Chapter 22

Helminth Parasites of Laboratory Mice

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THE MOUSE IN BIOMEDICAL RESEARCH, 2ND EDITION

I. INTRODUCTION

Since the last publication of this volume, significant strides have been made in eradicating the infectious diseases of laboratory mice. Mice are, without a doubt, freer from disease than they have ever been. However, animals that reside in today's rodent facilities still frequently test positive for the presence of helminth parasites. In the prior edition of this chapter (Wescott 1982), the importance of the parasites addressed was based on their prevalence in the laboratory rodent population, a categorization that continues in the current version. The oxyurids, or pinworms, of mice remain important parasites in terms of the numbers of institutions with mice infected with these parasites and their potential impact on biomedical research (Boivin *et al.* 1996; Huerkamp 1990; Huerkamp *et al.* 2000; Jacoby and Lindsey 1997, 1998; Klement *et al.* 1996; Le Blanc *et al.* 1993; Lipman *et al.* 1994; Murphy-Hackley and Blum 1990; Pinto *et al.* 1994; Shibihara 1999; Zenner and Regnault 2000). This is likely due to the persistence of oxyurid eggs in the environment, their means of transmission, and their lack of overt symptomology. Of 68,110 samples submitted from outside sources to a large animal diagnostic laboratory for parastitological examination from February 2002 to November 2004, 206 tested positive for oxyurid parasites (Cosentino 2004). These samples represent biotech firms, universities, hospitals, pharmaceutical companies, and government institutions, with colleges and universities having the highest number of positives. This number probably underestimates the number of institutions harboring oxyurids, for many infections are discovered on routine health monitoring exams conducted in-house, treated, and cleared.

Hymenolepis (=Rodentolepis) nana was previously treated as a parasite of major importance in laboratory mice. Although it remains important in terms of zoonotic potential, a survey of approximately 68,000 samples submitted to a major diagnostic laboratory over three years (2001–2004) failed to show a single case (Cosentino 2004). Since *H. nana* has a more complex life cycle than the oxyurid parasites, this would suggest that modern "mousekeeping" methods have had an effect on the prevalence of this parasite. Many other helminths may parasitize mice, especially wild mice, but they are not found in a laboratory setting, unless these parasites are serving as a model of host–parasite interaction. For a more detailed treatment of less common parasites of laboratory or wild mice, the reader is directed to Flynn's *Parasites of Laboratory Animals* (Flynn 1973c). A second edition of this volume is currently in press.

II. HELMINTHS OF MAJOR IMPORTANCE

A. Oxyurids: Overview

Oxyurids, the family to which the pinworms of mice, *Syphacia obvelata* and *Aspiculuris tetraptera,* belong, are cosmopolitan monoxenous parasites that are transmitted through ingestion of embryonated eggs. Pinworms are routinely found in animals from modern animal facilities, even in facilities free of viral and bacterial diseases of mice (Jacoby and Lindsey 1998; Zenner and Regnault 2000). Oxyurids are also a common parasite of wild mice (Behnke *et al.* 1999; Derothe *et al.* 1997; Pisanu *et al.* 2001; Singleton *et al.* 1993).

Mice may be infected with both species of pinworms concurrently (Agersborg *et al.* 2001; Bazzano *et al.* 2002; Eaton 1972; Goncalves *et al.* 1998; Hoag 1961; Jacobson and Reed 1974; Macarthur and Wood 1978; Nicklas *et al.* 1984; Pinto *et al.* 2001; Scott and Gibbs 1986; Taffs 1975, 1976b; Zenner 1998). The common finding of two species of pinworms in an infection can be explained by the species predilections for slightly different portions of the gastrointestinal tract. Since they do not compete directly for resources, they are able to maintain simultaneous infections. In concurrent infections, *A. tetraptera* may have higher worm numbers because its longer lifespan may allow it to accumulate in the host (Scott and Gibbs 1986). The prevalence of pinworms in an infected rodent population depends on many factors, including gender, age, strain, immune status, and the concentration of parasite ova in the environment. Male animals tend to have higher parasite burdens than female animals (Behnke 1975a; Derothe *et al.* 1997; Eaton 1972; Mathies 1959a, b). Studies suggest that this is not entirely due to the tendency of male mice to exhibit more exploratory behavior, and thus become infected at a higher rate through greater exposure to eggs. Rather, it may be attributed to some innate resistance in female mice resulting in a greater rate of parasite expulsion (Behnke 1975b). Young animals tend to have higher oxyurid burdens than older animals (Behnke 1976; Eaton 1972; Mathies 1959b; Panter 1969), a fact that is important to consider when designing sentinel programs to detect pinworm infection. In wild populations endemically infected with *A. tetraptera,* susceptibility to infection peaks in animals between 10 and 17 grams (or approximately 4 to 7 weeks of age) and subsides as animals age and apparently become increasingly immune to reinfection (Behnke 1976). In the laboratory setting, animals were shown to develop resistance to infection with *S. obvelata,* regardless of previous infection status, between 4 and 9 weeks of age (Panter 1969). Laboratory mice are more resistant to experimentally induced infection than wild mice (Derothe *et al.* 1997) and hybrids of two populations of wild mice are more susceptible to infection than either of the parent species (Sage *et al.* 1986). The "Columbia" and CF1 strains of mice differed in their susceptibility to pinworm infection (Chan 1952). AKR/LwNIcr, DBA/2J, DBA/2An, and C3H/Cum mice were shown to be more susceptible to pinworm infection than other inbred strains of laboratory mice (Eaton 1972; King and Cosgrove 1963). Athymic mice have an increased susceptibility to infection (Clarke and Perdue 2004; Jacobson and Reed 1974), but the susceptibility of other immunocompromised animals has not been examined.

Rodent pinworms are not a significant zoonotic hazard, with only *S. obvelata* reported to infect humans, and then only rarely

(Flynn 1973a). A more recent source states that rodent pinworms are not transmissible to humans and vice versa (Marx 1991). The two common pinworms of mice, *Syphacia obvelata* and *Aspiculuris tetraptera,* are described next. A summary of morphologic and reproductive data may be found in Table 22-1. A comparison of the appearance and size of the ova of *S. obvelata, A. tetraptera,* and *S. muris* may be found in Fig. 22-1. *Syphacia muris,* the pinworm of rats, is occasionally found in mice, but it will not be discussed in detail.

