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Suboptimal Ability of Dirty-bedding Sentinels to Detect *Spironucleus muris* in a Colony of Mice with Genetic Manipulations of the Adaptive Immune System

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***Spironucleus muris* is an unacceptable infectious agent for most rodent colonies. Exposure of sentinel mice to dirty bedding and examination of sentinel intestinal smears was not sufficient for identification of the extent of spironucleosis within 1 mouse room. Clinical abnormalities were not reported in the animals housed in the room despite extensive breeding and a preponderance of mice genetically engineered to have nonfunctional T and B cells. In addition, researchers reported that the infection had not altered their research data. During investigation of the outbreak, direct intestinal smears performed on related animals (conspecifics, offspring, or siblings) revealed that immunodeficient mice often tested negative whereas the immunocompetent cohort tested positive. In this study, we used culled colony animals and compared direct intestinal exam test results with histologic results. The comparison showed the extent of false negatives that may occur when direct intestinal exam alone is used to detect this protozoon. Sensitivity of the direct intestinal exam for detection of *S. muris* was calculated to be 71%, while histology sensitivity was 91%. In light of the study results and an extensive literature review, we revised our health surveillance plan so that the age and duration of exposure to dirty bedding among sentinel mice is varied at the time of testing.**

Spironucleus muris (Figures 1 and 2) is classified as a parasitic diplomonad flagellate in the family Hexamitidae. Originally described in 1881 and called *Dicercomonas muris*,[19] the organism was renamed, first as *Hexamita muris*[44] and later as *Spironucleus muris*.[8] In 1969, an article[29] was published implicating *Spironucleus* as a cause of disease in mice; prior to that time, the protozoan was considered to be nonpathogenic. Other reports[12,17,27,36,43] corroborating the parasite's ability to cause clinical illness quickly followed. In the 1970s and early 1980s, a considerable amount of research was done to characterize the parasite, and its presence was shown to interfere with immunology research in mice.[6,7,21,34] Consequently, *S. muris* has been considered an unacceptable pathogen for research animals, and currently vendors and most rodent research facilities, including the National Institute of Allergy and Infectious Diseases, exclude this protozoan from their rodent colonies.

Rodent colonies at the National Institute of Allergy and Infectious Diseases were monitored for protozoa by screening sentinel mice exposed to dirty bedding from research mouse cages. Sentinel mice were submitted to the National Institutes of Health, Division of Veterinary Resources, Animal Health Surveillance Laboratory approximately every 6 wk. Screening for intestinal protozoa was performed by direct examination of intestinal contents.

In March 2006, 1 of 28 sentinel animals from 1 room of a facility was reported as positive for *S. muris*. This room had been depopulated and the research colonies embryo-rederived 3 y earlier to eradicate the protozoa. Therefore, this result generated great concern. Research use of the mice in the room involved extensive breeding, with a goal of having multiple targeted and nontargeted mutations of the immune system on single mice. Many of the strains in the room were not commercially available and required homozygous rederivation to preserve their engineered genetics.

According to program standard operating procedure, the room was quarantined. *S. muris* infection was confirmed by testing a cagemate of the index positive sentinel animal. Subsequently cull mice from the research colonies were sent to the Health Surveillance Laboratory to determine the extent of infection; 93 of 267 mice (35%) submitted were found to be positive, with positive animals identified on all racks in the room. The researchers, dreading the lost research time involved with eliminating the parasite again, expressed concern about the inconsistency of the direct smear findings as well as the need to eradicate the organism. The direct smears showed positive and negative animals in the same cage. However, the investigators stated that sometimes the animals reported as positive were immunocompetent, whereas their immunocompromised cagemates reportedly were uninfected. In addition, the investigators' expected research results, involving both T- and B-cell derangements,[25,26] had not changed since the last room depopulation, nor had colony breeding parameters or incidence of illness in the various lines changed. Therefore the researchers questioned the reliability of using direct intestinal examination to identify the organism, as well as the conclusions reached in the early

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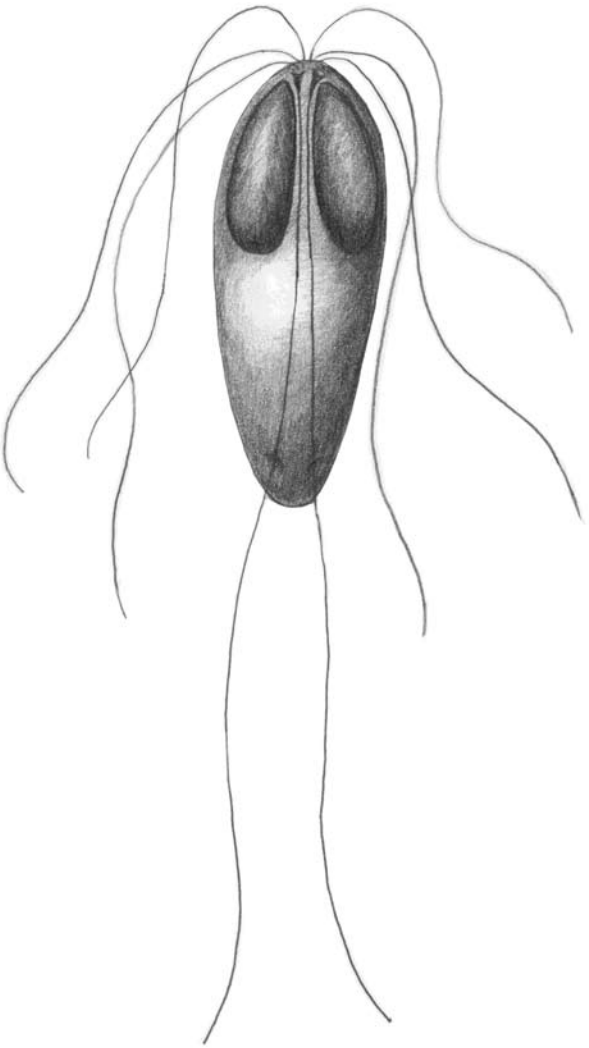

