Persistent infection with *Strongyloides venezuelensis* in the Mongolian gerbil (*Meriones unguiculatus*)

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Abstract: To examine the fate of *Strongyloides venezuelensis*, Mongolian gerbils (*Meriones unguiculatus*) were orally infected with 1,000 L_3 larvae per animal. Altogether, 50 gerbils divided into 5 groups of 10 each were monitored for a period of 570 days to document the kinetics of faecal egg output, adults worm population, morphological development, fecundity, and hematological changes including peripheral blood eosinophilia. This study chronicled a life long parasitism of *S. venezuelensis* in the gerbil host, and showed that *S. venezuelensis* infection was quite stable throughout the course of infection and the worms maintained their normal development as evidenced by their body dimension. A progressive loss of body condition of the infected gerbils was observed as the level of infection advanced. However, no detectable pathological changes were observed in the gastrointestinal tract. The present findings indicate that an immunocompetent host, such as the Mongolian gerbil, can serve as a life long carrier model of *S. venezuelensis* if the worms are not expelled within 570 days after infection.

Key words: *Strongyloides*, chronic disease, eosinophils, larva, fertility, life cycle

INTRODUCTION

Strongyloides venezuelensis, a gut dwelling nematode of murines, has been a suitable parasite model for the study of human and/or animal strongyloidiasis (Sato and Toma, 1990; Taira et al., 1994). The Mongolian gerbil is considered a fascinating laboratory animal model for biomedical research on parasites (Horii et al., 1993), because of its high degree of susceptibility to a variety of infectious agents including helminthic parasites such as *Brugia*

pahangi (Ash and Riley, 1970) *S. stercoralis* (Nolan et al., 1993) and *S. ratti* (Horii and Nawa, 1992). The latter species are very closely related to *S. venezuelensis* (Tsuii et al., 1993). The expulsion mechanisms of intestinal helminths have been demonstrated by many workers (Abe and Nawa, 1988). In rats and mice, intestinal mastocytosis occurs in *Strongyloides* species infection, and a substantial proportion of mast cells migrate into the epithelial layer around the time of immune-mediated expulsion suggesting that mast cell derived mediators are the effector molecules (Nawa et al., 1994). Horii et al. (1993) infected Mongolian gerbils either with *S. venezuelensis* alone or concurrently with *Nippostrongylus brasiliensis* and demonstrated that the *S. venezuelensis* infection persisted for

over 10 weeks, whereas *N. braziliensis parasites* were expelled by 3 weeks. They concluded that gerbils have selective functional defects in the expulsive mechanisms, commonly directed against *Strongyloides* species. Khan et al. (1993) reported that the inability of Mongolian gerbils to expel *S. venezuelensis* adult worms from the small intestine was due to defects in effector/regulatory cells, presumably mast cells, but not due to immune unresponsiveness to parasite antigens. Most of the previous studies have demonstrated the importance of the host's immune mechanisms operating against *S. venezuelensis* in the rodent host. These observations prompted us to use the gerbil to investigate the course of infection, kinetics of faecal egg output and worm populations, their morphological development and fecundity. Hematological changes were also monitored.

MATERIALS AND METHODS

Parasite strain

The strain of *S. venezuelensis* used throughout this study was isolated by Professor Saeki from the Kagoshima Prefecture, Japan and designated as the Kagoshima strain.

Serial passage, culture and preparation of infective doses

The parasites were maintained in male Sprague-Dawley rats by monthly passage. The L_3 stage larvae cultured in 0-12% nutrient broth in polyvinyl bags (Baek et al., 1998) were isolated by Baermann's device and washed several times in phosphate buffered saline (PBS) containing 600 IU penicillin, 500 IU streptomycin sulfate and 200 IU amphotericin B (Sigma, USA). The suspension of L_3 was pipetted onto glass slides and numbers counted. The final concentration was adjusted to 1000 L_3 in 1 ml PBS and used as an oral inoculum for each animal.

