Journal of the American Association for Laboratory Animal Science
Copyright 2008
by the American Association for Laboratory Animal Science

Vol 47, No 5
September 2008
Pages 39–43

Detection of *Spironucleus muris* in Unpreserved Mouse Tissue and Fecal Samples by Using a PCR Assay

Michele A Fain[1,†] Zuzana Karjala,[2,†] Kathy A Perdue,[1,*] Michelle K Copeland,[1] Lily I Cheng,[1] and William R Elkins[1]

The purpose of this study was to develop a rapid DNA isolation method and a sensitive and specific PCR assay for detecting *Spironucleus muris* in mouse tissue and fecal samples. A PCR assay based on the carboxy terminus of the *elongation factor 1*α gene was developed; the PCR product was confirmed by nucleic acid sequencing and nested PCR. The new PCR assay then was used to test feces from animals that had been screened for *S. muris* by using direct intestinal examination and histology. The PCR assay was determined to be a more sensitive test than either direct intestinal examination or intestinal histology. To our knowledge, this assay represents the first use of a PCR-based diagnostic screening method to confirm the presence of *S. muris* in murine tissue and fecal samples.

Abbreviation: bp, basepair

Spironucleus muris is a flagellated protozoan found in the intestinal lumen of mice, rats, and hamsters.[23,25] The elongated, bilaterally symmetrical trophozoites measure 3 to 4 × 10 to 15 µm and have 6 anterior and 2 posterior flagella. The trophozoites are found in the small intestine, mainly the duodenum, whereas 4 × 7 µm cysts appear in the large intestine and feces.[6,15] The organism has a prepatent period of 5 d.[24]

Although numerous mouse strains have been infected with *S. muris*,[2,5,15] often spironucleosis does not result in clinical signs. Instead, the infection is identified histologically as accumulations of trophozoites in the intestinal lumen, between villi, and in the crypts of Luberkuhn.[8] Trophozoites have also been detected in the glands of the pyloric region of the stomach.[19,20] A variety of intestinal lesions have inconsistently been reported, including damaged, degenerating and hyperplastic villus epithelial cells and occasional crypt abscesses.[9,25,26] One study using electron microscopy to evaluate mice with severe spironucleosis revealed that protozoa coated the mucosal surface of the small intestine, perhaps leading to malabsorption and severe malnutrition in the affected mice.[8]

Spironucleus muris reportedly has interfered with research by causing deaths among irradiated and cadmium-exposed mice,[9,16] altering macrophage activity and metabolism,[4,12] and depressing lymphocyte responsiveness to tetanus toxoid in the mouse.[18,22] As a result of these findings, *S. muris* is now considered a facultative pathogen.

In the past, several techniques have been used to detect *S. muris*, including direct smear of intestinal contents (direct examination), histologic examination of intestinal samples, fecal smears, fecal smear immunochemical techniques, and light and electron microscopy. With the exception of direct smears of intestinal contents for trophozoites, these techniques are impractical for screening large numbers of animals due to time constraints, expense, or the need for highly trained personnel. In addition, individual research mice could not reliably be shown to be free of infection because the techniques required euthanized animals or had poor sensitivity.[20] The purpose of the current study was to develop a sensitive and specific PCR technique for detection of *S. muris* in fecal and tissue samples so that large numbers of and individual live mice could be screened rapidly.

Materials and Methods

Sample collection and trophozoite and cyst count. Experimental cull mice from a room naturally infected with *S. muris* were used. Animals were euthanized with carbon dioxide in accordance with applicable guidelines[1] and the animal study protocol. The pyloric region of the stomach and proximal duodenum were recovered, minced with iris scissors, and placed in a culture dish; 1 ml PBS (pH 7.4) was mixed with the sample. A 150-µl drop of the resulting suspension was placed on a slide and examined under light microscopy at ×250 and ×400 magnification. Protozoa were identified by their size, shape, and motion and were speciated according to Flynn.[11] A grid coverslip was used to count the number of trophozoites, and the number of trophozoites per 1 ml was calculated.

From each mouse positive for *S. muris*, 3 fecal pellets were collected and mixed with 1 ml PBS. A 200-µl drop of the suspension was placed on a slide, dried, fixed with formalin, and then stained with Masson trichrome to differentiate and enumerate the cysts.[3] The number of cysts per 1 ml was determined by using a grid coverslip.