Figure 1. Artist depiction of *S. muris*. The trophozoite is teardrop-shaped and has 6 anterior and 2 posterior flagella. The posterior flagella arise internally from the anterior region of the cell and emerge from the 2 subterminal openings or cytostomes on the posterior region of the cell. The body measures 3 to 4 µm across and is 7 to 9 µm long.[9,19]

published articles about the organism's effect on animal health and immunology research.

Cull mice from the involved room were studied to explore the apparent inconsistencies seen with direct intestinal examination for the parasite. Histopathology of the upper small intestine and gastric pylorus, a method considered to be superior to other diagnostic methods,[32] was chosen as a secondary assessment method. An extensive literature review of *S. muris* was completed, and sentinel mice with variable durations of exposure to dirty-bedding were tested for the protozoa in an effort to improve the ability of the program to detect *S. muris*. The rationale for the redesigned program plan is presented.

Materials and Methods

Husbandry. Mice were housed on 7 stainless-steel shelving units that held as many as 98 filter-top static polycarbonate microisolation cages (Allentown, Allentown, NJ) each. Hardwood chip bedding (7086G, Harlan Teklad, Madison, WI), nesting material (Nestlet, Ancare, Bellmore, NY), and autoclavable pelleted feed (2018SX, Harlan Teklad) were placed in the cages

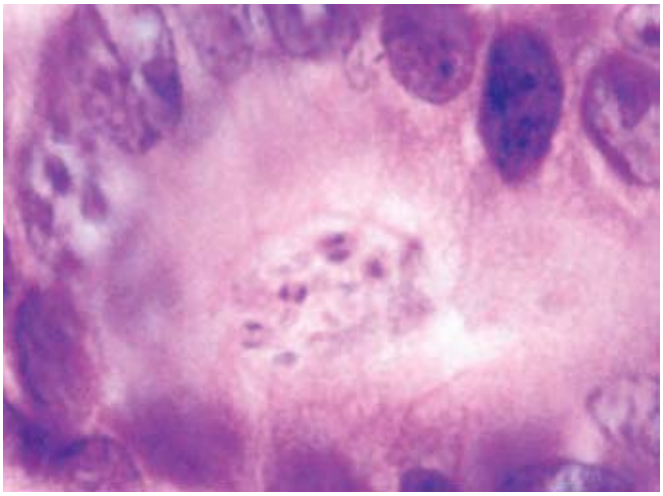

Figure 2. Cross-section of a pyloric crypt containing numerous *S. muris* organisms with prominent paired anterior nuclei that give the protozoan a '2-eyed' appearance. Hematoxylin and eosin stain; magnification, ×1000.

before autoclaving. Acidified water (pH, 2.9) was provided ad libitum in sterile bottles, which were added to the cages during cage changing. Cages were changed at least once weekly in a Class II biological safety cabinet, by using 1:18:1 chlorine dioxide (Clidox, Pharmacal, Naugatuck, CT) to spray off the cages and the handler's gloves; excessively soiled cages were changed more frequently. Personnel entering the quarantined room wore a hair bonnet, surgical mask, gloves, a Tyvek (DuPont, Richmond, VA) jumpsuit over either dedicated facility clothing or their personal clothing, and disposable shoe covers donned over either dedicated facility shoes or street shoes already covered by shoe covers that were put on at the facility entrance. This layer of disposable clothing was removed and left in the room on exiting. The daily lighting scheme provided 14 h of light and 10 h of dark, the room air pressure was negative relative to the corridors, the room temperature was maintained between 20 and 23°C, and relative humidity was maintained between 30% and 70%. All mice were on protocols approved by the animal care and use committee in accordance with applicable federal regulations.

Bedding-exposed routine sentinel mice. Taconic Swiss–Webster mice (Tac:SW; Taconic Farms, Germantown, NY) were used routinely as sentinel animals. Three 4- to 6-wk-old, female Tac:SW mice were maintained in each sentinel cage, with the oldest animal in the cage sent for testing and replaced every 6 to 7 wk. Each of the 7 racks housed 1 sentinel cage per active rack quadrant; rack quadrants held as many as 21 (upper quadrant) or 27 (lower quadrant) cages depending on the current population of the room at the time of sampling. The sentinel cages received 15 cm^3 of dirty bedding weekly from all cages within the respective quadrant. Routinely sentinels were exposed to dirty bedding once weekly for 18 wk before being sent to the Health Surveillance Laboratory for testing when 22 to 24 wk of age. Over the study period, 363 routine sentinels were tested for protozoa. Testing was limited to direct intestinal examination.

