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CONTROL OF *HYMENOLEPIS NANA* INFECTION AS A MEASURE TO IMPROVE MOUSE COLONY WELFARE

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After cannibalism had appeared in the reproductive units of a white mouse colony, treatment against confirmed *Hymenolepis nana*, a tapeworm with zoonotic potential, was performed on 67 mice in the reproductive and nursery units. Faecal droppings were evaluated by flotation and sedimentation methods. The sedimentation method revealed a higher number of positive results before, during and after the treatment, but the flotation method yielded some additional positive cases. In the reproductive unit, *H. nana* eggs were confirmed in 50% of the tested mice by the flotation and in 70% by the sedimentation method. In the nursery units, *H. nana* eggs were detected in 10.5% of the tested mice by the flotation and in 24.6% by the sedimentation method. A colony of mice was treated against the tapeworm *H. nana* with praziquantel and emodepside in doses of 2.574 mg praziquantel/100 g body mass and of 0.642 mg emodepside/100 g body mass. The content of the original pipettes (Profender®) was applied as a spot-on on the back of the neck in the area between the shoulders. The application was repeated three times at 14-day intervals. Seven days after the third therapy no *H. nana* was found in any of the tested mice in the reproductive or the nursery units. After the treatment, cannibalism was no longer observed. This treatment represented one of the steps aimed at improving animal welfare and preventing potential zoonotic disease. The public health significance of this cestode should receive more attention, especially among people who take care of mice, have them as pets, or feed them to reptiles.

Key words: Endoparasites, *Hymenolepis nana*, mice, treatment, welfare

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This paper describes a successful treatment against infection with *Hymenolepis (H.) nana* in a large group of mice and the prevention of reinfection with this cestode. Concern for the welfare of mice kept as laboratory animals is growing (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Unsatisfactory health (e.g. cestodosis) in large breeding groups of mice can be one of the factors leading to cannibalism and unnecessary pain. In mouse breeds intended for keeping as pets or for use as food for captive reptiles, all of the five aspects of animal welfare should be followed. One of these aspects is freedom from pain, injury, and diseases.

The dwarf tapeworm *H. nana* (synonyms: *Rodentolepis nana*, *Vampirolepis nana*, *Taenia nana*) (Macnish et al., 2002; Sadaf et al., 2013) is one of the smallest tapeworms and is the most common tapeworm infecting humans (Arcari et al., 2000; Bisen et al., 2012). Hymenolepiosis is usually asymptomatic in adult humans, but prolonged infection or multiple tapeworms, especially in children, can cause severe symptoms (Arcari et al., 2000; Bisen et al., 2012). It is estimated that approximately 50 to 75 million of people are infected globally (Macnish et al., 2002; Roble et al., 2012). Infection is more common in living conditions with poor hygiene and also in temperate zones (Macnish et al., 2002).

Hymenolepis nana is a slender worm, usually about 25–40 mm long and less than 1 mm wide. The scolex bears four suckers, and the rostellum is armed with a single ring of 20–27 small hooklets. Mature proglottids are more wide than long and trapezoidal (Baker, 2008).

The eggs of *H. nana* are colourless, almost transparent, spherical or ovoid, they measure 30–47 μm in diameter and have polar filaments. When shed in droppings, they are immediately infective and survive for up to 10 days in the environment. They are embryonated and have a six-hooked oncosphere inside the shell, which consists of two distinct membranes. On the inner membrane, there are two small 'knobs' or poles from which 4–8 filaments arise and spread out between the two membranes (Arcari et al., 2000). The embryo is spherical and thin-walled with a knob at each pole, from which six fine filaments emerge. The oncosphere (hexacanth embryo) possesses three pairs of small hooks (Baker, 2008).

Diagnosis of infection with *H. nana* can be made using direct microscopy, when the eggs are present in the faeces or adult tapeworms are found in the intestine at necropsy. Cysticercoids with a scolex having typical suction cups and rostellum are present in the lamina propria of the intestinal villi (Roble et al., 2012).

A morphologically identical variant, *H. nana* var. *fraterna,* infects rodents (Macnish et al., 2002). In the 1920s and 1930s, various authors experimentally demonstrated that tapeworm infection with *H. nana* in rodents can be transmitted to humans, and *vice versa* (Woodland, 1924; Kiribayashi, 1933; Ogura, 1936). Macnish et al. (2002) reported that attempts to infect mice with human isolates of *H. nana* were unsuccessful. Only in one out of 24 mice did a single cysticercoid develop from an egg, but even this failed to develop to an adult tapeworm.