S. muris-positive tissue and fecal samples, containing 70,933 trophozoites/ml and 38,080 cysts/ml respectively, were frozen at –80 °C and used for PCR assay development and testing.

DNA extraction and quantification. DNA was extracted from intestinal tissue samples by using a commercial kit (Puregene Tissue Core Kit A, Gentra Systems, Minneapolis, MN). Frozen samples were placed in a culture dish on wet ice and minced in lysis buffer with sterile scalpel blades. The crude homogenate

Received: 12 Feb 2008. Revision requested: 12 March 2008. Accepted: 9 May 2008.
[1]Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, [2]Health Surveillance Laboratory, Diagnostic and Research Services Branch, Division of Veterinary Resources, Office of Research Services, National Institutes of Health, Bethesda, MD
[†]These authors shared equally in the work for this project.
[*]Corresponding author. Email: kperdue@niaid.nih.gov

then was transferred to a 1.5-ml microfuge tube and digested overnight with 0.03 mg proteinase K at 55 °C in a rotating mixer (Thermomixer-R, Eppendorf, Westbury, NY) at 300 revolutions per min. DNA was isolated from the resulting fully digested tissue according to the manufacturer's instructions. DNA from *S. muris* cysts in feces was isolated by using a commercial kit (QIAmp DNA Stool Mini Kit, Qiagen, Valencia, CA) with modifications: 2 or 3 fecal pellets with an average weight of 25 mg per pellet were used as starting material for the DNA extraction process with half of an Absorbex solid-phase extractor tablet (Qiagen). To obtain more concentrated DNA, 60 to 100 µl of elution buffer was used in the final elution step rather than the recommended 200 µl.

The concentration of DNA isolated from feces or tissue was determined spectrophotometrically (ND1000, NanoDrop Technologies, Wilmington, DE), and the quality of each DNA sample was assessed by electrophoresis of 1 µg in a 0.65% Trevigel in TAE Matrix (Trevigen, Helgerman, CT) at 65 V. If the 260:280 optical density ratio of a sample was below 1.5, the sample was purified further by using the reagents of a DNeasy Tissue Kit (Qiagen).[21] When additional cleanup was performed, the concentration and quality of the resulting product was determined as just described.

PCR primer development. We designed 2 sets of PCR primers based on the carboxy terminus of *S. muris elongation factor 1*α (EF1α; Genbank accession no., U94405). The primary primer pair (forward, 5′ CTC CAG GAC GTG TAC AAG ATC 3′; reverse 5′ CGA GAA GTT CCT GTA GAA GAT C 3′) spanned nucleotides 1 to 393 of the deposited sequence. A nested primer pair (forward, 5′ TTC GCC CCC TCT GAC GAG TCC 3′and reverse, 5′ ATA CCC TTC TTC AGG TTC TTG G 3′) was designed for nucleotides 90 to 229. Both sets of primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). Nested primers were used to confirm the first amplicon produced by the primary primer set and to retest replicate samples for which conflicting PCR results were obtained.

PCR controls. Two types of positive controls were used. The first was a primer pair targeting exons 3 and 4 of the mouse 36B4 acidic ribosomal phosphoprotein gene (*Arbp*; GenBank accession no., AC159539; forward, 5′ GTG TGA GGT CAC TGT GCC AGC T 3′; reverse, 5′ A GCT GGC ACA GTG ACC TCA CAC 3′), which were developed and are used commonly in our laboratory. These primers were used to confirm sample suitability for PCR amplification when a sample was negative by using the *S. muris* primers. The second positive control samples were pyloric tissue and feces from mice heavily infected with *S. muris* as determined at necropsy by direct examination.

Negative control samples were developed from feces harvested from a Tac:SW (Taconic Farms, Germantown, NY) mouse.

PCR. A HotStarTaq kit (Qiagen) was used for the assay, except that the 5× buffer from the rtPCR kit (Qiagen) was used because it resulted in a more robust reaction. Each primary PCR reaction contained 200 ng DNA and 0.2 nM of each primer in a 50 µl total reaction volume. A 1-µl aliquot of PCR product from the primary reaction was used in each nested PCR reaction. Amplification conditions in the thermocycler were set at: HotStarTaq polymerase activation, 15 min at 95 °C; 40 cycles of denaturation (60 s at 95 °C), annealing (60 s at 62.5 °C), and extension (60 s at 72 °C); and final extension, 10 min at 72 °C. The number of cycles was reduced to 35 cycles for nested primer assays. PCR products were separated on a 1.8% Trevigel 500 (Trevigen). PCR products were visualized by using either GelRed (Biotium, Hayward, CA) dye incorporated into the gel or, for maximal sensitivity, SYBR Gold Nucleic Acid Gel Stain (Molecular Probes, Eugene, OR) applied after electrophoresis. SYBR Gold-stained gels were photographed by using a SYBR photographic filter (S7569, Molecular Probes), whereas GelRed stained gels were photographed by using a standard ethidium bromide filter.

