reaction. The diagnostic sensitivity of these primers was compared with traditional postmortem direct exam of cecal/colon contents and antemortem fecal floatation and perianal tape test methods. The PCR assay was the most sensitive antemortem test evaluated and was only slightly less sensitive than direct examination of cecum/colon contents for detecting pinworms in mice and rats. Additionally, restriction enzyme analysis of the PCR amplicon allowed genus-specific identification of *Aspiculuris tetraptera* and *Syphacia* spp. pinworms. Together, these newly developed assays may prove to be valuable antemortem tests for detection of pinworm infections in mice and rats, negating the need to sacrifice animals for pinworm detection.

Design of pinworm generic PCR



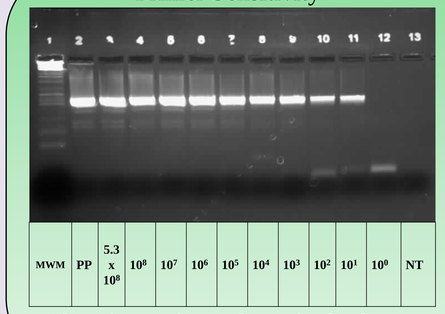
A unique region of the 18s rRNA gene was identified, common to *A. tetraptera*, *S. muris*, and *S. obvelata*. Primers were designed, using NCBI Primer Blast, to amplify this 364 base pair region.

Restriction Enzyme digest to differentiate pinworm genera



Restriction Enzyme digest of the generic PCR product with the restriction enzyme Mbo II results in distinct size fragments that differentiate *Syphacia* from *Aspiculuris*.

Primer Sensitivity



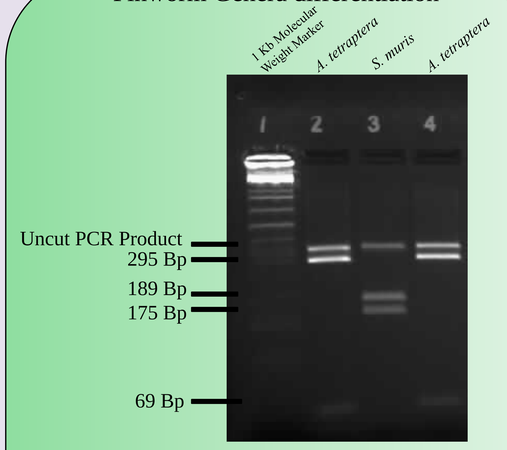
MWM = Molecular Weight Marker of 1Kb

PP = Pure Plasmid Prep

10^X = number of template copies per PCR reaction

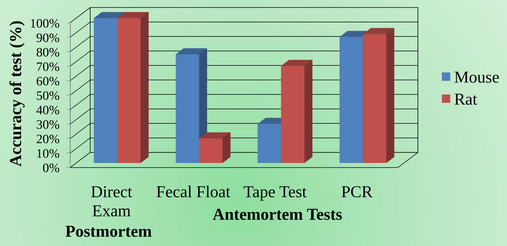
The primer pair was determined to have a sensitivity of 10 copies per reaction.

Pinworm Genera differentiation



This gel shows how to differentiate the genera after a restriction enzyme digest using PCR product. A band at 295 and 69 indicates an infection of *Aspiculuris tetraptera*. A band of 175 and 189 in a mouse indicates an infection with *Syphacia obvelata*. A band of 175 and 189 in a rat indicates an infection with *Syphacia muris*.

Comparison of traditional assays with PCR for pinworm detection



This bar graph shows the results when the post-mortem direct exam assay was compared with the antemortem tape test, fecal float, and the newly-developed PCR assay. Using the direct exam as the "gold standard" with 100% accuracy, the tape test was accurate only 27% of the time in the mouse and 67% of the time in the rat. The fecal float was accurate 75% of the time in the mouse and only 17% of the time in the rat. The PCR assay was accurate 87% of the time in the mouse and 89% of the time in the rat.

n = 15 mice; n = 9 rats * Fecal float had n = 12 mice; n = 6 rats

Materials & Methods

Animals

Both mice and rats were used in this project. They were of variable age with variable backgrounds. All procedures performed on these animals were approved by IACUC.

Direct Exam

During necropsy, samples of the tip of the cecum and the proximal loop of the colon were collected. These samples were placed in saline and observed under a dissection microscope, looking for adult worms.

Fecal Float

Approximately 2-4 fecal pellets were placed in sodium nitrate to concentrate ova at the surface. A microscope cover slip was set on top of the tube for 10 minutes, transferred to a microscope slide and examined under the microscope for ova.

Tape test

A piece of scotch tape was placed around the perianal area, transferred to a microscope slide, and then examined under a microscope for ova.

Polymerase Chain Reaction

DNA was extracted from the fecal pellets using the Standard Operating Procedure at RADIL. A reaction mixture of 10X Buffer with $MgCl_2$ (5 µl), dNTPs at a 5mM concentration (8 µl), Faststart Taq (0.25 µl), forward and reverse primers at a 20 mM concentration (2.5 µl) and 5 µl of DNA was made up with a final volume of 50 µl. This reaction was amplified for 45 cycles with cycling parameters of 94 C for 15 seconds, 62 C for 15 seconds, and 72 C for 30 seconds, run on a 3% agarose gel and stained with ethidium bromide.

Restriction Enzyme Digest

PCR product (15 µl) was added to 2 µl of buffer and 1µl of Mbo II with a final volume of 20 µl. The reaction was incubated at 37 C for 60 minutes and heat inactivated at 65 C for 20 minutes. The products were then run on a 3% agarose gel, stained with ethidium bromide.

Conclusions:

- The pinworm PCR assay was the most sensitive antemortem pinworm assay evaluated, being more sensitive than the perianal tape test and fecal floatation test for detecting *Syphacia* spp. and *Aspiculuris teraptera* in mice and rats.
- These data suggest that the PCR assay is nearly as sensitive as the direct exam and has the advantage that the animal does not need to be euthanized for evaluation.
- This PCR assay combined with a restriction enzyme digest allows for differentiation between *Syphacia* spp. infections and *Aspiculuris tetraptera* infections.

Acknowledgements:
This project was funded by GlaxoSmithKline, the American Society of Laboratory Animal Practitioners, and the Research Animal Diagnostic Laboratory at The University of Missouri - Columbia.