Once yearly in October, 4-wk-old Tac:SW mice are used in our sentinel program to provide adjunct screening animals for mouse parvovirus and pinworms. These mice were distributed 1 per rack and housed separately from the other sentinels. Once all cages on a rack were changed, a handful of dirty bedding was removed from each of the 4 sentinel cages on the rack and added to the cage housing the single mouse. This task was

done for 4 wk and then the mouse was submitted to the Health Surveillance Laboratory for mouse parvovirus serology and endoparasitology. Hoping to better identify the level of *S. muris* infection in the room, we added examination for *S. muris* by direct intestinal examination to the surveillance request. This type of testing was performed only once during the study period.

Sentinel mice from the room have tested negative for mouse hepatitis virus, pneumonia virus of mice, Sendai virus, Theiler murine encephalomyelitis virus, mouse rotavirus, lymphocytic choriomeningitis virus, *Ectromelia* virus, mouse cytomegalovirus, minute virus of mice, polyoma virus, reovirus 3, mouse adenovirus, Hantaan virus, mouse parvovirus, cilia-associated respiratory bacillus, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Salmonella* spp., *Streptobacillus moniliformis, Mycoplasma pulmonis*, and common endo- and ectoparasites for 2 y before manuscript submission. Murine norovirus, *Helicobacter* spp., *Chilomastix bettencourti*, and *Tritrichomonas* spp. were known to be highly prevalent in the room.

Sentinel program variations. To optimize *S. muris* detection, we added available surplus mice to the room's sentinel program. At different time periods, mice lacking 1 of the recombinant activating genes (RAG mice) were housed individually, 1 per rack, and exposed to dirty bedding by using the procedure outlined for the annual 4-wk-old Tac:SW sentinels. The RAG mice were excess animals from a breeding contract with Taconic Farms held by the National Institute of Allergy and Infectious Diseases and were 1 of the following genotypes: B10.AAi-Rag2^{tm1Fwa},[39]C57BL/10SgSnAi-Rag2^{tm1Fwa},[30] or C57BL/6JAi-Rag1^{tm1Mom}.[31] The first RAG mice placed were euthanized and tested after 2 wk of exposure to dirty bedding; a second group was exposed for 4 wk before being tested.

A total of 28 surplus Tac:SW mice were added, 1 per sentinel cage, to the room. The mice were 5 wk old at the time of placement and were screened for protozoa 5.4 wk (38 d) after initial exposure. Occasionally other sentinels that had been exposed to dirty bedding for less than the 18-wk standard period were submitted to the Health Surveillance Laboratory for parasite screening. These sentinels were submitted for various reasons: follow-up testing after an initial positive result, illness, or to determine whether a shorter exposure time would result in a greater number of positive animals. Over 18 mo, 29 shortened-exposure sentinels were submitted; the period of dirty bedding exposure for these mice varied from 3.6 wk (25 d) to 16.6 wk (116 d; Table 1). None of the groups described were housed in the room at the same time. In all cases, testing for protozoa was limited to direct intestinal examination.

Research colonies. The mice in the involved room were used for studies of T-cell differentiation, selection, and development. Most of the mice were genetically engineered (that is, transgenic or knockout) and then bred to produce mice with multiple genetic manipulations of the adaptive immune system. During a 5-mo period, 18 different transgenic, knockout, transgenic–knockout combination strains culled from 32 cages were obtained from the investigator and examined; 29 of the cages housed more than 1 animal. Colony mice were on a mixed 129–C576BL/6–C57BL/10 background in various combinations. The animals ranged in age from 10 to 36 wk old when euthanized and had normal, compromised (decreased numbers of T cells), severely compromised (no B or T cells other than T cells with highly restricted specificity), or deficient (no T or B lymphocytes) immune systems. A total of 87 research mice were tested for *S. muris* by both direct intestinal examination and histology.

Table 1. Dirty-bedding sentinels exposed for less than 18 wk

Exposure time in wk (d)	No. positive for *S. muris* (no. tested)	Age in wk when placed (d)	Reason submitted
3.6 (25)	0 (4)	9.4 (66)	↓ exposure
5.4 (38)	2 (28)	4.0 (28)	↓ exposure
6.1 (43)	1 (4)	7.4 (52)	↓ exposure
7.9 (55)	0 (1)	6.6 (46)	ill
8.0 (56)	2 (2)	4.0 (28)	follow-up
9.0 (63)	0 (1)	9.6 (67)	ill
10.6 (74)	0 (1)	9.6 (67)	ill
10.7 (75)	1 (1)	9.6 (67)	ill
11.0 (77)	1 (1)	9.6 (67)	ill
11.3 (79)	0 (1)	4.0 (28)	follow-up
11.4 (80)	0 (3)	4.0 (28)	follow-up
13.7 (96)	1 (1)	5.0 (35)	follow-up
14.1 (99)	0 (1)	6.0 (42)	ill
15.6 (109)	0 (1)	5.0 (35)	follow-up
15.9 (111)	4 (6)	5.0 (35)	follow-up
16.6 (116)	0 (1)	7.6 (53)	ill
	Total: 12 (57)		

Control mice. Three naïve surplus Tac:SW mice from the sentinel program, assumed to be negative for *S. muris*, were sent to pathology for direct intestinal examination and histology sample collection.