TABLE 22-1
DIFFERENTIATION OF *SYPHACIA OBVELATA* AND *ASPICULURIS TETRAPTERA*

	S. obvelata	*A. tetraptera*
Physical Characteristics		
Cervical alae	Subtle	Prominent
Shape of tail of female	Long and pointed	Conical
Location of vulva	Anterior of body	Middle of body
Mamelons in male	Present	Absent
Spicule	Present	Absent
Ova size	134 x 36 µm, one side flattened	86 µm x 37 µm, ovoid, symmetrical
Life Cycle		
Location in host	Cecum and colon	Colon and cecum
Prepatent period	11–15 days	21–25 days
Location of ova	Perianal skin	Fecal pellet
Time to infectivity of ova	5–20 hours	5–8 days

2. Syphacia obvelata

A. MORPHOLOGY *Syphacia obvelata* was first described in 1801 (Rudolphi 1801), but it would be several years until it was distinguished from *Aspiculuris tetraptera* and placed in the genus *Syphacia* (Seurat 1916). In the genus *Syphacia*, adult parasites have three fleshy lips, a round esophageal bulb, and small

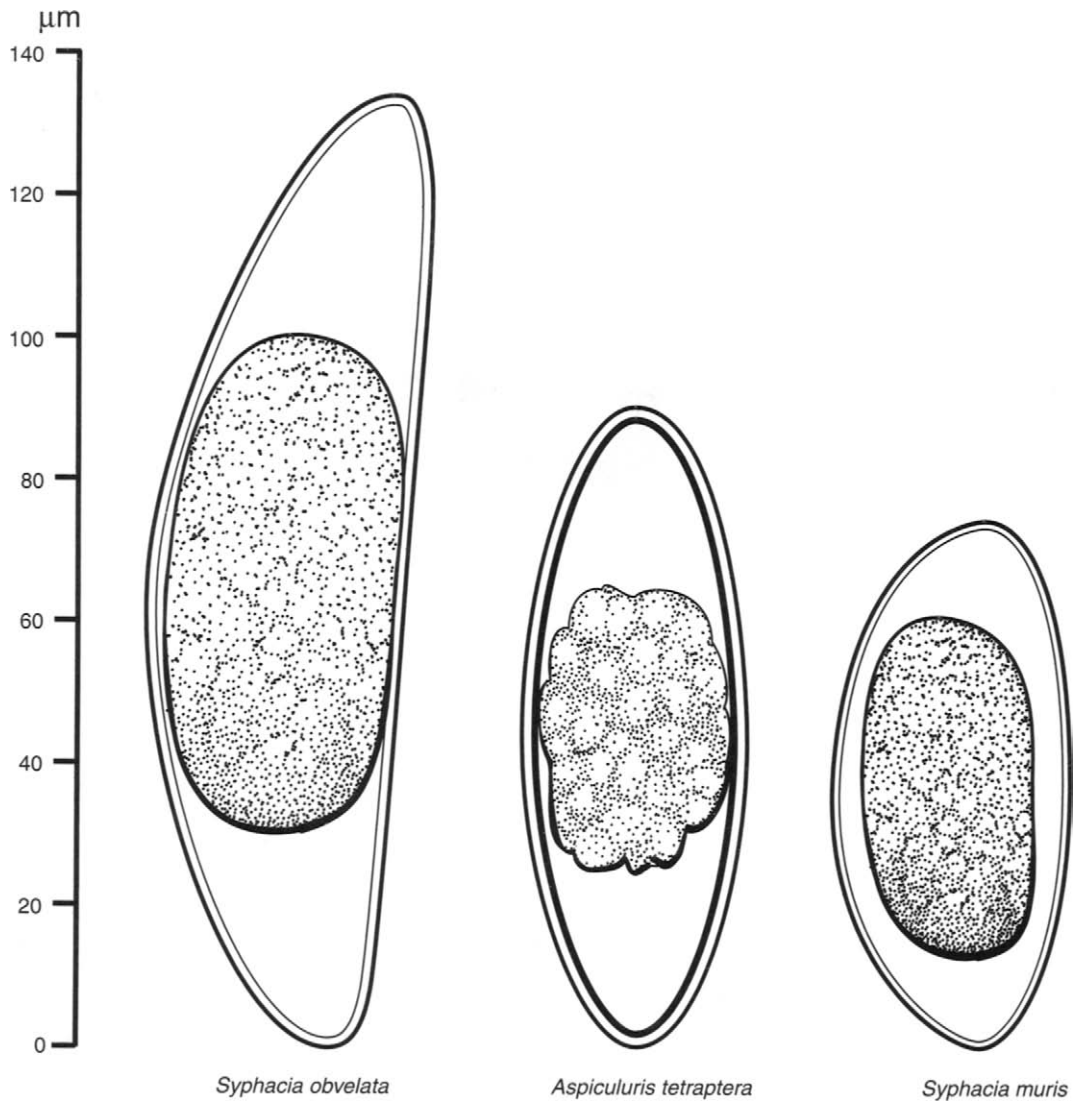

Fig. 22-1 Appearance and relative size of the ova of the oxyurid parasites of mice, *Syphacia obvelata*, *Aspiculuris tetraptera*, and *Syphacia muris*.

cervical alae, or clear, cuticular flanges found on the anterior lateral margins of the body. Female *S. obvelata* are 3.4 to 5.8 mm long and 240 to 400 μm wide, with a 530 to 675 μm tail. The vulva is found in the anterior part of the body, behind the excretory pore. *S. obvelata* exhibit marked sexual dimorphism, and the males measure only 1.1 to 1.5 mm long with a 130 μm long tail. Males have a prominent spicule that measures 68 to 90 μm and two to three rounded protuberances, or mamelons, on their caudal ventral surface. These mamelons allow the male to grasp the female during copulation (Fig. 22-2). The ova of *S. obvelata* are pointed ovals that are flattened on one side and measure approximately 75 x 29 μm. Frequently, the eggs have embryonated before leaving the female, and larva may be seen in newly laid eggs.

B. LIFE CYCLE *S. obvelata* has a direct life cycle and a prepatent period of 11 to 15 days (Levine 1968). *S. obvelata* eggs are infective once embryonated, which often occurs 5 to 20 hours after release from the female (Chan 1952; Taffs 1976a). When the infective eggs are ingested by a suitable host, the eggs hatch and the larvae migrate to the cecum over a 24-hour period (Chan 1952). *Syphacia* spp. reside in the cecum or anterior colon, where they feed on bacteria present in the lumen. Two-thirds of the females are fertilized by 6 days after hatching (the usual lifespan of the male), and the females remain in the cecum for another 10 to 11 days while they produce ova. Gravid females migrate to the anus to lay their eggs on the perianal area of the host. *S. obvelata* females release an average of 350 eggs per female, after which they die (Chan 1952). Animals are usually infected through contact with surfaces or substances contaminated with embryonated eggs. Due to the short time necessary for eggs to embryonate and become infective, it is theoretically possible for *S. obvelata* ova to embryonate on the host and retroinfect the animal by migrating back into the body (Prince 1950), although this is considered an uncommon route of infection (Chan 1952).