Whether humans and mice have their own strains of *H. nana* which do not cross-infect is under debate. Regardless of this, the Centers for Disease Control and Prevention (2009) recommends taking precautions when handling infected rodents, because of the possibility of human infection (Roble et al., 2012). In mice, clinical signs are rarely observed. In less severe cases the segments of *H. nana* are only passed with the faeces, but in heavy infections retarded growth, weight loss, severe catarrhal enteritis, and intestinal obstruction are seen. Chronic infections can produce abscesses and focal granulomatous lymphadenitis of the mesenteric lymph nodes (Baker, 2008).

Different species of rodents have varying susceptibility to infection with *H. nana* (Wagner, 1987). Investigating the development of adult parasites in various species of rodents, Duclos and Richardson (2000) observed inter-species differences. Eleven days after infection the length of the parasites was 17.5 mm in hamsters and 7.5 mm in mice. The intensity of tapeworm infection in host animals also differed by species: the highest number of worms was observed in rats (66 worms) and lower worm counts were found in hamsters (15 worms) and mice (14 worms).

The life cycle of *H. nana* does not necessarily require an intermediate host. Direct life cycle can result in complete development within the intestinal villi of a single host, but the worm can also utilise arthropods (mostly insects) as an intermediate host (Arcari et al., 2000). This is the only cestode whose larval form (named cysticercoid) can also be transmitted directly. Eggs can pass in the faeces of the definitive host and are infective to another definitive host (Baker, 2008).

There are two forms of the direct type of life cycle in susceptible animals (mice, rats, gerbils, chinchillas). One occurs by the ingestion of eggs passed in the faeces of infected individuals. The other involves internal autoinfection, where eggs hatch in the gut without leaving it. When eggs are in the intestine, some of the oncospheres enter the villi. Development proceeds to completely formed cysticercoids that break out of the villi, attach themselves to the intestinal mucosa, and develop into the strobilar form. Other oncospheres enter the lymphatic and blood vessels, to be transported to the various organs. In the lymph nodes, liver, lungs, and pancreas, they lodge and develop into cysticercoids, which can migrate back to the small intestine. Adult cestodes in the lymph nodes of infected mice fed to clean mice can produce intestinal infections with strobilating and egg-producing worms. Thus, mice may serve both as a definitive and an intermediate host (Heyneman, 1953). As early as in 1953 Heyneman described that auto-reinfection in mice existed for a single parasite generation only. The cysticercoids developing in the intestinal villi stimulate an immune response that guards the host against the continual internal multiplication of the tapeworm by a direct cycle (Olsen, 1974).

In human infections, the larvae can develop to sexual maturity without ever leaving the host. The life span of adult worms is 4 to 6 weeks, but internal

autoinfection can lead to persistence for several years (Sadaf et al., 2013). Slightly oval eggs released from mature proglottids in the upper ileum are usually passed in the faeces. If these are swallowed by another human, hexacanth oncospheres burrow into the villi of the small intestine where they develop into tailless cysticercoids and then migrate towards the ileum. After five to six days, they attach to commence the formation of proglottids (Peters and Gilles, 1977; Arcari et al., 2000).

In the indirect life cycle, the eggs are ingested by intermediate hosts, and larvae develop inside flour beetles, fleas, cockroaches or other coprophagous insects. The eggs hatch to form tailed cysticercoids which remain unmodified as long as they are inside the insect. If they are accidentally swallowed by rodents or humans, the cysticercoid larvae pass down to the ileum and develop into adults (Peters and Gilles, 1977; Arcari et al., 2000). Infection of the definitive host occurs when cysticercoid-bearing beetles are eaten, digested, and the parasites liberated. They reach sexual maturity in about 10 days. Senescence begins after 14 days of productivity, and then the parasites start to die (Heyneman, 1953).

Praziquantel is very effective against *H. nana* in mice (Arther et al., 1981; Marshall, 1982; Campos et al., 1984). In humans, hymenolepiosis is also treated with a single dose of praziquantel, which has an efficacy of 96%. If praziquantel is not available, albendazole, fenbendazole or mebendazole can be used instead (Beck and Pantchev, 2006; Bisen et al., 2012; Li et al., 2012).