Sample collection. Health Surveillance Laboratory personnel (ZK) performed the necropsies and tissue collections. Compressed carbon dioxide gas was used to euthanize the mice. Gastrointestinal tracts were removed and sectioned to include a portion of the glandular stomach, the pylorus and the proximal duodenum. Each sample then was cut bilaterally lengthwise into 2 identical halves. One tissue half was placed into PBS (pH 7.4) and minced with surgical scissors. A 20-µl drop of liquid was removed from the resultant suspension for observation under a light microscope. The remaining minced tissue suspension was frozen and stored at –80°C for later evaluation with a PCR assay; an assay has been developed and will be reported separately. The remaining intact half of the gastrointestinal tract was placed in 10% formalin.

Direct examination. The tissue suspension droplet was placed on a glass slide and examined by light microscopy under ×250 and ×400 magnification. *Spironucleus* organisms were identified by their morphology and typical pattern of movement.

Histology. Formalin-fixed tissues were processed by Histoserv (Germantown, MD). The resultant hematoxylin- and eosin-stained slides were read by veterinary pathologists in the Comparative Medicine Branch, Infectious Diseases Pathogenesis Section, for the presence of *Spironucleus* organisms and evidence of inflammation.

Results

Sentinel animal results in the affected room. Sentinel animals were examined by direct intestinal exam only. Over a 16-mo period, only 1 of 363 (0.73%) routine sentinels, exposed to dirty bedding from the affected colony mice for approximately 18 wk, tested positive for *S. muris.* None of seven 4-wk-old Tac:SW mice exposed for 4 wk were found to be positive for *S. muris*. Among the RAG sentinels, 2 of the 7 (29%) exposed for 2 wk and 1 of the 7 (14%) exposed for 4 wk tested positive. Among Tac:SW mice tested 5.4 wk (38 d) after exposure, 2 of 28 were positive

for *S. muris*. The direct intestinal examination test results for these mice and 29 other sentinels with exposure times shorter than 18 wk are shown in Table 1. Of the 57 animals tested, 12 mice (21%) exposed for 3.6 to 16.6 wk were identified as *S. muris* positive.

Identification of infected experimental animals by direct examination and histology. Cull mice from all 7 racks in the room were tested, and positive animals were identified on all racks. Findings from the 2 detection methods are presented in Table 2. Eight of the 29 multianimal cages showed mixed results, that is, both positive and negative animals were in the same cage, when tested by direct exam. The combination of direct exam and histology results showed that all of the mice in 6 of these cages were infected with *Spironucleus*. In 5/6 mixed result cages, histology identified all mice as positive. In a sixth cage 2/4 mice were positive by direct exam while 3 of 4 mice were positive by histology. However the mouse negative by histology was positive by direct exam. (Data not shown.)

Histologic examination of the animals from the 29 multianimal cages resulted in mixed results in 7cages. Three of these cages were cages that had been found to have mixed results by direct exam. By direct exam, the other cages were identified as positive in 2 cases and negative in the other 2. Histology identified 5 additional positive cages (3 multianimal and 2 single) that had been reported as negative by using the direct exam.

Only 1 cage contained a mouse that was negative according to both detection methods as well as mice confirmed positive by 1 or both detection methods; the mice in the cage had normal immunocompetency. Another cage held mice with differing immunocompetencies; all 3 of the mice in the cage were negative. Twenty-nine of the 87 cull mice studied were negative by both detection methods.

Confirmation of control mice negativity. All 3 naïve Tac:SW mice were negative for *S. muris* by both direct exam and histology (Table 2).

Statistical sensitivity and specificity of the direct intestinal and histological exams. To compare the ability of each test method to identify positive animals (test sensitivity), test specificity (the ability to identify negative animals) was set at 100%. The 32 samples that were negative according to both detection methods were considered to be true negatives.[18] A total of 90 samples (87 research mice and 3 naïve sentinels) were examined and included in the computation. Any animal identified as infected by either assay method was considered to be truly infected with *S. muris*; therefore there were no false positives. Given that 32 samples were considered true negatives, 58 samples (90 – 32= 58) were true positives. The direct intestinal examination identified 41 positives, leaving 17 samples (58 – 41 = 17) that were false negatives. Histology identified 53 of the 58 positive samples, whereas the remaining 5 samples were false negatives. Assay sensitivity was determined as:

no. of true positives (TP) ÷ (no. of true positives + the number of false negatives) × 100%

Assuming 100% test specificity, sensitivity for the direct intestinal exam was calculated to be 71% compared with 91% for histologic exam (Figure 3).