Nucleotide sequence confirmation of PCR products. Pyloric tissue from a mouse with a high parasite load, determined by direct examination and histology, was chosen for initial verification of the PCR assay. After amplification and electrophoresis, the PCR product was confirmed by amplification with the nested primers. The primary and nested PCR products then were purified (Qiaquick PCR Purification Kit, Qiagen) and sequenced (Lofstrand Labs), and resulting sequence data were analyzed (Lasergene Sequence Analysis Software, DNAStar, Madison, WI). The resulting nucleotide sequence then was used as a query sequence against the GenBank core nucleotide database by using nBLAST.[7]

Evaluation of limits of detection and specificity of PCR assay. The limits of detection of the assay were evaluated by using the tissue and fecal samples provided by the parasitologist. *S. muris* DNA was extracted as described; DNA from sources other than *S. muris* was considered negligible in these highly positive samples. With this assumption and taking into account that cysts contain 2 trophozoite genomes, serial dilutions of the extracted DNA were made to give the equivalent of 10, 50, 100, and 150 trophozoites or cysts per 5µl of DNA suspension. A 5-µl aliquot of each dilution was used in the PCR assay.

To demonstrate primer specificity for *S. muris*, appropriate tissues were collected from mice determined to be negative for *S. muris* but positive for *Chilomastix bettencourti*, *Tritrichomonas muris*, or *Entamoeba muris* by direct examination; in some cases, the mice were infected with more than 1 protozoan species. DNA was isolated from the samples as outlined, with the exception that sections from the cecum, positive for *Entamoeba* and *Chilomastix*, were treated as feces rather than as tissue samples. Formalin-preserved intestinal tissue, collected from a *Peromyscus leucopus* mouse demonstrated to be heavily infected with *Giardia muris* by histopathology, was the sample used to test for *Giardia* protozoa; fecal samples were not available from this animal. For the PCR reaction, the total DNA concentration of all samples was adjusted with diethyl pyrocarbonate-treated water to 200 ng DNA per 5.0 µl.

Diagnostic screening comparison. Samples (n = 14) were selected from a sample pool for which we had previously determined results for the presence, or lack, of *S. muris* by 2 other commonly used diagnostic methods. All of the samples had been collected from mice housed in a room known to be endemically infected with *S. muris*.[20] The criteria set for sample selection were: availability of direct examination and intestinal histology results; a range of samples positive by 1, both, or neither method; samples from mice with either normal or severely compromised immune systems (that is, mice had only T cells with highly restricted specificity and no B cells); and availability of fecal samples stored at –80 °C. Typically, 2 fecal pellets were used for the isolation process, although samples as small as half of a pellet were sometimes used. PCR reactions were performed in triplicate for all samples, in light of the conflicting histopathology and direct examination results for some samples. Fecal pellets from the naïve Tac:SW mouse were used as the PCR mouse DNA negative control, in addition to an H_2O negative control.

Results

PCR product obtained from the highly infected sample. Both the primary and nested primers amplified a PCR product of the expected size. An nBLAST search of GenBank confirmed that the nucleotide sequence products obtained from the primary and nested primer pairs aligned 100% with the targeted regions of the carboxy terminus of *S. muris EF1*α. No mouse genes were present in the top 50 nBLAST alignment results for either PCR amplicon (data not shown).

PCR limits of detection and specificity. The serial dilution experiment confirmed the accuracy of the parasitologist's counts and our estimation of the number of cysts per 5-µl aliquot. The PCR assay was able to detect an estimated 10 copies of the *EF1*α carboxy terminus in both pyloric tissues and feces when stained with SYBR Gold (Figure 1). No positive amplification was detected in the samples positive for *Tritrichomonas* spp., *C. bettencourti*, *G. muris*, or *E. muris*, except for a single sample with an unanticipated weak band at the appropriate 400-basepair (bp) size, corresponding to *S muris EF-1*α, in 1 of the triplicate assays (Figure 2, sample 2A). The band was purified, sequenced, and was used as a query in an nBLAST search against GenBank. The results aligned 100% with the target region of *S. muris* EF1α.

