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▣ ORIGINAL ARTICLE

Susceptibility of Laboratory Rodents to *Trichinella papuae*

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Abstract: Members of the genus *Trichinella* are small nematodes that can infect a wide range of animal hosts. However, their infectivity varies depending on the parasite and host species combination. In this study, we examined the susceptibility of 4 species of laboratory rodents, i.e., mice, rats, hamsters, and gerbils to *Trichinella papuae*, an emerging non-encapsulated *Trichinella* species. *Trichinella spiralis* and *Trichinella pseudospiralis* were also included in this study for comparison. Fifteen animals of each rodent species were infected orally with 100 muscle larvae of each *Trichinella* species. Intestinal worm burden was determined at day 6 and 10 post-inoculation (PI). The numbers of muscle larvae were examined at day 45 PI. The reproductive capacity index (RCI) of the 3 *Trichinella* species in different rodent hosts was determined. By day 6 PI, 33.2-69.6% of the inoculated larvae of the 3 *Trichinella* species became adult worms in the small intestines of the host animals. However, in rats, more than 96% of adult worms of all 3 *Trichinella* species were expelled from the gut by day 10 PI. In gerbils, only 4.8-18.1% of adult worms were expelled by day 10 PI. In accordance with the intestinal worm burden and the persistence of adults, the RCI was the highest in gerbils with values of 241.5±41.0 for *T. papuae*, 432.6±48 for *T. pseudospiralis*, and 528.6±20.6 for *T. spiralis*. Hamsters ranked second and mice ranked third in susceptibility in terms of the RCI, Rats yielded the lowest parasite RCI for all 3 *Trichinella* species. Gerbils may be an alternative laboratory animal for isolation and maintenance of *Trichinella* spp.

Key words: *Trichinella spiralis*, *Trichinella pseudospiralis*, *Trichinella papuae*, rodent, susceptibility

INTRODUCTION

Members of the genus *Trichinella* are small nematodes that can infect a wide range of animals, including mammals, birds, and reptiles [1,2]. This genus comprises 9 species and an additional 3 genotypes from undefined species [2,3]. The infectivity of each *Trichinella* species can be determined by the number of larvae recovered from muscles of the host species after experimental inoculation of a known number of infective larvae and varies among host animals [4]. The reproductive capacity index (RCI) has also been used to demonstrate infectivity in rats and pigs [5]. Adult worms are expelled from the small intestine of hosts at around days 9-12 post-inoculation (PI) in rats, days 11-14 in mice, and by day 15 in golden hamsters [6]. The degree of expulsion and the time of initiation varies according to the host animal and the species of *Trichinella* [7-9]. *Trichinella papuae*, a recently found non-encapsulated species of *Trichinella*, was responsible for outbreaks and sporadic cases in humans in Thailand [10]. However, its infectivity to laboratory animals has not yet been studied in detail. In this study, we measured the infectivity of *T. papuae* to mice, rats, hamsters, and gerbils using the adult worm expulsion rate and the parasite RCI as the parameters. *Trichinella spiralis* and non-encapsulated *Trichinella pseudospiralis* were also studied for comparison. The knowledge obtained in this study will be useful for the development of animal models for trichinellosis.

MATERIALS AND METHODS

Parasites

T. spiralis (ISS62) [11] and *T. papuae* (ISS4120) [10] isolates used in this study were obtained locally. *T. pseudospiralis* (ISS13) [12] was a generous gift from the Department of Parasitology, Gifu University Graduate School of Medicine, Japan. All *Trichinella* species were maintained in our laboratory by serial passage through mice.

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Animals

All laboratory rodents used in the study were 6-8 week-old males and consisted of Swiss albino mice, 20-35 g, Sprague-Dawley rats, 292-390 g, Syrian Golden hamsters, 95-140 g, and Mongolian gerbils, 47-66 g. All animals were outbred and produced by the Animal Unit of Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. The study protocol was approved by the Animal Ethics Committee of Khon Kaen University (AE 50/2553), and the animal care was in compliance with the Ethics of Animal Experimentation Guideline of the National Research Council of Thailand.

Experimental infection

To obtain *Trichinella* muscle larvae, infected mice were euthanized, skinned, and eviscerated. The carcass was minced and digested by conventional pepsin-HCl solution (1% pepsin, 1% HCl in 0.85% NaCl solution; NSS). The muscle larvae were collected using a modified Baermann technique [13]. Each animal (15 per group) was inoculated orally with 100 larvae using a tuberculin syringe equipped with a blunt 16-gauge needle. Five animals in each group were sacrificed at day 6 and 10 PI for counting intestinal adult worms, and the remaining 5 animals were sacrificed at day 45 PI for counting muscle larvae.