Necropsy and histology findings. No gross abnormalities of the intestinal tract were noted in any of the mice at the time of tissue collection. Protozoa were identified on histologic slides by their size, location, and typical binucleate morphology (Figures 4 through 6); the pathologist scored the stomach fundus, pylorus, and proximal duodenum for the organism's presence (data not shown). *S. muris* was found in all 3 tissues in only 2 of 58 positive mice; 6 positive mice had *Spironucleus* in the stomach fundus; 5 of these mice were severely immunocompromised. Examination of the pyloric and duodenal sections identified 50 and 24 of the 58 positive animals, respectively. *S. muris* organisms were found most commonly within crypts at the pyloric–duodenal junction.

Inflammation, characterized by lymphocytes, plasma cells, and occasional neutrophils or eosinophils, was segmentally present in the stomach or duodenum of some animals with normal immune systems (Figure 4). However, inflammation was not consistently associated with the presence of *Spironucleus* organisms. Likewise, there were no consistent changes in the epithelium in contact with the organism or in crypt depth.

Effect of infection on research. Experimental animals continued to thrive in the infected colonies. No clinical disease was reported, and no adverse effects on research were identified by the investigators housing mice in the room.

Discussion

Our findings add to the growing body of literature that points out pitfalls of dirty-bedding exposure sentinel programs.[1,11,13,41] The current study also highlights the importance of devising health surveillance programs that by their design successfully identify mice contaminated with undesirable infectious agents. The infectiousness of an agent and its lifecycle along with the age, immune status, and exposure period of the sentinels used must be considered carefully.

The addition of histology increased the ability to detect infected mice and helped clarify shortcomings of the direct examination. Although histology was a more sensitive test, it is time-prohibitive for large health surveillance programs. Unlike histology, the direct method is relatively easy to learn, simple and expeditious to perform, and gives prompt results. However, the sensitivity of both test methods varies with the experience of the individual performing the test.

The current prevalence of *S. muris* in research colonies has been estimated to be high.[2] Previous to our experience, we would have disagreed with this assertion based on health surveillance information compiled at the National Institutes of Health as well as reports received by the Comparative Medicine Branch for domestic and foreign rodent imports. Unfortunately, according to our experience, lack of positive findings may be due to flaws in the design of the health surveillance program. Programs that rely on testing dirty-bedding-exposed animals may miss adventitious infections. The detection problem may be compounded by rotating animals in and out of the sentinel cage when there are a number of months between first exposure to an agent and testing for that agent.[3,7,10]

Surveillance of Tac:SW sentinels exposed to dirty bedding for 18 wk and tested by direct examination of intestinal contents inadequately represented the health status of the room. Despite extensive and severe *S. muris* infection of colony mice, only 1 routine sentinel animal was found to be positive over a 16-mo period. Similarly, Tac:SW mice exposed to dirty bedding for 4 wk and submitted for testing when 8 wk old did not reveal the infection. Although RAG mice exposed in the same manner for 2 wk did identify 2 out of 7 racks as harboring *S. muris* infected mice, use of these mice routinely as sentinels would likely be cost-prohibitive unless a free source was available, as in our case. Results from Tac:SW sentinels exposed to dirty bedding for 6 to 16 wk suggest that establishing an exposure period between 6 and 16 wk in duration may benefit *S. muris* detection.

Considering the life cycle of *S. muris*, the inability of Tac:SW sentinels, exposed for 4 wk, to detect the organism was surprising. A study in which stocks and strains of mice were orally

Table 2. Comparison of the results of direct and histologic examination

Immune system	No. of mice in cage	Direct exam[a]	Histology[a]	Age at euthanasia (wk)
compromised	1[b]	negative	negative	10
compromised	1	negative	+	31
compromised	4	+	+	31
compromised	3	2	+	32
compromised	2	+	+	32
compromised	3	2	+	36
deficient	3	+	+	29
deficient	2	+	+	31
deficient	3	negative	+	32
deficient	1	negative	+	32
deficient	2	+	+	32
deficient	1	+	+	32
deficient	2	negative	negative	33
normal	3	negative	negative	10
normal	3	negative	negative	10
normal	3	negative	negative	10
normal	2[b]	negative	negative	10
normal	3[c]	negative	negative	26
normal	4	negative	negative	31
normal	4	3	3	31
normal	3	1	+	32
normal	4	2	3	32
normal	3	2	1	33
normal	3	negative	1	33
normal	3	2	+	33
normal	3	1	+	33
normal	3	negative	2	33
normal	4	negative	negative	33
severely compromised	4	+	+	30
severely compromised	2	+	+	32
severely compromised	2	negative	negative	34
severely compromised	2	+	1	34
severely compromised	2	+	+	34
severely compromised	2	+	1	36
Totals:	90	41	53	

[a]A + indicates that all animals in the cage were positive; otherwise the number of positives is given.
[b]Mice in the same cage but differed in immune competency.
[c]Tac:SW control mice

infected with *S. muris* cysts showed peak levels of trophozoites and cysts occurred 2 wk after infection, with trophozoite counts higher but paralleling cyst output. Cyst output ceased within 10 wk for most of the strains studied. Two strains and a Swiss–Webster-derived Theiler Original (TO Swiss) mouse stock continued to excrete cysts at the end of the 13-wk study, with cyst output highest (more than 10^5 cysts excreted every 2 h) in the TO Swiss.[7] Assuming similar results for Tac:SW mice, our sentinels should be susceptible to and maintain infection for more than 13 wk, although the infection could have been overcome between 13 and 18 wk. Curiously, the RAG and 4-wk-old Tac:SW mice with a 4-wk exposure time did not result in more positive findings. The dilution effect of taking a handful of dirty bedding from each of 4 sentinel cages, which represents exposure of the 4-wk-old and RAG mice to bedding from as many as 94 cages, may have been a factor. Controlled studies are being developed to further explore the use of Tac:SW mice of different ages to detect *S. muris* by using dirty-bedding transfer.