Direct examination, histology, and PCR assay comparison. In all cases the PCR assay identified as positive, samples found to be positive by either direct examination or histology. In addition the PCR assay revealed 2 samples, previously identified as negative by these methods, to be positive. Three samples from mice with normal immune systems were positive by PCR in 2 of the triplicate tests; these samples were confirmed to be positive for *S. muris* by nested PCR (Table 1).

Fecal PCR sensitivity. Calculation of the PCR assay's sensitivity rested on 2 assumptions: that any sample identified as positive for *S. muris* by either direct intestinal examination or histology was a true positive (that is, that there were no false-positives) and that samples testing positive by PCR in all 3 triplicate assays (or in 2 replicates with a positive nested PCR result) were true positives. Because all 14 samples met the PCR criteria, all samples were considered to be truly positive. Assay sensitivity was calculated as:

$$\text{Sensitivity} = \text{no. of true positives} / (\text{no. of true positives} + \text{no. of false negatives}) \times 100\%$$

An example calculation is shown in Figure 3.

The sensitivity calculated for direct intestinal examination was: $7/(7+7) \times 100\% = 50\%$; the sensitivity of the fecal PCR when all assumptions were held to be true was $14/(14+0) \times 100\% = 100\%$. However, using the primary PCR results from a single PCR run and disregarding the nested PCR confirmatory results gives the primary fecal PCR assay a sensitivity of $13/(13+1) \times 100\% = 93\%$.

Discussion

S. muris is a facultative pathogen that can impair experiments in rodents. However, the last article that reported sporadic death putatively due to *S. muris* infection in several types of mice was published in 1993.[26] Reports on the adverse effects of the protozoan on research are also dated. The apparent disappearance of severe infections may result from a lack of reporting, changes in mouse microbial status or husbandry that have eliminated cofactors necessary for overt disease, or the prior existence of a more pathogenic strain of *S. muris* that is now uncommon. A recent report[20] also raises questions about the actual effect of *S. muris* infection on immunology research.

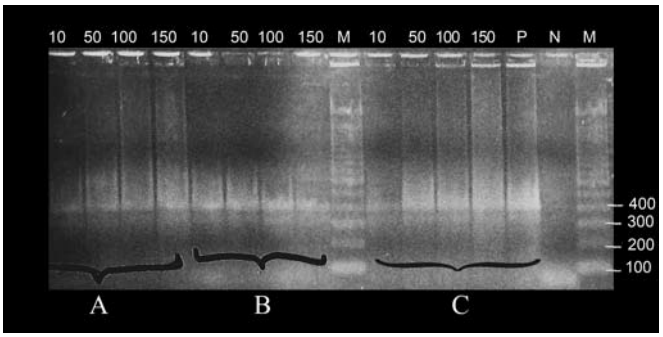

Figure 1. PCR limits of detection. Product of 393-bp *S. muris* carboxy terminus of *EF1*α. M, DNA molecular weight marker (100-bp ladder); P, positive control; N, negative control. Group A represents template amounts of 10, 50, 100, and 150 trophozoites per PCR reaction. Group B represents 10, 50, 100, and 150 cysts per PCR reaction. Group C samples 10 and 50 are template dilutions of the 100-trophozoite sample and represent 10 and 50 trophozoites, respectively. Samples labeled 100 and 150 are repeats of the B group 100 and 150 samples. SYBR Gold stain.