Intestinal worm burden

Adult worms were recovered using the procedure described by Bell et al. [7] with modifications. Infected animals were fasted 1 day prior to sacrifice. The small intestine was removed and transferred into a Petri dish containing normal saline, cut into 5 cm-segments, and slit open longitudinally. The dish was left standing at room temperature for 30-60 min. The adult worms that migrated into the solution were counted under a stereomicroscope. The intestines were transferred to another Petri dish containing pepsin solution, and placed in a 37℃ incubator. These dishes were examined every 30 min for 3-4 times until no additional worms were found. The numbers of worms counted before and after the pepsin treatment were added together to produce a total number of adult worms per animal.

Muscle larvae count

The muscle larvae collected from each pepsin-digested animal carcass were pooled in a 50 ml centrifuge tube and then transferred to a glass dish with internal area of 3.75×3.75 cm. The animal tissues were carefully removed under a stereomicroscope. The larval suspension was transferred to a 1.5 ml Eppendorf tube, and the volume was adjusted to 1,350 µl. The larval suspension was mixed well, and 3 samples of 50 µl were each placed into a glass dish for counting under a stereomicroscope. An average number of larvae per 50 µl was calculated. Then, the total number of larvae per carcass was finally calculated from the total volume of the larval suspension. The RCI was calculated as the total number of larvae per carcass divided by 100 (100 being the number of larvae in the initial inoculum).

Statistical analysis

Descriptive statistics were used. Data were expressed as the mean±SD. Worm expulsion was expressed as the percent reduction of the intestinal worm burden from day 6 to day 10 PI.

RESULTS

Establishment and expulsion of worms in the small intestine

Animals were each infected with 100 larvae of 1 of the *Trichinella* species, and intestinal worm burdens were determined for 5 animal of each rodent species at days 6 and 10 PI. Table 1 shows the worm count at days 6 and 10 PI for each *Trichinella* species and each animal host. All 3 *Trichinella* species were successfully established in the small intestine of the 4 rodent hosts, and 33.2-69.6% of the inoculated muscle larvae were recovered as adult worms at day 6 PI. The lowest and the highest worm counts for *T. papuae* were in the hamster and mouse, respectively. However, at day 10 PI, the expulsion of worms was the greatest in rats, with >96% reduction for all 3 species of

Table 1. Numbers of adult *Trichinella* in the small intestine of animals at day 6 and 10 PI[a]

	Mouse	Rat	Hamster	Gerbil
Trichinella spiralis				
Day 6	65.2±3.9	45±8.6	55.6±18	58.6±5.2
Day 10	43.2±8.4	1.6±0.9	42.0±5.1	48.0±2.9
	(33.7%)	(96.4%)	(24.5%)	(18.1%)
Trichinella pseudospiralis				
Day 6	55.2±5.9	40.8±3.6	53.8±4.6	55.4±5.5
Day 10	42.6±6.1	1.6±1.5	28.0±3.7	48.6±12.4
	(22.8%)	(96.1%)	(48.0%)	(12.3%)
Trichinella papuae				
Day 6	69.6±3.4	48±1.6	33.2±1.8	46.2±6.4
Day 10	4.2±4.3	0.4±0.5	26.6±6.0	44±6.2
	(94.0%)	(99.2%)	(19.9%)	(4.8%)

[a]Data are expressed as the mean and SD from 5 animals.
Numbers in parentheses indicate percent reduction from the count of day 6 PI.

Table 2. Reproductive capacity indices (RCI) of *Trichinella* in laboratory rodents inoculated orally with 100 muscle larvae[a]

	Mice	Rats	Hamsters	Gerbils
Trichinella spiralis	78.8±20.7 (57-101)	63.6±6.6 (56-73)	247.4±97.2 (135-297)	528.6±20.6 (504-556)
Trichinella pseudospiralis	56.4±3.3 (50-58)	10.3±4.6 (5-14)	210.9±51.4 (150-267)	432.6±48 (380-489)
Trichinella papuae	36.1±11.7 (26-56)	9.8±2.3 (6-11)	59.9±3.8 (53-62)	241.5±41.0 (187-291)

[a]Data are expressed as the mean and SD from 5 animals.
Numbers in parentheses indicate range of RCI.

Trichinella. A high level of expulsion was also seen in mice infected with *T. papuae* (94% reduction). In contrast, gerbils exhibited the least degree of worm expulsion for all 3 species of *Trichinella* (4.8-18.1% reduction). A moderate degree of worm expulsion was observed in hamsters infected with *T. spiralis* and *T. papuae* (24.5% and 19.9% reduction, respectively) and in mice infected with *T. pseudospiralis* (22.8% reduction).