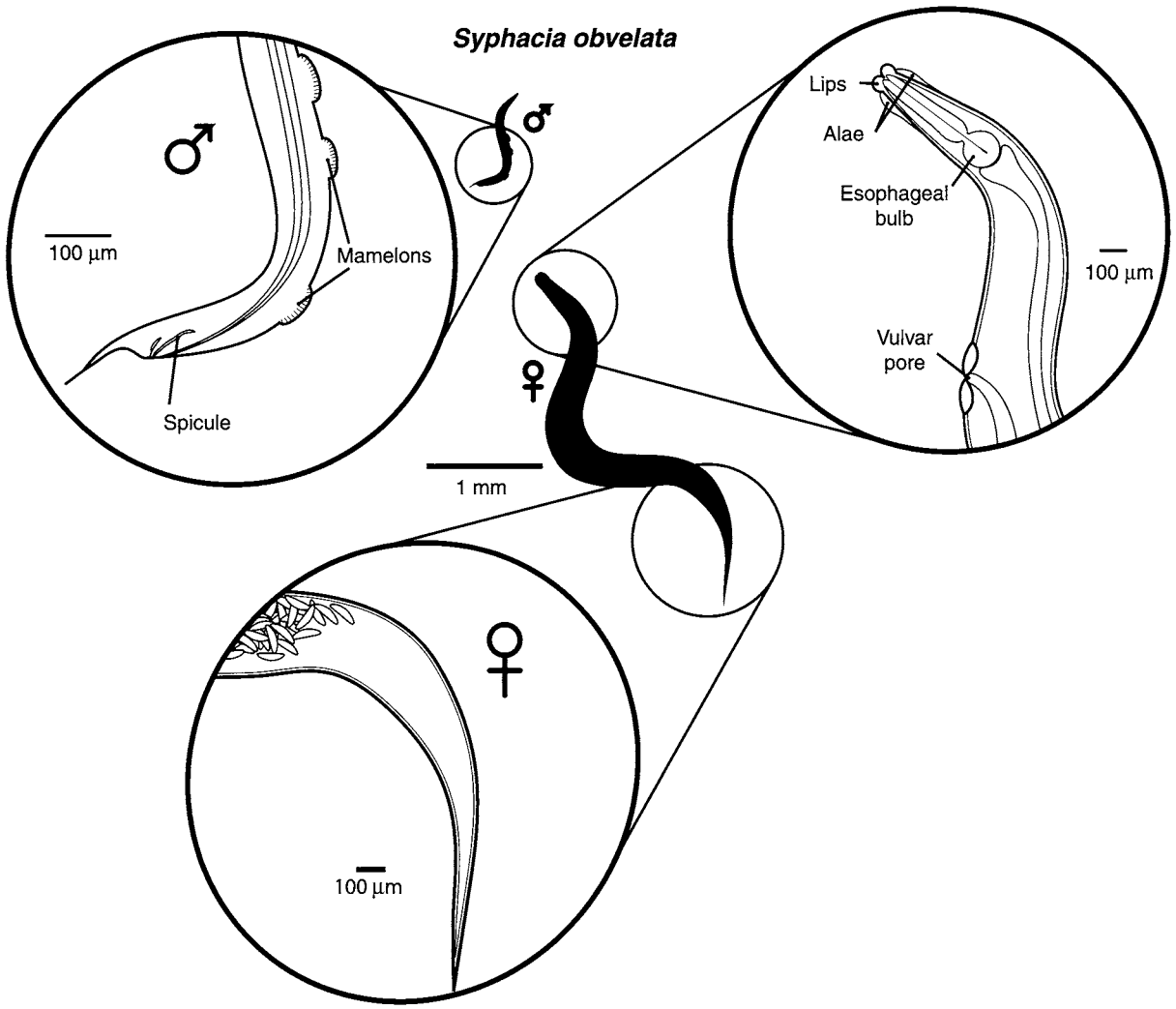

Fig. 22-2 Distinguishing features of *Syphacia obvelata*. Note the sexual dimorphism and the mamelons and spicule present in the male. In the female, the characteristic round esophageal bulb is illustrated, as are the small cervical alae and the fleshy lips. The vulvar pore is seen in the illustration of the head of the female.

C. DIAGNOSIS Since the eggs of *S. obvelata* may be found on the perianal skin and hair, the usual method of diagnosing infection is the perianal cellophane tape test and variations thereof (Eguiluz *et al.* 2001). In this test, a strip of clear cellophane tape is pressed firmly to the perianal region of the mouse, then mounted on a glass microscope slide. The slide is examined for the presence of pinworm ova using a microscope. This test relies on the presence of oxyurid eggs on the perineum. For that to occur, the infection must have reached a point where gravid females are present and releasing ova. Mice are frequent, assiduous groomers and may remove evidence of eggs before they are found, or the mouse may be infected with a very light worm burden and very few eggs may be present. The "tape test" is therefore less sensitive than a test in which the animal's gut contents are directly examined for the presence of parasites. *S. obvelata* ova may also be found using standard fecal flotation techniques, but this is much less common, since the animals release their eggs at the anus. An anal swabbing technique has been described in live mice and has been shown to be effective in diagnosing infection with *S. obvelata* (Goncalves *et al.* 1998). While the "tape test" and the anal swab technique are excellent tests for evaluation of treatment success when the mouse must remain alive, examination of animals at necropsy should include both the evaluation of cecal and colonic contents for adult worms using a dissecting scope and flotation of the cecal and colonic contents (Klement *et al.* 1996; West *et al.* 1992). Although the ova of *S. obvelata* are present mainly on the perineal skin, maceration of worms present in the contents of the large intestine may yield positive results.

2. Aspiculuris tetraptera

A. MORPHOLOGY *Aspiculuris tetraptera* was first described by Schulz in 1812. This pinworm of mice may be differentiated from *S. obvelata* by its broad cervical alae, oval esophageal bulb, and striated cuticle. Female *A. tetraptera* are 3 to 4 mm long and 215 to 275 μm wide, with a tail that is 445 to 605 μm long. The vulva of the female *A. tetraptera* is found anterior to the middle of the body, but the vulva is more posterior than in *S. obvelata*. The ova of *A. tetraptera* are symmetrical, oval, and approximately 86 x 37 μm. The eggs are at the morula stage at the time of release and the inner cell mass does not fill the shell. Males are 2 to 4 mm long and 120 to 190 μm wide, with a 117 to 169 μm tail. The male *A. tetraptera* has neither spicules nor mamelons (Fig. 22-3).