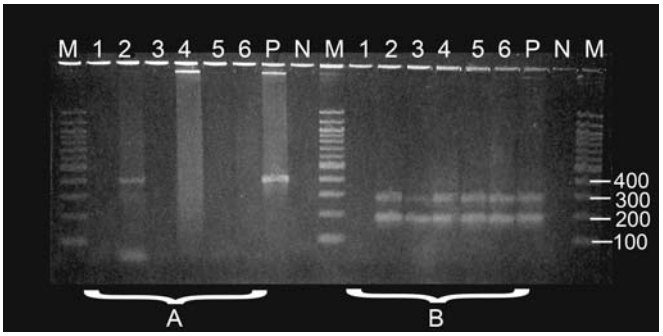

Figure 2. Assay of PCR specificity. Result of *S. muris* PCR assay performed on gut tissues infected by other common protozoa; *S. muris EF1*-α primers were used in the amplification process for the samples to the left of the center M, DNA marker (100-bp ladder); 1-6 tissue samples are bracketed by A; P, *S. muris* positive control (PCR product of 393 bp); N, negative control. Murine 36B4 primers (PCR product of 182 and 300 bp) were used in the amplification process for the tissue samples labeled 1-6 to the right of the center marker (M) and bracketed by B, to verify sample suitability for PCR amplification. Tissue samples were positive by direct examination for: 1. *Giardia*, 2. *Entamoeba*, 3. *Chilomastix*, 4. *Tritrichomonas*, lane 5. *Entamoeba* + *Tritrichomonas* + *Chilomastix*, 6. *Entamoeba* + *Tritrichomonas*. P positive control and N negative control (blank). SYBR Gold stain. (Although in this PCR, the *Giardia* sample (bracket A, lane 1) failed to amplify with the 36B4 primers; in previous assays it was positive.)

Previously to obtain *S. muris* DNA from mouse fecal samples, cysts were isolated on sucrose gradients[14] and then the trophozoites induced to excyst.[10] Here we report a rapid DNA isolation method from protozoan cysts in fecal pellets and the detection of *S. muris* by performing PCR using primer pairs specific for the carboxy terminal portion of *EF1*α of this flagellate.[13] The DNA isolation method and the PCR assay greatly simplify and improve the ability to detect this organism. Increased sensitivity of the PCR assay, as compared with direct examination and histopathology, was exemplified by the confirmed *S. muris*-positive finding in a sample thought to be negative according to the traditional methods as well as the identification of 2 additional positive samples during the diagnostic screening comparison study.

Although *S. muris* should not be ignored by investigators who use laboratory rodents, more work is needed to substantiate the effect of infection on research. This assessment can be done only if the organism's presence can be detected reliably. The current

Table 1. Comparison of results of direct examination, histology, and PCR assay for detection of *S. muris*

			Triplicate PCR assay of fecal sample			
Immune system	Direct examination	Histology	I	II	III	Nested PCR[a]
Severely compromised	positive	positive	positive	positive	positive	not done
Severely compromised	positive	positive	positive	positive	positive	not done
Severely compromised	positive	negative	positive	positive	positive	not done
Severely compromised	positive	positive	positive	positive	positive	not done
Severely compromised	positive	positive	positive	positive	positive	not done
normal	negative	positive	positive	positive	positive	not done
normal	positive	positive	positive	positive	positive	not done
normal	positive	negative	positive	positive	positive	not done
normal	negative	positive	negative	positive	positive	positive
normal	negative	positive	positive	positive	positive	not done
normal	negative	positive	positive	positive	positive	not done
normal	negative	positive	positive	negative	positive	positive
normal	negative	negative	positive	positive	positive	not done
normal	negative	negative	positive	positive	negative	positive
Total positive	7	10	13	13	13	3

Mice with severely compromised immune systems had no B cells, and any T cells present had highly restricted specificity.
[a]Performed on any sample with variable primary PCR results.

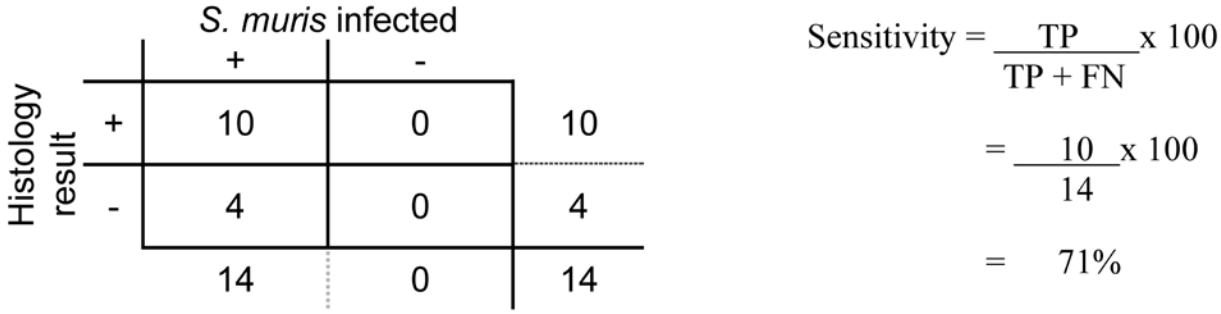

Figure 3. Example sensitivity calculation by using histology as a *S. muris* detection method.