Materials and methods

Mouse colony

The colony of white mice involved in this study consisted of six large cages with groups of 500 mice in each (reproductive units). The ratio of bucks and does was about 1:20 in favour of the females. A few days before giving birth, mice were moved into small terrariums (nursery units). Ten to twenty females resided together with their offspring until their removal after the age of 21 days in units intended for sale in pet stores or for food for captive reptiles.

From one reproductive unit, samples from 10 mice were randomly taken and examined for the presence of *H. nana*. From different nursery units, 39 adult mice (females, weighing 30 g) and 18 young mice (3 weeks old, weighing 10 g) were randomly chosen and examined for the presence of *H. nana*.

Identification of animals and sampling methods

At the routine examination, the presence of ectoparasites (Dovč et al., 2015), endoparasites and cannibalism was confirmed. All tested mice were followed individually during the treatment. Females were housed in different cages and fol-

lowed by cutting fur on the head, neck and/or tail. Additionally, the serial number was written on the tail. At sampling, mice were put in a separate box for a few minutes while 8 to 15 droppings were collected individually.

Laboratory testing

Faecal droppings from each tested mouse were evaluated by flotation with NaCl solution and the sedimentation method. Technical procedures for flotation and the sedimentation method were implemented as described by Thienpont et al. (1979).

Treatment

In this paper, only the treatment against *H. nana* with a spot-on solution (Profender®) is described in detail. All breeding mice were treated, but only 67 animals (10 mice from the reproductive unit and 57 mice from the nursery units) were monitored individually throughout the experiment: before, during (three times) and seven days after the last treatment. The result of treatment was therefore checked prior to the treatment and then 7, 14, 21, 28 and 35 days after the first application of Profender®, primarily intended for cats, which contains 85.8 mg/ml praziquantel and 21.4 mg/ml emodepside (European Medicines Agency, 2008).

The original solution was placed as a spot-on application on the back of the neck in the area between the shoulders. Between applications, each mouse was isolated in a small container and kept separately for about 3 to 5 min. Does were given 9 µl (0.7722 mg praziquantel/mouse and 0.1926 mg emodepside/mouse) and young mice 3 µl (0.2574 mg praziquantel/mouse and 0.0642 mg emodepside/mouse). The application was repeated three times at 14-day intervals. Pipettes and tips (Biohit pipette 5–25 µl) were used to enable the precise dosing of the drug.

Statistics

Raw data were transferred to MS Excel 2010 software and exported to the IBM SPSS Statistics program version 20. Differences among groups were analysed using chi-square and Fisher's exact tests where values of $P < 0.05$ were considered to be significant.

Results

Reproductive units

Before the treatment with Profender® in all six reproductive units, the presence of *H. nana* eggs was confirmed in one unit in 50% (5/10) of mice tested by the flotation method and in 70% (7/10) of mice tested by the sedimentation method. Intense cannibalism was present in all reproductive units. Mice were

Table 1

Flotation and sedimentation results in nursery units before, during and after treatment with Profender®

Time of collection	Number (%)				Chi-square test***		
	Negative	Positive F	Positive S	Positive F & S	Chi^2	df	P
Before the treatment – at the first application*	42 (73.7)	1 (1.8)	9 (15.8)	5 (8.8)	10.612	1	0.001
Between the first and second application**	43 (75.4)	3 (5.3)	7 (12.3)	4 (7.0)	5.862	1	0.015
At the second application*	46 (80.7)	0 (0.0)	4 (7.0)	7 (12.3)	28.042	1	< 0.001
Between the second and third application**	52 (91.2)	0 (0.0)	0 (0.0)	5 (8.8)		NC	
At the third application*	53 (93.0)	1 (1.8)	3 (5.3)	0 (0.0)		NC	
After the treatment**	57 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)		NC	

F = flotation; S = sedimentation; F & S = both methods together; NC = not calculated; *samples taken immediately before the application of Profender®; **samples taken seven days after the first, second and third application; ***differences between detection methods within individual testing

Table 3

Separate flotation and sedimentation results for young and adult mice in nursery units before, during and after treatment with Profender®