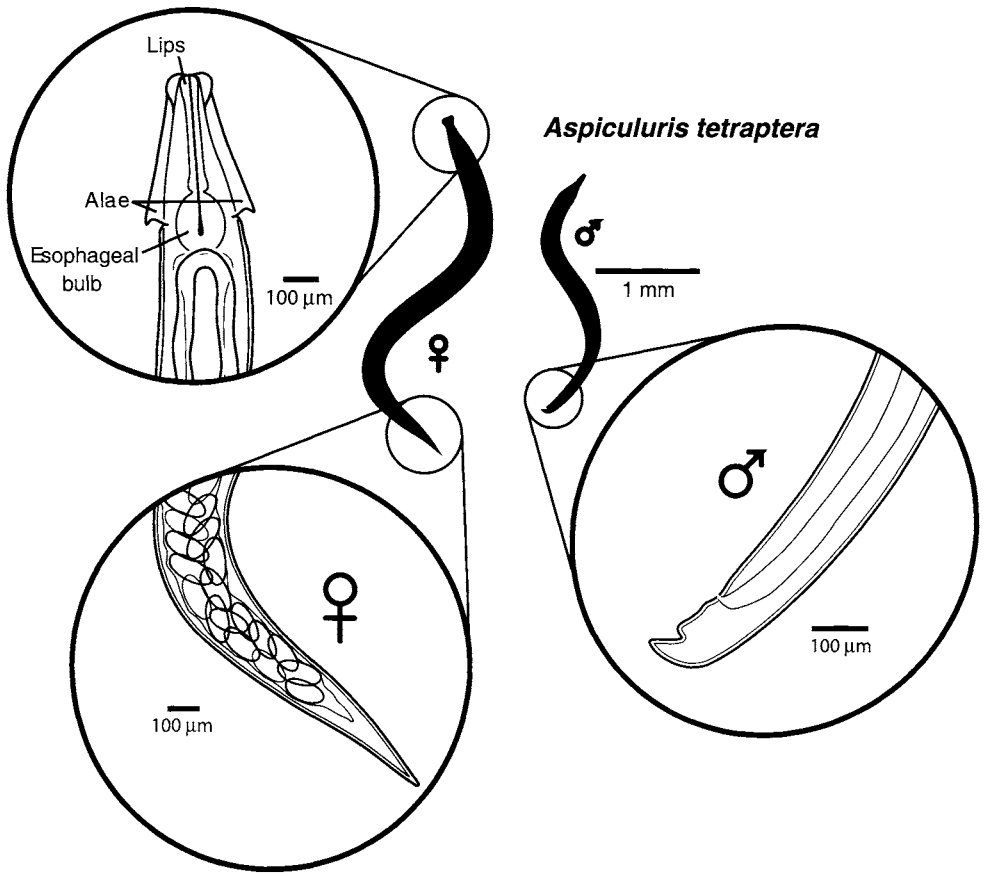

Fig. 22-3 Distinguishing features of *Aspiculuris tetraptera*. There is less sexual dimorphism in this species, and the male lacks spicules and mamelons. The illustration of the female head does not show the vulvar pore since it is slightly lower on the body than in *S. obvelata*. The broad cervical alae and oval esophageal bulb of *A. tetraptera* are highlighted.

B. LIFE CYCLE *A. tetraptera* has a 21- to 25-day prepatent period (Behnke 1974). After hatching in the cecum, *A. tetraptera* larvae may be found in the crypts of Lieberkühn in the proximal colon, where they remain for 3 to 5 days (Anya 1966a). Adult *A. tetraptera* reside in the colon and migrate from the proximal to distal colon to deposit eggs (Chan 1955). Each *A. tetraptera* female releases an average of 17 eggs/day, usually at night (Phillipson 1974). The eggs are excreted in the mucus layer of the feces, and are not infective for 5 to 8 days. Unlike *S. obvelata,* females live another 21 to 24 days after their first egg release, for a total lifespan of 45 to 50 days (Hsieh 1952). Due to the location of egg release in *A. tetraptera* and the extended time necessary for the eggs to reach infectivity, retroinfection is not thought to be a means of reinfection in *A. tetraptera* infections.

C. DIAGNOSIS The eggs of *A. tetraptera* may be found on the perianal skin and hair, but it is not a common occurrence. The perianal tape test is not an effective diagnostic tool when dealing with a single-species infection with *A. tetraptera*. The anal swabbing technique described by Goncalves has been shown to be effective in diagnosing infection with *A. tetraptera* (Goncalves *et al.* 1998). Evaluation at necropsy of cecal and colonic contents for the presence of adult worms is the most certain way to diagnose *A. tetraptera* (Klement *et al.* 1996; West *et al.* 1992). Ova may also be found on fecal flotation. Table 22-2 describes a fecal concentration and centrifugation technique effective in isolating these ova.

3. Effects on Research

When compared to the devastating effects of infection with certain murine viruses still in circulation, such as mouse hepatitis virus, mouse parvovirus, or ectromelia, oxyurid infection is more a nuisance than a life-threatening situation. Infections with pinworms are considered to be clinically silent in animals with normal immune systems (Harkness and Wagner 1995; Levine 1968; Taffs 1976a). Pinworms only rarely penetrate the mucosa of the gut, unlike other helminths, and reside mainly in the lumen of the intestines, where they feed on bacteria. A single report describes small (1 to 2 mm) granulomas in two mice (of an unrecorded number examined) caused by the penetration of the colonic wall by an adult *A. tetraptera* (Mullink 1970). Undoubtedly, this is a rare occurrence. A variety of nonspecific signs have been attributed to heavy oxyurid infections: poor condition, rough hair coats, reduced growth rate, and rectal prolapse (Eaton 1972; Harwell and Boyd 1968; Hoag 1961; Taffs 1976a). The reports that describe these signs fail to exclude agents such as *Citrobacter* and *Helicobacter,* both of which may cause similar signs in susceptible mice (Foltz *et al.* 1998; Maggio-Price *et al.* 1998; Vallance *et al.* 2003; Ward *et al.* 1996).

Despite the lack of clinical signs usually associated with infection, pinworms may interfere with research in a number of ways. One of the most important of these is by modification of the immune system. Host–parasite interaction is a complicated system that involves both T and B cell-mediated immunity. Infections with *S. obvelata* or *A. tetraptera* have been shown to increase the host humoral response to nonparasitic antigenic stimuli in AKR/J mice (Sato *et al.* 1995). Infection with *S. obvelata* was associated in B6AF1/J neonates with termination of the tolerance state and induction of a Th2-associated eosinophilic autoimmune oophoritis (Agersborg *et al.* 2001). In athymic mice, pinworm infection may induce proliferation of T and B lymphocytes in the spleen (Beattie *et al.* 1981) and cause the development of a lymphoproliferative disorder that eventually leads to lymphoma (Baird *et al.* 1982; Beattie *et al.* 1980). Pinworm infections also result in the inhibition of diabetes formation in the nonobese diabetic (NOD) mouse (Gale 2002), presumably through antigenic stimulation of the immune system. The prevalence and consistency of these effects are difficult to evaluate as few researchers are able to pinpoint pinworms as the cause and then publish work that directly explains why oxyurid infection produced unexpected experimental results.