Animals and infection

Equal numbers of male and female Mongolian gerbils, 8-10 weeks old and weighing 60-65 g, were used. They were provided by the Korean Food and Drug Research Center and were maintained in a clean environment and fed with commercial rodent feed with water ad libitum. A total of 50 gerbils divided into 5 groups of 10 gerbils each were used. The gerbils were sacrificed on the 7th, 21st, 35th, 105th and 570th day post-infection (PI).

Clinical monitoring of the experimental Mongolian gerbils

Careful examination of Mongolian gerbils was initiated immediately after infection on a routine basis. Any abnormalities/deviation found in relation to the animal's demeanor, appetite, resting and defecation patterns were recorded. Infected gerbils were also monitored for any pathological conditions on footpads, eyeballs, nose, skin, etc. on the designated days and until the final day of the experiment.

Monitoring of faecal egg outputs

Faecal egg output in terms of eggs per gram (EPG) of faeces was examined starting on day 3 PI and subsequently on the 5th, 7th, 9th, 21st, 35th, 105th, and 570th day PI. EPG was determined on freshly passed faeces according to the methods of Islam et al. (1999). Briefly, the individual gerbil was placed in a 100 ml beaker and allowed a few minutes to discharge faeces. The faeces were then suspended in 10 ml of distilled water in a 50 ml beaker. The eggs in the suspension were counted under a low power objective and the EPG was calculated.

Examination of adult worm populations

Adult worm populations were recovered from the small intestines of five gerbils sacrificed at various time intervals (i.e., 7th, 21st, 35th, 105th and 570th day) following the methods of Islam et al. (1999). Briefly, the entire small intestine of each sacrificed gerbil was dissected longitudinally and incubated at 37°C for five hours in Baermann's device. The whole length of the small intestine was examined carefully under a dissecting microscope, and worms attached to the mucosa were counted. Mongolian gerbils that died were similarly processed.

Fecundity and morphological development of worms

Long-term parasitism of *S. venzuelensis* was monitored in a group of ten gerbils until their

death on day 570 PI. Throughout this period, fecundity of 50 adult worms recovered at various intervals was measured (Baek et al., 1998). Morphological dimensions with respect to body length and width of the adult worms recovered on designated days were also recorded.

Hematological profiles and peripheral blood eosinophilia

Blood of infected and uninfected gerbils was obtained by cardiac puncture using a 1.0 ml disposable plastic syringe prior to sacrifice on the designated days to measure the hematological values. Blood was transferred immediately into a 2.0 ml vial containing anticoagulant and mixed thoroughly. Total erythrocyte and leukocyte counts as well as hemoglobin concentration were determined using an auto analyzer. The presence of eosinophils in the peripheral blood was examined according to the method described by Korenaga et al. (1995). Briefly, 5 : 1 of blood was taken from the tail vein and mixed with 20 : 1 of Hinkelman's solution (0.5% W/V cosin, 0.5% W/V phenol and 0.185% V/V formaldehyde in distilled water).

RESULTS

Quantitation of EPG of S. venezuelensis

The course of S. venezuelensis infection in a group of Mongolian gerbils infected subcutaneously each with 1000 L_3 was observed over a period of 570 days. Eggs in the faeces were first detected on day 5 PI, reaching a peak on day 9 PI and stabilizing thereafter until the end of the observation period. As shown in Table 1, the kinetics of faecal egg output were monitored starting from the 5th day PI. The level of egg output was 4,189 ± 670 at the 5th day, stabilizing on the 9th day after PI at 111,000 ± 225 to 106,200 ± 786 until day 570 PI.

Recovery of adult worms in the small intestine of gerbils

The kinetics of adult worm populations in the small intestine of gerbils sacrificed on the designated days are summarized in Table 1. The recovered adult worms were 361 ± 53.4. on the 7th day PI and 549.6 ± 95.8 on the 21st day PI. There was a significant difference (P < 0.05) between the 7th and 21st day PI, however there was no significant difference (p = 0.5) between the 35th and 105th day PI.