use of dirty-bedding sentinels, with direct examination of intestinal contents to detect *S. muris*, poorly identifies the presence of the protozoan in monitored research colonies and requires euthanasia of the animal. In addition, direct examination can be affected by rapid deterioration of collected samples. In our study, we noted that the vegetative form of *S. muris* survives, depending on ambient temperature, for 0.5 to 2 h after removal from the body; samples must be processed rapidly to detect live trophozoites. Other techniques used for detection of the protozoan, such as fecal smear followed by immunochemical staining and light and electron microscopy, can be done on live animals but are not feasible for screening a large number of samples, because these methods are time-consuming, insensitive, or require highly skilled microscopists.[17]

By contrast, the PCR assay developed in this study is a rapid tool for detection of *S. muris* in either mouse fecal or tissue samples and can easily be incorporated into a health surveillance program that is already performing diagnostic PCR tests. With a detection limit of 10 cysts per 5-µl aliquot (Figure 1, group B, lane 1), the fecal PCR assay likely can reliably detect *S. muris* shortly after and throughout infection. Within a week of experimental infection, S. *muris* cysts are shed. Shedding is continuous throughout the course of infection, and counts of cyst output parallel small intestine trophozoite counts.[5]

As a note of caution, the PCR assay was tested only on samples from mice. The nucleic acid sequence of *EF1*α is highly conserved across diverse species – mammals, protozoa, bacteria, and fungi – and the primary PCR forward primer aligns 100% with a number of non-*S. muris EF1*α sequences deposited in GenBank. Use of the PCR to test for *S. muris* in other rodents requires optimization of the PCR reaction and sequencing of the PCR product to confirm production of the anticipated 393-bp sequence. An nBLAST search of the primary PCR reverse primer sequence resulted in 100% homology with a sequence found in normal rat tissue as well as with a *Bacteroides* spp. commonly found in the human lower intestinal tract. Although the cull mice used in this study were known to be contaminated by other common protozoa (Figure 2) and bacteria, to date in laboratory mice, the PCR has proven to be specific for *S. muris* by the absence of multiple bands in the PCR gels and by confirmation that the resulting PCR product sequence aligns with the sequence for the *S. muris* carboxy terminus of *EF1*α deposited in GenBank. Suspected false-negative PCR samples should be retested by amplification of an aliquot of the primary PCR reaction solution with the nested primers.

While diethyl pyrocarbonate-treated water was used to adjust the concentration of the DNA for the PCR reaction, any reliable source of molecular biology grade H_20 would work. Trevigel

500 was used as the matrix for electrophoresis of PCR products because of its superior clarity compared with that of agarose. In addition, during development of the assays, the use of SYBR Gold as the intercalating DNA dye, in lieu of ethidium bromide or Biotium Red Dye, proved to increase sensitivity of the assay to low copy numbers, obviating the need for confirmatory nested PCR assays.

In conclusion, we have developed a sensitive and specific PCR method for rapid detection of *S. muris* trophozoites from mouse tissues and feces. PCR testing allows for specific diagnosis, epidemiologic studies, and routine screening and is recommended for screening of a large number of mice in a short time period. The availability of a fecal PCR test for *S. muris* is especially beneficial, because colonies can be screened, and the parasite detected, without euthanizing the animals.

Acknowledgments

We wish to thank Dr BJ Fowlkes, Dr Karen Laky, Ms Sharron Evans, RSSIII, for providing cull animals for testing; Ms Elizabeth M Williams for tissue processing assistance; Mr Jeremy Dathe of Lofstrand Labs for his superior service; Ms Roman Tesfaye for her help with the figures; and Dr Gerry Shea for his scientific assistance. This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Disease (National Institutes of Health, Bethesda, MD). The views and opinions provided are those of the authors and do not reflect the official policy or positions of the National Institutes of Health, the Department of Health and Human Services, or the United States Government.