Time of collection	Number of young mice (%)				Number of adult mice (%)			
	Neg.	F	S	F & S	Neg.	F	S	F & S
Before the treatment – at the first application*	18 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (61.5)	1 (2.6)	9 (23.1)	5 (12.8)
Between the first and second application**	9 (50.0)	1 (5.6)	4 (22.2)	4 (22.2)	34 (87.2)	2 (5.1)	3 (7.7)	0 (0.0)
At the second application*	11 (61.1)	0 (0.0)	1 (5.6)	6 (33.3)	35 (89.7)	0 (0.0)	3 (7.7)	1 (2.6)
Between the second and third application**	15 (83.3)	0 (0.0)	0 (0.0)	3 (16.7)	37 (94.9)	0 (0.0)	0 (0.0)	2 (5.1)
At the third application*	16 (88.9)	0 (0.0)	2 (11.1)	0 (0.0)	37 (94.9)	1 (2.6)	1 (2.6)	0 (0.0)
After the treatment**	18 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	39 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)

Neg. = negative; F = flotation; S = sedimentation; F & S = both methods together; *samples taken immediately before the application of Profender®; **samples taken seven days after the first, second and third application

continuously scratching themselves and one another with the consequent formation of scabs all over the skin. Other clinical signs were not seen. The combined results of the two methods confirmed the presence of *H. nana* in 80% (8/10) of mice in the reproductive unit. *Aspiculuris tetraptera (A. tetraptera)* was detected by at least one of the methods (flotation and sedimentation) in five samples. Seven days after the third therapy (35 days after the first treatment) no *H. nana* or other parasites were found in any of ten mice tested.

Nursery units

Cannibalism was not seen in the nursery units. Thirty-nine adult mice and 18 young mice were randomly chosen, and the presence of *H. nana* was followed individually before, during, and after the treatment. Results for both methods are shown in Tables 1 and 2.

Before the treatment with Profender®, 26.3% of the mice were carriers of *H. nana* (both testing methods included). More positive mice were detected with the sedimentation (24.6%) than with the flotation method (10.5%). After each treatment, the percentage of positive mice was lower, and at the last checking all mice were negative. Chi-square analysis of both methods together showed statistically significant differences in the data distribution between measurements (chi-square = 52.724, df = 15, $P < 0.001$) (Table 1). Furthermore, chi-square results separately for the flotation method and for the sedimentation method showed statistically significant differences in the data distribution between measurements (chi-square = 16.722, df = 5, $P = 0.005$, and chi-square = 23.370, df = 5, $P < 0.001$, respectively; Table 2).

Table 2

Positive results in nursery units before, during and after treatment with Profender® with respect to the method used

Time of collection	Number of positive F (%)	Number of positive S (%)	Number of positive F & S (%)
Before the treatment – at the first application*	6 (10.5)	14 (24.6)	15 (26.3)
Between the first and second application**	7 (12.3)	11 (19.3)	14 (24.6)
At the second application*	7 (12.3)	11 (19.3)	11 (19.3)
Between the second and third application**	5 (8.8)	5 (8.8)	5 (8.8)
At the third application*	1 (1.8)	3 (5.3)	4 (7.0)
After the treatment**	0 (0.0)	0 (0.0)	0 (0.0)

F = flotation; S = sedimentation; F & S = both methods together; *samples taken immediately before the application of Profender®; **samples taken seven days after the first, second and third application

Separate results for young and adult mice are shown in Table 3 and Figure 1. After each treatment, the percentage of positive mice was lower in both groups with one exception. Before the treatment, all the results in young mice (aged 21 days) were negative, while *H. nana* was found in 38.5% of adult mice. Between the first and the second application (one week later) *H. nana* was detected in 50% of young and 12.8% of adult mice. All the subsequent tests showed lower positive results in young than in adult mice.