In addition to effects on the immune system, pinworms may affect other systems. Infection with *Syphacia* has been shown to accelerate the development of the hepatic monooxygenase system in young C57BL/6N and WHW/HOM mice (Mohn and Philipp 1981). *S. obvelata*, but not *A. tetraptera,* infections have been shown to inhibit exploratory behavior in C57BL/6NHsd mice (McNair and Timmons 1977). Retarded growth in a colony of C57BL/6N and HOM mice was attributed to heavy pinworm infection, but no further information on the health status of this colony was given (Mohn and Philipp 1981).

A final consideration when evaluating the effect of pinworms on research programs is financial. Research programs and animal care facilities incur increased costs associated with treatment and environmental decontamination. Pinworm infection may also preclude the movement of animals between facilities or between portions of one facility, thereby delaying experiments.

TABLE 22-2

FECAL CONCENTRATION AND CENTRIFUGATION TECHNIQUE

1. Soften up to 5 cm^3 of feces in a 15-ml conical tube. A small amount of water may be used, but using the flotation solution is better.
2. Fill the tube with the flotation solution (a zinc sulfate solution at 1.18 sp gravity).
3. Put the filled tube into a centrifuge and add more flotation solution to the tube until there is a small positive meniscus.
4. Place a coverslip on each tube, ensuring that contact is made with the entire lip of the tube.
5. Spin at 616–760 RCF for 10 minutes.
6. Place coverslip on glass slide and evaluate under a microscope at 100x.

4. Treatment

Any treatment regimen considered for pinworms must take into account the conditions under which the animals are housed and the potential for environmental persistence of the pinworm ova.

Heroic sanitation measures are often employed to rid animal rooms of contamination; however, few reports directly document the presence of oxyurid eggs in the environment (Boivin *et al.* 1996; Le Blanc *et al.* 1993; Macy 2000). In conventionally housed mouse colonies infected with both *A. tetraptera* and *S. obvelata*, Hoag described eggs in dust and air intake filters and on equipment, but did not say from which species the eggs originated (Hoag 1961). Cellophane tape impressions from the interior surfaces of filter-top cages housing *S. obvelata*-infected mice failed to reveal eggs (Lipman *et al.* 1994). In a colony of *A. tetraptera*-infected mice housed in individually ventilated caging, tape tests of the environment were negative for eggs (Boivin *et al.* 1996). The addition of a simple bonnet-type filter top to colonies of outbred mice was able to substantially reduce transmission between cages (Wescott *et al.* 1976). With many modern rodent facilities using individually ventilated cages and laminar flow changing stations to effectively completely isolate the mouse from the environment, the distribution of eggs around an animal room may be minimal. Treatment failure in current rodent housing conditions may be due less to environmental contamination than to failure to rid mice of immature worms, resulting in small pockets of infected mice, mice avoiding treatment through normal movement for breeding or experimental purposes, or persistent infection in permissive hosts, such as immunocompromised genetically modified animals.

Recent work on the susceptibility of *Syphacia* ova to common disinfectants has focused on the ova of the rat pinworm, *Syphacia muris*. *S. muris* eggs may be less fastidious than *S. obvelata* ova as they were found to embryonate in tap water and saline while the eggs of *S. obvelata* did not (Stahl 1961; van Der Gulden and van Aspert-van Erp 1976). *S. obvelata* eggs do not embryonate in water, saline, moistened activated charcoal, agar, or glycerin (Chan 1952; Grice and Prociv 1993; Philpot 1924). Eggs harvested from gravid *S. obvelata* females and kept on a moistened slide at room temperature began to degenerate after only 24 hours (Grice and Prociv 1993). Although humidity was not measured in either Chan's or Grice's studies, eggs allowed to become wet either prematurely opened their opercula and/or ruptured, while eggs that dried out collapsed and were not viable (Chan 1952; Grice and Prociv 1993). Despite these differences, and the apparent delicacy and fastidiousness of *S. obvelata* ova, the results of disinfectant studies on the ova of *S. muris* should probably be extrapolated to the ova of *S. obvelata* until studies show otherwise.

Miyaji *et al.* showed that *S. muris* eggs were embryonated after exposure to several common disinfectants, but that exposure to 80°C for 30 minutes killed all ova (Miyaji *et al.* 1988). Work by Dix *et al.* showed that either 100°C heat for 30 minutes or ethylene oxide exposure produced a 100% kill rate in *S. muris* eggs. Formaldehyde gas was 94% effective, and chlorine dioxide was 96% effective under the conditions described by Dix *et al.*, which included both a technical failure in the formaldehyde trial that allowed for growth of the bacterial indicator used and a relatively brief (10-minute) exposure period to chlorine dioxide (Dix *et al.* 2004). Both formaldehyde gas and chlorine dioxide may have higher ova kill rates when applied in a different fashion. Dix *et al.* also stated that 41% of the ova left exposed to room air for 4 weeks hatched when exposed to suitable conditions (Dix *et al.* 2004). This resistance of *S. muris* eggs to many common cleaning and disinfection chemicals emphasizes the importance of including rigorous environmental decontamination as part of the treatment plan for a pinworm-infected area, despite the fact that the eggs may not have left the immediate cage area.

Little, if any, work has been done on the environmental persistence of *A. tetraptera* eggs, probably because they are excreted in the feces, and removal of feces should suffice to remove the eggs from the environment. However, removal of fecal matter from the environment may be difficult in situations such as open-topped caging environments or the dirty side of a shared cage wash. In 1952, Hsieh described hatching *A. tetraptera* eggs in distilled water at 27°C, which may indicate that if allowed to persist in the environment, *A. tetraptera* ova are not as fastidious as *S. obvelata* ova (Hsieh 1952). Anya described a similar experiment in which the ideal hatching temperature appeared to be 30°C (Anya 1966b). Regardless of which species of oxyurid infects the animals in a particular facility, environmental decontamination as part of a pinworm eradication program is controversial (Gaertner 2000). One cycle of decontamination and removal of potentially infective materials and fomites, preferably after the first week of treatment is completed, is probably sufficient. While potentially both expensive and time- consuming, environmental decontamination is an important part of any parasite eradication effort, if for no other reason than the perception of making a "clean sweep." However, at least one author has shown success in ridding a colony of rats of *S. muris* through treatment without environmental decontamination (Barlow *et al.*, 2005).