Fecundity in the uterus

The fecundity was monitored until the last day of observation. Fecundity profiles of worms recovered from day 7 through day 105 PI are shown in Table 1. At the 7th day PI the fecundity was 6.8, decreasing to 5.8 at 21 days PI and thereafter remaining stable until the end of observation period.

Morphological features of S. venezuelensis in gerbils

Morphological dimensions with respect to body length and width of adult worms recovered at various time intervals are shown in Table 2. The mean body length at 7 day PI was

Table 1. Kinetics of the faecal average egg output, number of adult nematodes and fecundity indices in the uterus of Strongyloides venezuelensis parasites in Mongolian gerbils infected subcutaneously with 1,000 third stage larvae

Parasite	Days post inoculation						
	5	7	9	21	35	105	570
EPG of feces (× 10^3)	4,189	98,700	111,000	102,800	104,800	107,400	106,200
	(± 670)	(± 560)	(± 225)	(± 987)	(± 1,034)	(± 946)	(± 786)
Average number of parasites	ND[a]	361 (± 53.4)	600.5 (± 69.5)	549.6 (± 95.8)	548.4 (± 86.5)	76.38 (± 76.4)	340 (± 45.1)
Average fecundity index[b]	ND	6.8	7.3	5.8	5.9	6.3	7
Survival rate of animals	ND	50	49.0	40	30	20	1

[a]ND = Not done.
[b]According to Baek et al. (1998).

Table 2. Morphological dimensions of adult *Strongyloides venezuelensis* recorded at various time intervals in the Mongolian gerbil model

Days post infection	Morphological dimensions (μm)[a]	
	Body length	Body width
7	2,685.0 ± 175.7	41.4 ± 1.8
21	2,765.0 ± 145.6	41.5 ± 1.9
35	2,775.0 ± 253.8	41.9 ± 1.7
105	3,033.8 ± 96.1	43.1 ± 1.4
570[b]	3,243.8 ± 79.8	45.3 ± 0.1

[a]Mean ± SD of 20 adult nematodes.

[b]Adult nematodes recovered immediately after death of the gerbils.

Table 3. Selected blood profiles of gerbil infected with *Strongyloides venezuelensis*

Days after infection	RBC (10⁶/mm³)	WBC (10³/mm³)	Hemoglobin (gm%)
0	7.9	12.6	15.4
7	7.4	12.8	14.6
21	7.5	13.1	14.2
35	7.3	11.5	14.0
105	8.1	10.0	16.3

2,685 ± 175.7 μm, later increasing to 3,244 ± 79.8 at 570 day PI. Worms showed an increasing body dimension with increasing parasite load. Worms recovered from dead animals at 570 PI at high infection level showed significantly higher morphological dimensions as compared with those recovered earlier (p = 0.001).

Hematological profiles and peripheral blood eosinophilia

Hematological findings such as hemoglobin concentration, and total erythrocyte and leukocyte counts from day 7 through day 105 PI are shown in Table 3. There were no significant changes in the blood profile. The total RBC count increased steadily from 7.9 × 10⁶/mm³ to 8.1 × 10⁶ mm³. Similarly, hemoglobin increased from 15.4 mg% to 16.3 mg%. There were no statistically significant differences in hematologic profiles. The WBC count, however, decreased from 12.6 to 10.1 × 10³/mm³.

The general health status of gerbils

There was reduced body weight and roughening of the hair coat, along with reduced feed intake, alopecia and lethargy as the course of infection advanced. All gerbils died between 324 and 570 days PI. At necropsy, 340 adult worms were recovered from two gerbils that died on days 510 and 570 PI (Table 1). At post mortem examination, no pathological changes were observed in the gut, lung or liver of any experimental animal.