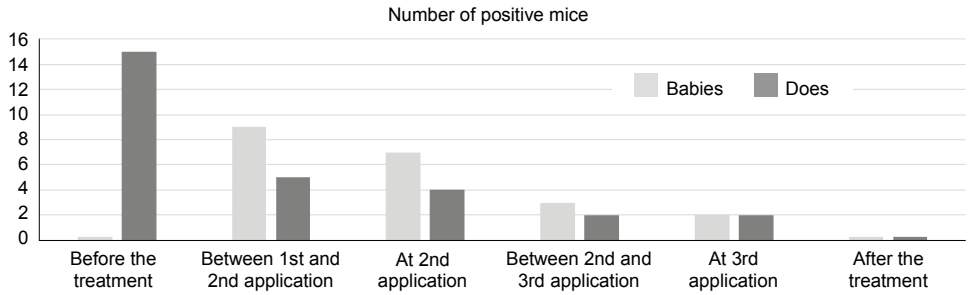

Fig. 1. Separate results for young and adult mice in nursery units using flotation and sedimentation method before, during and after treatment with Profender®

Before the treatment, eggs of *Trichuris* sp. in one mouse (1.8%), eggs of *Syphacia* sp. in four mice (7.0%) and eggs of *A. tetraptera* in 13 mice (22.8%) were found in the faecal droppings by both methods. Seven days after the last treatment the results were negative in all tested mice.

Discussion

Diagnosis

The important role of *H. nana* is in its unique direct life cycle and the consequent zoonotic potential for human infection. Breeders and people with compromised immune systems are more often infected. They can be infected via food, water, contaminated hands and objects contaminated with faeces. Epidemiological studies have shown that children who have pet rodents at home are more exposed, especially if there is poor hygiene (Duclos and Richardson, 2000). Infection with a small number of parasites may cause clinically inapparent infection. Severe infection may result in headaches, anorexia, convulsions, irritability, enteritis, diarrhoea, vomiting, abdominal pain, and pruritus (Lynne, 2007). Rodents with low infection rates do not show clinical signs. Severe infections are manifested as intestinal occlusion, catarrhal and chronic enteritis, abscesses of mesenteric lymph nodes, and hyperplasia of Peyer's patches; in exceptional cases,

infection may be fatal (Wagner, 1987). In our study the clinical signs described by Wagner (1987) were not seen, but cannibalism was present in all reproductive units with large groups of mice (about 500 animals in one terrarium) of both sexes. In these units, *H. nana* was confirmed in 80% while in the nursery units only in 26.3% of the mice. A similar situation was observed for other helminths: *A. tetraptera* was confirmed in 50.0% of the mice in the reproductive unit and in a lower percentage (22.8%) in the nursery unit. In the nursery units, eggs of *Trichuris* sp. in one mouse and eggs of *Syphacia* sp. in four mice were also confirmed. We assume that bigger groups of mice, the presence of males, and infection with parasites may severely affect the well-being of the animals, causing stress and leading to cannibalism.

For testing faeces, the flotation method is predominantly used in veterinary laboratories. By exploiting the density of the solution, parasite eggs float to the top of a dense solution (final specific gravity of about 1.20) and can then be skimmed from the top of the tube (Cuomo et al., 2009).

We tested only droppings of live mice using flotation and sedimentation methods. The sedimentation method yielded more positive results before, during and after the treatment. The flotation method revealed some additional positive cases that had not been confirmed by sedimentation, and *vice versa*. In our laboratory, a saturated solution of NaCl was used for the flotation method, although in other laboratories the most commonly used reagent is zinc sulphate.

Adult worms and proglottids are rarely seen in stool samples of humans (Cuomo et al., 2009). However, in our positive cases eggs of *H. nana* were found and in two cases proglottids were also confirmed (in one young and one adult mice at the second testing).

According to Duclos and Richardson (2000), the flotation results of faecal samples collected from different rodent species in pet shops showed the presence of infection with *H. nana.* The prevalence was 31.6% in rats, 22.2% in domestic mice, and 10.3% in golden hamsters. Overall, seven of the nine surveyed pet stores were selling infected rats, mice, or hamsters, indicating that pet store rodents pose a potential threat to public health (Duclos and Richardson, 2000). In 2012, a survey was conducted to study the prevalence of infection in pet stores (Roble et al., 2012). The majority (5 out of 6) of the surveyed pet stores were selling mice positive for the potentially zoonotic *H. nana*. This outcome is consistent with the previous findings of Duclos and Richardson (2000), according to which mice from 75% of the surveyed pet stores were positive for *H. nana*. The cited study found *H. nana* in several species, including mice, rats, and hamsters. Other authors reported infection with *H. nana* also in gerbils (Olsen, 1974; Duclos and Richardson, 2000), chinchillas (Olsen, 1974), prairie dogs and old world monkeys (Duclos and Richardson, 2000). In Slovenia, *H. nana* has been found in mice and hamsters from pet shops (unpublished data), and the mice from the present study were also intended for pet stores and food for captive reptiles.