The second part of a treatment plan for oxyurids should include the administration of anthelminthics to the infected mice. Mice have been treated with a variety of chemicals over the years in the quest to produce helminth-free research subjects. These have included gentian violet, crystal violet, sodium fluoride, hexylresorcinol, phenothiazine, terramycin, aureomycin, and bacitracin (Taffs 1976a). In addition, organophosphates such as dichlorvos and uredofos have been used in pinworm eradication programs (Tetzlaff and Weir 1978; Wagner 1970). Another family of agents occasionally used is the nicotinic agonist family, which includes levamisole and pyrantel (Brody and Elward 1971; Comley 1980; Scott 1988). These compounds are neither as safe nor as effective as the GABA-agonistic piperazine compounds, widely used and recommended throughout the 1960s and into the present, especially in combination with ivermectin or a benzimidazole (Lipman *et al.* 1994; Martin 1997; Owen and Turton 1979; Reiss *et al.* 1987; Taffs 1976a; Zenner 1998).

The most common agents in use today for pinworm eradication are avermectins and benzimidazoles. The avermectins are macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis*. Avermectins act by increasing muscle

Cl^- permeability through a glutamate-gated ion channel that paralyzes parasites (Martin 1997). In the laboratory animal literature, they are represented mainly by ivermectin. Ivermectin at an oral dose of 2 mg/kg/day was shown to be effective against *S. obvelata* in mice by removing 100% of gravid females, 94% of males, and 97% of immature worms (Ostlind *et al.* 1985). Early studies of the use of ivermectin in mice reported administration by gavage or subcutaneous injection (Flynn *et al.* 1989; Huerkamp 1990; Murphy-Hackley and Blum 1990; Ostlind *et al.* 1985). Treatments were either single or paired, given 7, 9, or 10 days apart. Animals remained parasite-free for 2 to 6 months (the length of published follow-up) after treatment (Flynn *et al.* 1989; Huerkamp 1990, 1993). Ivermectin applied between the scapulae of mice, using a micropipettor, at 2 mg/kg and administered 10 days apart has also been reported to be effective in treating pinworm infection in mice, with animals remaining free of parasites for 6 months (West *et al.* 1992). Ivermectin has also been administered topically through the use of a spray bottle and found to be effective in the treatment of pinworms, with animals remaining parasite-free for 6 months (Le Blanc *et al.* 1993). These methods of ivermectin administration involve direct handling of the affected mice and are relatively time-consuming and difficult for personnel, especially when dealing with large numbers of rodents.

Ivermectin has also been administered to mice in drinking water (Hasslinger and Wiethe 1987; Klement *et al.* 1996). Effective dosages were calculated to be 2 mg/kg/day, although, due to differences in water consumption, actual doses ranged from 1.7 to 4.8 mg/kg/day (Klement *et al.* 1996). The ivermectin formulation used by Klement was the liquid anthelminthic formulated for horses, Eqvalan® (Merial, Athens, Georgia), mixed in water (Klement *et al.* 1996). Klement examined several different treatment regimens, each of which consisted of 4 consecutive days of ivermectin treatment, spaced 3 days apart, but differed in total number of treatments (Klement *et al.* 1996). Animals were followed for 29 to 32 weeks, and pinworms were eradicated by the use of four or five treatment regimens, but no fewer (Klement *et al.* 1996). The combination of piperazine and ivermectin, both in drinking water, has also been shown to be effective in the elimination of oxyurid infections in mice (Lipman *et al.* 1994; Zenner 1998).

Unintended deleterious effects may result from ivermectin treatment, especially in animals with compromised blood-brain barriers (Didier and Loor 1995; Paul *et al.* 1987; Roder and Stair 1998). This effect has been demonstrated in a subpopulation of the outbred mouse stock, CF-1 (Jackson *et al.* 1998; Lankas *et al.* 1997), which is deficient in P-glycoprotein, a protein that functions as a drug transport pump across the blood-brain barrier. In addition, *mdr1a* (*Abcb4*) and *mdr1b* (*Abcb1*) knockout mice, which are also deficient in P-glycoprotein, are exquisitely sensitive to ivermectin (Schinkel *et al.* 1994; Schinkel *et al.* 1997). Toxicity has also been reported in young C57BL mice (Skopets *et al.* 1996). Young mice are more susceptible to ivermectin toxicosis due to postnatal blood-brain barrier closure and potential overdosing through receiving the drug via multiple routes, especially in milk, where concentrations are three to four times plasma concentrations (Lankas *et al.* 1989). Mice are more sensitive to the adverse effects of ivermectin than rats, and male mice are more sensitive than females (JEFCA 1991; Woodward 1993). An inadvertent overdose of ivermectin administered subcutaneously to BALB/cSim mice was shown to produce lesions in the liver and kidneys (Hamlen *et al.* 1994). Those lesions included mild to moderate diffuse microsvesicular fatty change of the liver and acute, diffuse tubular necrosis. Ivermectin may also affect some behaviors in rats and mice. In 129/SvEvTac, AKR/J, and C57BL/6J mice, ivermectin was not shown to affect swimming behavior or spatial learning, but had effects on more subtle behaviors, such as exploration of a novel open field (Davis *et al.* 1999). In Crl:CD1(SW) mice, ivermectin may also have immunomodulatory effects through the stimulation of helper T lymphocytes (Blakley and Rousseaux 1991).

New avermectins, especially selamectin, which is administered topically, have been shown to be safe and effective in cats, cattle, and dogs, including Collies, a subpopulation of which are sensitive to ivermectin toxicosis (Bishop *et al.* 2000; Jacobs 2000; Krautmann *et al.* 2000; McTier *et al.* 2000). Doramectin, one of the new avermectins, has been shown to be efficacious against *S. muris* in rats (Öge *et al.* 2000). A limited study demonstrating the efficacy of selamectin in the treatment of both *S. obvelata* and *A. tetraptera* in Crl:CD1(SW) mice has been performed, and the compound was both safe and efficacious (Winchester *et al.* 2004). Before initiating the widespread use of new avermectins in a potentially drug-sensitive colony of genetically manipulated rodents, pilot applications on an age range of animals from the colony of interest may prevent deleterious side effects.