The suboptimal ability of dirty bedding exposed sentinels to detect spironucleosis may be due to the lack of cyst hardiness after exiting the animal. *S. muris* trophozoites populate the gastric pylorus and intestinal lumen. Trophozoites that are carried into the lower intestine transform into cysts and are excreted in the feces.[9,23] Knowledge of the hardiness of excreted cysts has evolved. Unlike some oxyurid ova,[14,33] cysts are readily susceptible to desiccation. Initially cysts in feces were understood to retain infectivity for 2 wk at room temperature.[22] However, later transmission electron microscopy studies revealed that cysts darkened as they moved into the large intestine and rectum. As the cysts darkened, trophozoite flagellar movement stopped. After 2 h in feces, all of the cysts reached this 'old' stage, that is, they were no longer 'fresh.' In time the old cysts

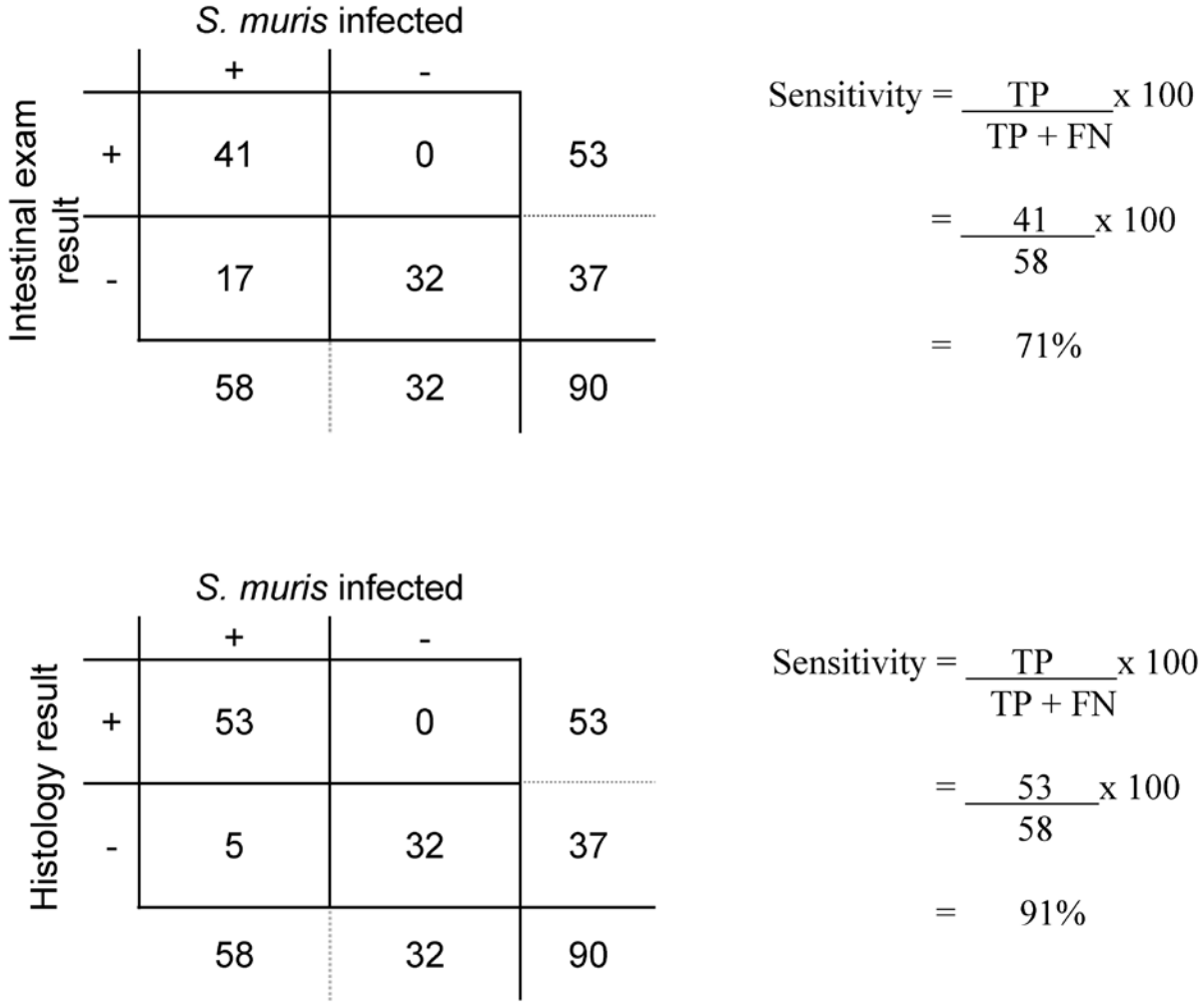

Figure 3. Estimated sensitivity of intestinal and histology examination for detection of *S. muris.*

slimmed and became noninfectious.[9] The time until noninfectiousness was not stated, but based on the earlier article,[22] at least some of the cysts must survive as long as 2 wk.