At the beginning, the results obtained in young mice before and after the first treatment seemed surprising. At the first sampling in less than 21 days old mice the results were negative, but at the second sampling 50% of the young mice were positive. Sexually mature forms of tapeworms were confirmed only in 21 days old or older mice. It is known that *H. nana* reaches sexual maturity in about 10 days; therefore, before this period the tapeworm did not release eggs in the environment (Heyneman, 1953). This means that the young mice were infected at the age of 10 to 12 days when they opened their eyes and began to crawl out of the nest.

Treatment

Arther et al. (1981) established that praziquantel blended into the feed at 140 ppm for seven consecutive days provides effective control of *H. nana* in mice. For complete elimination of this parasite, higher dosages may be necessary (Macnish et al., 2002). Infection may be eliminated by the oral application of praziquantel in three treatments at intervals of 14 days, administered at a dosage of 30 mg/kg body weight to mice, gerbils and rats (Ramsey, 2008).

The effects of different plant extracts (coconut, onion, garlic, fig, date tree, chicory, pineapple, and rockrose) on different intestinal cestodes were described by Abdel-Ghaffar et al. (2011). In all *in vitro* tests, the target parasites died. The treatment of mice and rats with a combination of onion and coconut extracts eliminated all cestodes from their final hosts. These results call for further research in this area.

Our results obtained after treating mice with emodepside in doses of 2.574 mg were negative for *Trichuris* sp., *Syphacia* sp. and *A. tetraptera*. Harder and von Samson-Himmelstjerna (2001) and Schmahl et al. (2007) successfully used emodepside in rodents against other nematodes.

In our study, the recommended and commonly used praziquantel was applied in doses of 25 mg/kg body weight. The drug was given in doses of 0.7722 mg/doe and 0.2574 mg/young mouse. The application was repeated three times at 14-day intervals. Campos et al. (1984) concluded that praziquantel should be given twice, 10 days apart, so that the second dose kills the larval and juvenile forms which survived the first one. The sexual maturity of *H. nana* is reached in about 10 days but oviposition is not yet started. Eggs can be present four days later. A larval form can survive the first seven days of treatment. The treatment is not yet satisfactory but sensitivity increases and as soon as cysticercoids reach sexual maturity the treatment is 100% efficacious (Campos et al., 1984). In our study treatment was performed every 14 days and the faeces was checked every 7 days, so false negative results could not be ignored. The last testing results of our study were satisfactory. In our opinion reinfection should be expected when other control measures are not implemented. Good hygiene, proper nutrition, good condition of the animals and the absence of cannibalism or other stressful

factors are very important for the success of treatment. No reinfection or cannibalism appeared after all preventive measures had been implemented. In the future, the correlation between invasion with parasites and cannibalism should be investigated. The high standard of welfare is of key importance for good reproduction results in mice colonies.

Prevention

In rodents, *H. nana* infection is difficult to control as this tapeworm spreads in different ways. An important component of prevention programmes against infection in mouse breeds is the high level of sanitation and disinfection. In the present study, flies were present in the colonies, so insect control was performed. Control of all newly purchased animals is needed before they enter the breeding facilities, and quarantine measures must be implemented. Public health and sanitation programmes must be considered. Feeding reptiles with *H. nana*-positive or anthelmintic-treated mice (withdrawal period) is not recommended.

Assigning roles and labels has a profound effect on ethical decision-making by typifying the roles designed for mice by a research laboratory. The mice used in research are offered government and institutional protective measures, while free-ranging pests are trapped and often suffer miserable deaths. Mice intended to be fed to other animals represent a grey area regarding moral status (Herzog, 1988). Many things are still to be done for the welfare of mice that fall in this grey area.

The systematic health control of rodents, with the special intention of providing a diagnosis of parasitic diseases and enabling their timely treatment, could significantly improve health and welfare. It could also be of vast importance as a measure to prevent the transmission of potentially zoonotic diseases among traders in pet shops, breeders and pet owners.

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