The benzimidazole class of anthelminthics binds to nematode β-tubulin and inhibits microtubule formation. Microtubule binding results in a drug that is adulticidal, larvicidal, and ovicidal, since microtubules are necessary for cell division (Kirsch 1978; Lacey *et al.*, 1987). Fenbendazole has been used since at least 1981 as a feed formulation to treat pinworm infection (Mohn and Philipp 1981). Fenbendazole is a relatively benign drug with no known teratogenicity and an acute oral LD_{50} of more than 10 g/kg in mice and rats. Toxicity occurred when rats were fed doses of 500 mg/kg/day (60 times the approximate dose of 8 mg/kg/day achieved by a 150 ppm feed level) for 14 days or longer. These changes included renal tubular hyperemia or hemorrhage, increased serum creatinine, and hepatocellular granular degeneration (Xu *et al.* 1992). In rats, fenbendazole appeared to promote liver tumor formation, but those changes were seen at doses 60 or more times the therapeutic dose (Shoda *et al.* 1999). In rats, at therapeutic dosages, over extremely long treatment periods (greater than 70 consecutive days, including pre- and postnatal exposure), fenbendazole was

found to have minimal behavioral effects and was deemed safe (Barron *et al.* 2000). Fenbendazole did not affect the hepatic monooxygenase system in C57BL/6N mice (Mohn and Philipp 1981), nor did it affect the immune response in BALB/cByJ mice (Reiss *et al.* 1987). Fenbendazole administered to mice at doses of 100 to 300 mg/kg was found to have no effect on pain perception, no influence on hexobarbitone anesthesia sleep times, and no effect on maximal electroshock convulsions (Keller 1991). In mice, fenbendazole given at a dose of 150 mg/kg of feed for 3 alternating weeks of treatment and combined with environmental decontamination proved efficacious against *A. tetraptera* for one year (Boivin *et al.* 1996). A standard protocol in use at many institutions in the United States is the administration of feed containing 150 ppm (or mg/kg) of fenbendazole for at least three 7-day periods over at least 5 weeks. Fenbendazole is recommended over ivermectin due to its lack of documented interference with research, its large margin of safety, and its ovicidal, larvicidal, and adulticidal effects. The effectiveness of this treatment should be evaluated by necropsy of colony and susceptible sentinel animals and by both gross and microscopic evaluation (via fecal flotation) of the gastrointestinal contents.

The treatment of an infection with *A. tetraptera* would seem to be substantially easier than the treatment of *S. obvelata* infections due to the difference in their life cycles. The egg of *A. tetraptera* is excreted in the feces and takes 5 to 8 days to reach an infective stage. Retroinfection through hatching on the perianal skin and migration through the anus is impossible. *A. tetraptera* infections would seem to be able to be controlled through the simple expedient of giving one treatment with an anthelminthic such as fenbendazole, cleaning the environment, and keeping treated animals from having access to the feces of infected animals. However, since many infections with pinworms are infections of more than one species, following the more rigorous treatment recommendations designed to remove *S. obvelata* from the environment is a wise choice. If facilities harbor wild-caught mice for research projects, these animals should be prophylactically treated for parasites to reduce the risk of zoonotic disease and to prevent contaminating other animals at the institution.

III. HELMINTHS OF MINOR IMPORTANCE

A. Nematodes

1. Syphacia muris

Syphacia muris is the pinworm of the rat. Mice can be infected with *S. muris* (Hussey 1957; Ross *et al.* 1980). Adult *S. muris* are slightly smaller than adult *S. obvelata* but the easiest way to differentiate the two *Syphacia* species is to examine the ova. The ova of *S. muris* resemble *S. obvelata* eggs but are smaller, measuring approximately 75 x 29 μm, and more symmetrical. Mixed infections of *S. obvelata* and *S. muris* in mice are uncommon. Treatment of *S. muris* would be as recommended above for the oxyurids of mice, plus the cessation of exposure to infected rats. The effects on research of *S. muris* infection in mice are probably similar to those seen with *S. obvelata.*

2. Trichuris muris

Trichuris muris is the whipworm of mice. Infection is common in wild mice but vanishingly rare in laboratory mice, unless the animals are deliberately infected with *T. muris* as a model of host/parasite interaction. Eggs are not infective until 30 days after they are laid, so good housekeeping practices should preclude the spread of infection in laboratory mice (Fahmy 1954). The relatively large (16 to 25 mm) worm may be found in the cecum. Immunity to *T. muris* is strain-dependent (Else and deSchoolmeester 2003). Effects on research may include a modulation of the immune system or, with heavy infections, typical whipworm pathologies, such as anemia. Treatment may be accomplished by a single administration of oxantel at 12.5 mg/kg or two doses of mebendazole at 50 mg/kg (Rajasekariah *et al.* 1991).

3. Heligmosomoides polygyrus

Heligmosomoides polygyrus is a trichostrongyloid nematode of mice with a strictly enteric life cycle. *H. polygyrus* is common in wild rodents and absent in laboratories unless animals are used for parasitology research. The worm resides in the anterior duodenum, where it penetrates tissues and feeds on tissue components (Bansemir and Sukhdeo 1994). Effects on research of infection with *H. polygyrus* are mainly related to the immune system (Barnard *et al.* 1998; Bashir *et al.* 2002). *H. polygyrus* appears to have some innate tolerance to ivermectin, requiring a dose of at least 1.7 mg/kg to remove fourth stage larvae from mice (Njoroge *et al.* 1997).

B. Cestodes

The characteristics of the three most common mouse cestodes are addressed in Table 22-3. A comparison of the relative size and appearance of their eggs may be found in Fig. 22-4. Infection with cestodes is extremely rare in modern mouse facilities, unless the animals are being used to study host/parasite interactions.

1. Rodentolepis (=Hymenolepis) nana

Mice are the definitive host of *Rodentolepis nana,* also known as the "dwarf tapeworm" and the most common cestode parasite of mice. The parasite attaches to the intestinal villi

TABLE 22-3
DIFFERENTIATION OF *RODENTOLEPIS* (=*HYMENOLEPIS*) *NANA*, *HYMENOLEPIS DIMINUTA*, AND *RODENTOLEPIS* MICROSTOMA

	R. nana	*H. diminuta*	*R. microstoma*
Physical Characteristics			
Length	25–40 mm	20–60 cm	8–50 mm (up to 120 mm)
Width	0.25–0.5 mm	4 mm	0.5–4 mm
Armed rostellum	Yes	No	Yes
Ova size	~40 x 50 µm	~70 µm	~85 µm
Life Cycle			
Requires intermediate host	No	Yes	Yes
Location in host	Small intestine	Small intestine	Bile duct
Prepatent period	14–16 d	19–20 d	16–17 d

using an armed rostellum and subsists on the host's interstitial fluids. Alone among cestodes, *R. nana* can reproduce in immunocompetent hosts using either a direct or indirect life cycle (Flynn 1973b). In the indirect life cycle, invertebrates such as the flour beetle, *Tribolium confusum,* ingest eggs, which hatch and develop into cysticercoids in the intestines. Mice consume these invertebrates and are infected with the cysticercoids. Mice may also become infected by directly ingesting *R. nana* eggs, as may humans. Cysticercoids will form in the villi of the small intestine and then hatch in 5 to 6 days to become active infections. The parasites excyst in the duodenum, but most parasites are found in the lower ileum of the mouse (the last 80 mm of small intestine), after the fourth day of infection (Henderson and Hanna 1987). As described by Henderson, the maximum mean worm length of *R. nana* is 51.5 mm but the worm is usually 25 to 40 mm long and 1 mm wide (Henderson and Hanna 1987). The eggs of *R. nana* are infective for 11 days outside of the host (Baskerville *et al.* 1988). Although *R. nana* is considered a zoonotic parasite, the human and rodent strains may be different and not cross-infective (al-Baldawi *et al.* 1989). Successful treatment for *R. nana* has been accomplished using benzimidazole compounds, but since zoonotic potential does exist, euthanasia or rederivation via hysterectomy or embryo transfer is recommended if a colony becomes infected (Baskerville *et al.* 1988; Taffs 1975, 1976b). Since the parasites attach to the mucosa and feed on host interstitial secretions, heavy infection of *R. nana* in mice may result in weight loss and retardation of growth (Flynn 1973b). This may have a negative effect on research projects, as may the antigenic stimulation inherent in parasitism.