Cysts are destroyed quickly when exposed to 70% ethanol (15 s) or 13% hypochlorite (1 d).[22] Susceptibility to drying, destruction by sanitizing chemicals along with use of microisolation caging, disposable protective clothing, and changing cages with aseptic technique in a biological safety cabinet could account for lack of introduction of an infectious dose from the research mice to the sentinels. Likewise, these factors could explain our ability to maintain some mouse lines free of *S. muris* (data not shown) on racks housing heavily infected mice.

Severe *S. muris* infection, with resultant diarrhea, weight loss, hunched posture, and in some cases a 20 to 50% mortality rate in weanling mice, in several stocks and strains has been reported.[4,5,15,27,29,36,43,45] Weaning has been suggested as a major stressor predisposing to severe disease.[27,29,36] The intestinal tracts of sick animals contained a watery, foamy, white to yellow material. In 1 case, histology revealed glands of Lieberkühn so full of flagellated organisms that the glands appeared cystic.[29] Several cases reported that organisms caused epithelial damage or invaded the lamina propria of the gastrointestinal tract.[16,17,27,29] Others reported no structural alteration to the tract and suggested that clinical signs were related to changes in gut flora[5] or decreased intestinal absorption leading to malnutrition.[28,29,42] Although *S. muris* is considered a facultative pathogen, the extent of infection discovered in the current study in the 11 mouse lines with altered immune systems, without any clinical signs, was unexpected. Perhaps the protozoa has subtle effects on breeding efficiency or weaning weight, but demonstrating such effects in the room would be difficult because of the genetic inconsistency of the mice held. The mice are on a mixed background and the transgenic–knockout gene combinations bred onto the various backgrounds change frequently. *Helicobacter* spp., other protozoa, and murine norovirus are other potential confounders.

Cystic crypts full of protozoa, as described previously,[29] were not found in any of the immunodeficient mice that were 36 wk old when euthanized. The exact age at which these mice were infected is unknown, but infection likely occurred prior to weaning because the mice came from breeder cages that consistently produced infected offspring. Without more definitive correlation with the *Spironucleus* organisms, changes seen in the histology sections could not be attributed to the protozoa.

Variable virulence of *S. muris* strains has been suggested,[9] and the possibility of multiple strains of *S. muris* in mice could account for the lack of clinical signs. *S. muris* organisms isolated from mice, rats and hamsters show variable host specificity.[24,35,37,38] Multiple species of *Spironucleus* are found in fish, with 1 species being pathogenic whereas the other is a commensal.[20] Perhaps the same is true in mice.

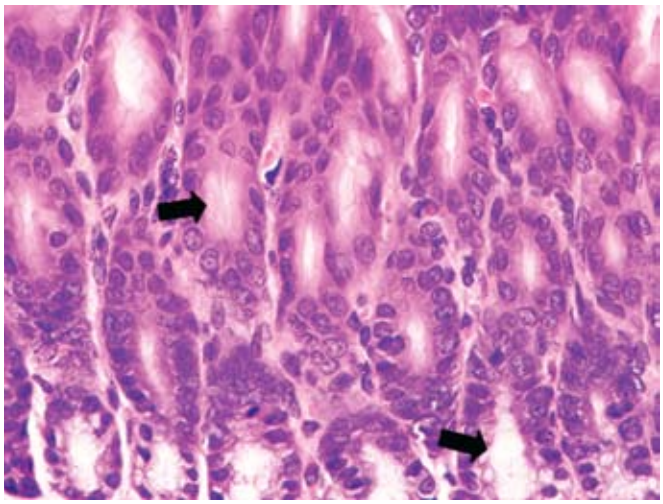

Figure 4. Pyloric crypts (arrows) of a 26-wk-old uninfected Tac:SW mouse. Hematoxylin and eosin stain; magnification, ×400.

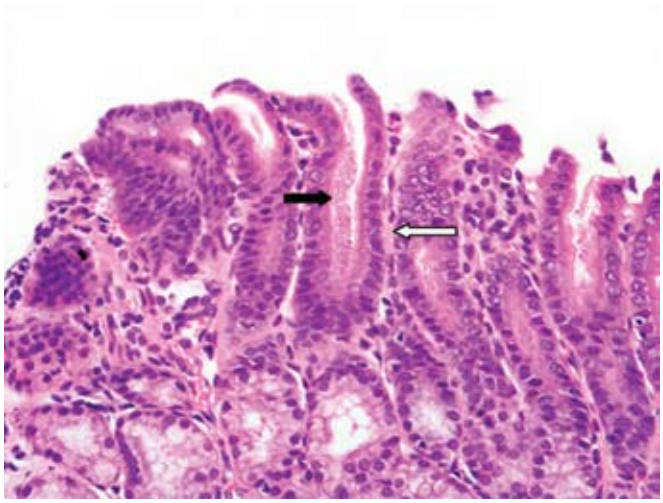

Figure 5. Cypts at the pyloric-duodenal junction of a 33-wk-old experimental mouse with a normal immune system and infected with *S. muris*. Note the crypt packed with protozoa (closed arrow) and the lack of an inflammatory response (open arrow). Hematoxylin and eosin stain; magnification, ×400.