Reproductive capacity indices

Muscle larvae were recovered from each infected animal by pepsin digestion at day 45 PI, and RCIs were determined. Table 2 shows the RCI of each *Trichinella* species in each rodent host. The RCIs of all 3 species of *Trichinella* were the highest in gerbils and hamsters (range 210.9-528.6). Low RCIs for all 3 *Trichinella* species were seen in rats and mice (range 9.8-78.8).

DISCUSSION

Rats and mice have long been employed for maintenance and study of *Trichinella* species. However, the low infectivity of *T. papuae* and *Trichinella murrelli* in laboratory mice and rats raised concern about the usage of these laboratory animals for studying *Trichinella* isolates from human and animal sources of infection [5]. A newly identified species, *Trichinella patagoniensis*, isolated from cougars has a much lower RCI than that of *T. spiralis* in mice and rats [3]. In this study, we re-examined the susceptibility of small laboratory rodents to 3 *Trichinella* species. Two parameters were used to determine the host susceptibility; the reduction rate of intestinal adult worms and the RCI. The expulsion of adult worms from the host intestine is mediated by the host immune response to the parasite [14]. The RCI reflects not only host susceptibility but also parasite characteristics. In our study, 33.2-69.6% of the initial dose of larvae were recovered as adult worms in the small intestines of the experimental rodents at day 6 PI. Worms were then expelled over the next 4 days, and the degree of expulsion varied between host species. Rats expelled more than 90% of all *Trichinella* species between days 6 and 10 PI. Larsh [6] reported that a significant loss of *T. spiralis* adult worms from the small intestines of rats occurred from day 9 to day 12 PI, and Bell et al. [7] stated that adult worm rejection did not begin until day 12 PI in Fisher rats [7]. The RCIs of *T. papuae* (RCI=9.8) and *T. pseudospiralis* (RCI=10.3) were very low in Sprague-Dawley rats in the present study, but Murrell et al. [5] observed a much higher RCI for *T. spiralis* (RCI=185-237) and *T. pseudospiralis* (RCI=47-62) in Wistar rats. Such variances might be, at least in part, due to the use of different animal strains and different isolates of the parasite.

In the present study, mice also strongly expelled *T. papuae* and moderately *T. spiralis* and *T. pseudospiralis*. The results from other studies revealed that the degree of expulsion depends on the strain of mice and species of *Trichinella*, e.g., 87% expulsion in Swiss mice [15] and 50% in NIH mice [8] infected with *T. spiralis*. The RCI of *T. papuae* in Swiss albino mice in the present study was 36.1, thus higher than the RCI of 5.3 reported for CD1 mice [16]. Hamsters showed the lowest degree of expulsion for *T. papuae* (19.9%), but the RCI was lower than those for *T. spiralis* and *T. pseudospiralis*. Over 50% of *T. spiralis* and *T. pseudospiralis* adult worms were found in the small intestine of hamsters at day 6 after infection. The percent worm recovery was higher than the 32% reported by Sadun and Norman [17] for 11-week-old golden hamsters infected with 250 *T. spiralis* larvae. Boyd and Huston [18] also recovered only 23% of the inoculum of 100 larvae as adult worms after day 6 PI in 120-day-old hamsters. This could be due to the use of older animals [6]. Others have reported RCIs for *T. spiralis* in hamsters of 125 [18] and 258 [17], and these are comparable with our results (RCI=247.4).

Sagar et al. [19] employed gerbils as an animal to study the immune response to enteric infections by *T. spiralis*. Our study, however, focused on maintenance of the parasite. Gerbils appeared to be the most susceptible rodent species to *T. papuae* in our study; 46% of adult worms were recovered at day 6 PI, and only 4.8% were expelled during the next 4 days. The RCI for *T. papuae* in gerbils (RCI=241.5) was much higher than that for hamsters, mice, and rats (59.9, 36.1, and 9.8, respec-

tively). Gerbils were also susceptible to *T. spiralis* and *T. pseudospiralis*, being indicated by a lower percentage of expulsion and a higher RCI than those of other rodents.

In conclusion, Mongolian gerbils appear to be the most susceptible laboratory rodents to *Trichinella* infection. Gerbils may be suitable for primary isolation of *Trichinella* species from animal and human sources. In case of animal houses which have difficulty in production and maintenance of small, fragile gerbils, it is suggested that mice, based on moderate RCI, can be used for long term maintenance of *Trichinella* following an initial passage in gerbils.

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