DISCUSSION

We have demonstrated that *S. venezuelensis* adults adapted rapidly and well in the small intestine of the Mongolian gerbil and persisted for over 570 days, by which time all the infected animals died. These observations are consistent with those of Tsuii et al. (1993) who also observed prolonged infection for over 450 days with *S. venezuelensis* in the Mongolian gerbil. However, in contrast to our approach, Tsuji et al. (1993) terminated their experiment before the death of the gerbils. Although the animals showed progressive loss of body condition as the stages of infection advanced, post-mortem examination did not demonstrate any detectable pathological changes in any part of the GI tract, lungs or liver. This might have been due to the low infective doses of *S. venezuelensis* used in this study, deliberately selected in order to prolong the infection status. Since massive infection with *S. venezuelensis* (10⁶ L₃) can induce severe itching, bleeding on the digital pads at the time of infection, listlessness, hemorrhagic pneumonia and death (Taira et al., 1995), we chose a low dose infection to facilitate prolonged infection.

An interesting observation is the high worm

populations in the small intestine and EPG counts in the faeces, which remained stable as long as the host animals survived. There was an apparent evidence of autoinfection, uniquely associated with S. stercoralis, which is a closely related species to S. venezuelensis, even at the advanced stage of infection. When freshly passed faeces were examined, no rhabditiform/filariform L_3 was found, even when the entire length of the GI tract was incubated at 37°C using the Baermann technique. It thus seemed unlikely that the population of adult worms in the small intestine was due to autoinfection. The phenomenon of autoinfection with S. venezuelensis has not yet been proven in any kind of rodent host including Mongolian gerbil (Sato and Toma, 1990; Tsuji et al., 1993) and our data confirm those observations.

The morphology of adult S. venezuelensis worms regularly showed tendencies of increasing dimensions with respect to length and width of the body among those recovered after death of the infected gerbils. The morphological changes of S. venezuelensis in the gerbil host appeared to be much greater than those reported in normal rats (Wertheim, 1970; Hasegawa et al., 1988; Sato & Toma, 1990; Islam et al., 1999), indicating that gerbil hosts favored extended development of S. venezuelensis worms in the small intestine. The fecundity of these worms was also found to be higher than those recovered from mice (5.5 ± 0.9) by Sato & Toma (1990) but lower than those in rats (7.2 ± 1.9) (Islam et al., 1999). These findings indicate that the Mongolian gerbil is a suitable model for Strongyloides species, particularly for S. venezuelensis, which has poor immunogenicity. This is also in agreement with the reports of Horii et al. (1993) and Khan et al. (1993) who observed that the Mongolian gerbil has functional defects in the effector/regulatory cells, presumably mast cells, but not due to immune unresponsiveness to parasite antigen.

The mechanism by which adult S. venezuelensis worms firmly establish in the gut epithelial layer is yet to be investigated. Recently, Maruyama and Nawa (1997) reported that the production/secretion of the adhesive substances by S. venezuelensis adult worms is a key step for the parasites to invade and

establish in the host's defense mechanisms. Hematological profiles such as CBC and hemoglobin concentration revealed no changes as compared with those recorded in normal animals prior to infection. Up to 500 adult worms in a gerbil S. venezuelensis (the worm burdens recorded) does not cause anemia.

The normal counts of leukocytes in S. venezuelensis infected gerbils also indicated that the defense mechanisms of the gerbils did not trigger the production of leukocytes including eosinophils. This was further supported by the complete absence of eosinophils in the peripheral blood of the gerbil hosts. It has been demonstrated that low levels of peripheral blood eosinophilia (PBE) in S. venezuelensis infected rats increases the host's susceptibility to the parasite, while a high level enhances worm expulsion (Islam et al., 1999). The association between increased production of eosinophils and worm expulsion has also been reported with other gut dwelling nematodes such as S. stercoralis (Rotman et al., 1999) and N. brasiliensis (Shin et al., 1997). These studies also elucidated the concept that the absence of elevated PBE favors persistent parasitism of S. venezuelensis in the gerbil host. The persistent infection of S. venezuelensis in the gerbil may be prolonged beyond 570 days under controlled environmental conditions, thus making the gerbil an excellent laboratory model.

Given the success of our experiment in inducing persistent infection in this model we feel that a foundation has been laid to precisely define the life cycle and pathogenesis of strongyloidosis based on lifelong infection attained by the dosage we have optimized. Such an approach also offers promise for studies in chemotherapy and vaccinology.

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