In vitro studies showed that the activity of macrophages from *S. muris*-infected mice was increased whereas their response to foreign antigen was impaired.[21] T-helper and B-cell functions were attenuated,[34] particularly the immune response to thymus-dependent antigens.[6] Mortality of infected nude mice could be diminished with thymic transplants.[4] Although it seems clear that spironucleosis could affect research results, the investigators holding infected animals in the room continue to claim that they have not experienced unusual alterations in their control data since the last time the colonies were rederived due to *S. muris* infection. Most of the immunology studies referenced were conducted more than 30 y ago, and in many of the clinical cases, the health status of the animals was questionable or they were multiply infected with other undesirable agents[5-7,12,27-29,36,45] that are uncommon or nonexistent in modern research animal facilities.

Unrecognized cofactors may be needed to provoke the severe clinical signs previously reported. Irradiation, surgery,[29] changes in feed and bedding type,[4,12] and high dietary cadmium levels[16] have been associated with increased mortality of infected mice. Despite a continual weanling population and a diet change 3 mo before the study started, no increase in small, malnourished mice or mortality occurred in the room. Improvements in diet, housing, husbandry, and adventitious infections in modern research facilities may have eliminated the conditions needed to trigger lethal infections.

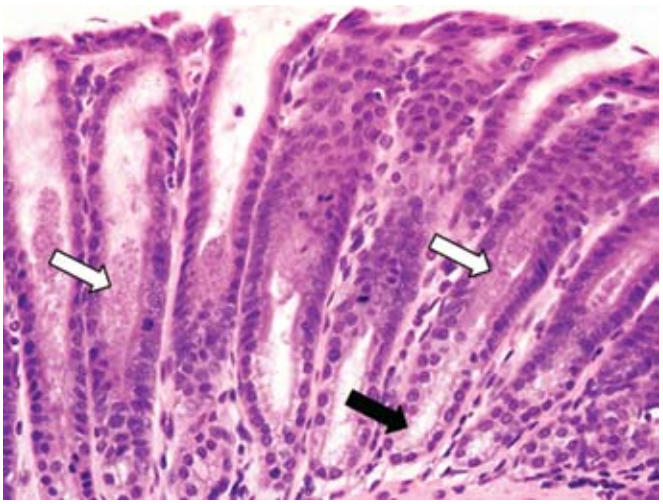

Figure 6. Pyloric crypts from a 32-wk-old mouse with a compromised immune system. While *S. muris* is abundant (open arrows), the bases of the crypts do not appear cystic (closed arrow). Hematoxylin and eosin stain Magnification, x400.

The poor ability of dirty-bedding transfer to reveal *S. muris* infection is likely multifactorial. Staff noncompliance to transfer dirty bedding, insufficient volume of bedding transferred, number of fresh cysts in the feces, the type of sentinel used, sentinel age and exposure time, sample collection and handling, the method used to examine the samples, and the experience of laboratory personnel to detect *S. muris* in submitted samples should all be considered.

Hoping to increase the ability to pick up *S. muris* infection, we have changed the rotation and submission schedule of our health surveillance program. Animals to be submitted for surveillance will enter the cage at 3 to 4 wk of age and will have been exposed to dirty bedding for varying amounts of time, from 4 to 16 wk, at the time of testing. The results of this change will be reported at a later date. In addition we are developing a PCR test for *S. muris* detection.

In conclusion, original studies should be consulted when inconsistencies exist between what is seen in the facility and what is written in review literature. Even though some references state that only 1 cyst is needed to infect a mouse and that cysts remain infective for 14 d,[2,32] these statements are more informative in the context of the original studies. The 1 cyst had to be a 'fresh cyst'[40] and the cysts that remained infective for 14 d were within feces.[22] The minimal infectious dose criterion was established in athymic nude mice that received cysts by gavage.[40]

S. muris likely was reintroduced via mice from another National Institutes of Health facility from which we accept animals. However, dirty-bedding sentinels in that facility continue to test negative by direct examination for the protozoan. That facility was not depopulated in conjunction with our room depopulation and rederivation of all lines 3 y earlier. The investigators' mice are once again being embryo-rederived, not because of an effect on their research but the unwillingness of other facilities to accept animals from a room known to harbor *S. muris*. However, after this rederivation, the mice will be housed in a facility that accepts mice only from a limited number of vendors or as embryos.

More studies are needed to determine whether the *Spironucleus* spp. found in our facility differs from those reported to cause severe disease and affect research. Studies of the small subunit ribosomal *RNA* gene would provide insight.

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