2. *Hymenolepis diminuta*

Despite its name, *Hymenolepis diminuta* is not the smallest of the cestodes infecting laboratory mice, with an average length of 20 to 60 cm and a width of 3 to 4 mm (Flynn 1973b). This cestode has an indirect life cycle, in which arthropods such as flour beetles, fleas, or moths are the intermediate hosts. *H. diminuta* adults reside in the small intestines of mice,

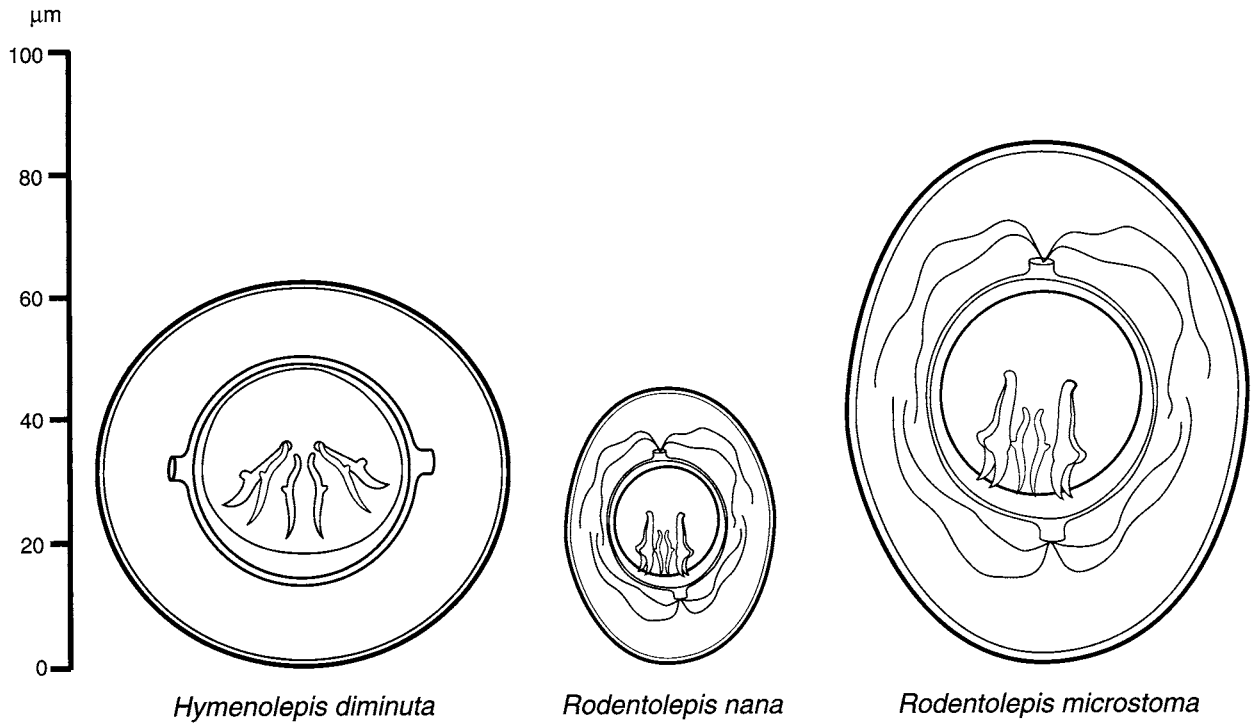

Fig. 22-4 Appearance and relative size of the ova of *Hymenolepis diminuta*, *Rodentolepis nana*, and *Rodentolepis microstoma*.

where they feed on the interstitial fluid of the host. Zoonotic infections have been described, but this requires that a human ingest the intermediate host (Flynn 1973b). This tapeworm is often described as a tapeworm of rats, and infection may be difficult to establish in laboratory mice (Flynn 1973b; Read and Voge 1954). The treatments effective against *R. nana* would also be effective for *H. diminuta* (McCracken *et al.* 1992).

3. Rodentolepis (=Hymenolepis) microstoma

Rodentolepis microstoma is found in the bile duct of its definitive hosts, which include mice. This cestode, which is similar in size to *R. nana,* has an indirect life cycle, in which arthropods such as flour beetles, fleas, or moths serve as intermediate hosts. *R. microstoma* may, however, exhibit a direct life cycle in immunocompromised hosts (Andreassen *et al.* 2004). After ingestion of the intermediate host, the parasites excyst in the duodenum and migrate to the bile duct in 5 to 7 days (Macnish *et al.* 2003). Mature proglottids are found at 15 to 16 days postinfection (De Rycke 1966). Treatment of mice with either mebendazole or albendazole at a dose of 50 mg/kg did not clear infection (McCracken *et al.* 1992), perhaps because of the parasite's protected location in the bile duct. Cholangitis is associated with infection with *R. microstoma* (Percy and Barthold 2001). This parasite was recently described as a zoonotic agent (Macnish *et al.* 2003).

4. Taenia taeniaeformis

The mouse is the intermediate host of this feline tapeworm. Approximately 30 days after ingesting eggs shed by an infected cat, tapeworm larvae, or strobilocerci, begin to form in an infected mouse's muscle tissue or liver. This tissue phase of *T. taeniaeformis* may be found in older sources such as *Cysticercus fasciolaris*. The strobilocerci are white or clear and approximately 4 to 10 mm in diameter. The strobilocercus contains a scolex and a segmented strobila that appears exactly as an adult tapeworm, but there is a bladder on the end. There are usually only 1 to 2 per host (Owen 1992). Different strains of mice are more susceptible to infection than others (Conchedda and Ferretti 1984). If this parasite is found in animals in a facility, a thorough investigation as to how the mice are gaining access to and ingesting cat feces should be conducted (Balk and Jones 1970). Effects on research may be minimal, other than antigenic stimulation occurring with the development of the parasite, but the discovery of strobilocerci in the liver or muscle of affected mice is alarming and indicates a breakdown in sanitation procedures.

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