References

1. **AVMA Panel on Euthanasia.** 2000. 2000 Report of the AVMA Panel on Euthanasia. J Am Vet Med Assoc **218:**669–696.
2. **Baker DG, Malineni S, Taylor HW.** 1998. Experimental infection of inbred mouse strains with *Spironucleus muris*. 1998. Vet Parasitol **77:**305–310.
3. **Bancroft JD, Stevens A.** 1996. Theory and practice of histological techniques, 4th ed. Oxford (UK): Churchill Livingstone. p 129.
4. **Brett SJ.** 1983. Immunodepression in *Giardia muris* and *Spironucleus muris* infections in mice. Parasitology **87:**507–515.
5. **Brett SJ, Cox FE.** 1982. Immunological aspects of *Giardia muris* and *Spironucleus muris* infections in inbred and outbred strains of laboratory mice: a comparative study. Parasitology **85:**85–99.
6. **Brugerolle G, Kunstyr I, Senarud J, Friedhoff KT.** 1980. Fine structure of trophozoites and cysts of the pathogenic diplomonad *Spironucleus muris*. Z Parasitenkd **62:**47–61.
7. **Dumontier M, Hoque CW.** 2002. NBLAST: a cluster variant of BLAST for N×N comparisons. BMC Bioinformatics **3:**13.
8. **Eisenbrandt DL, Russell RJ.** 1979. Scanning electron microscopy of *Spironucleus (Hexamita) muris* infection in mice. Scan Electron Microsc **3:**23–27.
9. **Exon JH, Patton NM, Koller LD.** 1975. Hexamitiasis in cadmium-exposed mice. Arch Environ Health **30:**463–464.
10. **Feely DE, Gardner MD, Hardin EL.** 1991. Excystation of *Giardia muris* induced by a phosphate–bicarbonate medium: localization of acid phosphatase. J Parasitol **77:**441–448.
11. **Flynn RJ.** 1973. Parasites of laboratory animals. Ames (IA): Iowa State University Press. p 17.
12. **Keast D, Chesterman FC.** 1972. Changes in macrophage metabolism in mice heavily infected with *Hexamita muris*. Lab Anim **6:**33–39.
13. **Keeling PJ, Doolittle WF.** 1997. Widespread and ancient distribution of a noncanonical genetic code in diplomonads. Mol Biol Evol **14:**895–901.
14. **Koudela B, Karr CD, Meyer EA, Meyer B, Jarroll L.** 1996. In vitro excystation of *Spironucleus muris.* J Eukaryot Microbiol **43:**61–64.
15. **Kunstyr I, Ammerpohl E, Meyer B.** 1977. Experimental spironucleosis (hexamitiasis) in the nude mouse as a model for immunologic and pharmacologic studies. Lab Anim Sci **27:**782–788.
16. **Meshorer A.** 1969. Hexamitiasis in laboratory mice. Lab Anim Care **19:**33–37.
17. **Morgan UM, Thompson RC.** 1998. Molecular detection of parasitic protozoa. Parasitology **117 Suppl:S**73–S85.
18. **Mullink JW, Ruitenberg EJ, Kruizinga W.** 1980. Lack of effect of *Spironucleus (Hexamita) muris* on the immune response to tetanus toxoid in the rat. Lab Anim **14:**127–128.
19. **National Research Council.** 1991. Infectious diseases of mice and rats. Washington (DC): National Academy Press. p 148.
20. **Perdue KA, Copeland MK, Karjala Z, Cheng LI, Ward JM, Elkins WR.** 2008. Suboptimal ability of dirty-bedding sentinels to detect *Spironucleus muris* infection in a colony of mice with various genetic manipulations of the adaptive immune system. J Am Assoc Lab Anim Sci Forthcoming.
21. Qiagen Technical Support Line. 2007. Technical support: referred to page 44 of the kit manual.
22. **Ruitenberg EJ, Kruyt BC.** 1975. Effect of intestinal flagellates on immune response of mice. Proceedings of the British Society for Parasitology; 1975 April 15–17; Norwich, England. Cambridge (UK): Cambridge University Press.
23. **Sebesteny A.** 1974. The transmission of intestinal flagellates between mice and rats. Lab Anim **8:**79–81.
24. **Stachan R, Kunstýr I.** 1983. Minimal infectious doses and prepatent periods in *Giardia muris*, *Spironucleus muris*, and *Tritrichomonas muris.* Zentralbl Bakteriol Mikrobiol Hyg [A] **256:**249–256.
25. **Wagner JE, Doyle RE, Ronald NC, Garrison RG, Schmitz JA.** 1974. Hexamitiasis in laboratory mice, hamsters, and rats. Lab Anim Sci **24:**349–354.
26. **Whitehouse A, France MP, Pope SE, Lloyd JE, Ratcliffe RC.** 1993. *Spironucleus muris* in laboratory mice. Aust Vet J **70:**193.