Massive Experimental Infection with *Strongyloides venezuelensis* in Rats and Absence of Sudden Death

Noriyuki Taira, Yoshio Nakamura, Maria Angela O. Almeida, and Hideharu Saeki

First Research Division, National Institute of Animal Health, Kanonnaide, Tsukuba, Ibaraki 305, Japan; Federal University of Bahia, Ondina, Bahia 40.210, Brazil; and Nippon Veterinary and Animal Science University, Kyonan-cho, Musashino, Tokyo 180, Japan

(Received 9 January 1995/Accepted 4 June 1995)

**Abstract.** Ten rats were divided into five groups, A-E, to determine the larval dose-effect of infection with *Strongyloides venezuelensis* (SVZ). The rat groups were exposed to SVZ infective larvae as follows: (A) 100, (B) 1,000, (C) 10,000, (D) 100,000 and (E) 1,000,000. The eight rats in Groups A-D survived the infection. Rats exposed to the higher doses of larvae had higher egg counts (EPG). The four rats in Groups C and D had EPG counts greater than 10,000 EPG during 3-4 days after infection. Maximum EPG values in Group C were 85,400 and 106,600; those in Group D were 134,000 and 346,000. The two rats in Group E showed severe itching and bleeding on their digital pads at the time of infection. They became listless thereafter and died with hemorrhagic pneumonia at 4 days after infection. The sudden death that has been demonstrated in calves infected with massive doses of *S. papillosus* was not observed in SVZ-infected rats. — Key words: experimental infection, fecal egg output, lethal dose, rat, *Strongyloides venezuelensis*.

---


Fatal strongyloidosis has often been reported for man infected with *Strongyloides stercoralis* [1] and in calves infected with *S. papillosus* (SPL). Strongyloidosis as a course of death is well known as a result of unusually massive infections of the larvae. Massive infection in human strongyloidosis is mainly caused by autoinfection in the intestine [1]. In calf strongyloidosis under natural condition in Japan, large number of animals showed "sudden death" as a result of massive infections with SPL larvae which developed in the sawdust litter of confinement pens [10]. A similar sudden death syndrome was demonstrated by experimental infections in calves [4, 8, 11, 12] and sheep [2].

On the other hand, *S. venezuelensis* (SVZ) is well known that the developmental process and the larval migration in rats are similar in those of SPL in calves. Therefore, SVZ was considered to be a useful model for fundamental studies of strongyloidosis in calves [6]. However, critical experiments on the larval dose-effect in animals infected with SVZ had not been reported. The major objection of the present work was to determine if massive infection of SVZ in rats might result in sudden death similar to that found in SPL infected calves.

**Materials and Methods**

*Animals and experimental groups:* Ten parasite-free male rats (SD strain), aged 6 weeks, weighing 140-156 g in body weight were divided into 5 groups (A to E, 2 rats per group). Rats of Groups A, B, C, D and E were infected once with 100, 1,000, 10,000, 100,000 and 1,000,000 SVZ larvae per animal, respectively.

The infected rats were maintained in individual cages in an animal house at constant temperature of 25-26°C. On the fecal tray of each cage, an absorbent moistened paper sheet was placed to prevent fecal pellets from drying up. The paper and feces were removed every morning.

*Infective larvae and exposure to rats:* The SVZ Kagoshima strain [6] maintained in our institute by 40 serial passage through rats was used in this experiment. The infective larvae were obtained by a fecal culture technique described previously [7]; a suspension of feces in tap water containing eggs of SVZ was sieved through a 100 mesh net for elimination of debris, and the suspension was filtered through an absorbent cotton layer in order to recover the eggs on the cotton layer. The cotton layer was sealed in a polyethylene tube and incubated at 25°C for 3-4 days.

The larvae were used within 8 days after culture. The larvae on the cotton layer were eluted from the cotton with tap water and numbers of larvae in 0.5 ml samples of suspension was counted at 8 times to determine a mean value. Planned doses of larvae for each rat of the 5 groups was taken from the larval suspension. Doses of larval suspension were then filtered with a clean absorbent cotton pad. Subsequently, the cotton pad with larval dose was then layered on the bottom of a 500 ml beaker. Each rat was then kept in the respective beaker for 5 hr at 25°C.

*Clinical and fecal examinations:* Clinical signs of rats were observed at the time of exposure, and in daily management of animals. Feces on the moist paper sheet in the fecal tray of each cage were collected every morning. A modified McMaster technique for EPG counts, using saturated NaCl solution, was carried out every day during 5-15 days after infection (DAI), and 3 times per week during 16-40 DAI.

*Postmortem examinations:* Eight surviving rats (Nos.10-17) of Groups A-D were necropsied at 40 DAI. The two rats of Group E which died, were necropsied on the day of
death. Gross lesions in major organs and SVZ worm counts in the small intestines were examined in all animals.

Recovery of SVZ migratory larvae was carried out for the two rats of Group E which died by a previously technique [10]. Tissue sample weighing 0.2–5.0 g collected from various body areas such as the lungs, myocardium, eye, mandibular muscle, diaphragm, liver, kidney, trunk muscle, both fore-limbs, both hind-limbs and tail were examined.

The LD$_{50}$ of SVZ in rats was estimated by the Reed-Muench fifty percent end-point determination [5].

RESULTS

Clinical observation: All rats showed itching from 5 min after exposure to larvae. The itching continued for about 15 min in 6 rats (Nos. 10, 11, 12, 13, 14, 15) of Groups A, B and C. The itching continued for approximately 1 hr in 2 rats (Nos. 16, 17) of Group D, and for approximately 5 hr in 2 rats (Nos. 18, 19) of Group E. Four rats (Nos. 16, 17, 18, 19) of Group D and E showed the significant edema showing a red color of the digital pads at the time of exposure. Two rats (Nos. 18, 19) showed bleeding of the pads for one day, and were anorexia from the time of exposure. These 2 rats also showed hemorrhage from the eye orbit (so called “red tears”) and from the nose at 3 DAI, and died at 4 DAI.

Fecal egg output: Eggs appeared in the feces at 6.6 (6–8) DAI. Rats (Nos. 10, 11) of Group A had relatively low egg counts. However, the duration of egg output was the longest among the groups. The maximum EPG was 2,200 at 14 DAI (No. 10) and 800 at 9 DAI (No. 11). Rats (Nos. 12, 13) of Group B had counts of more than 3,200 EPG during 7–21 DAI. The maximum EPG was 12,200 at 9 DAI and 18,400 at 18 DAI, respectively. Rats of Group C (Nos. 14, 15) and D (Nos. 16, 17) had relatively high egg counts (Table 1, Fig. 1). The two rats of Group C had counts greater than 32,000 EPG during 9–19 DAI, and their maximum EPG were 85,400 and 106,600 at 9 DAI, respectively. The two rats of Group D had counts greater than 32,000 EPG during 7–20 DAI, and their maximum EPG were 134,000 and 346,000 at 12 DAI, respectively. Rats (Nos. 18, 19) of Group E died before infection patency.

Postmortem findings and worm counts: The surviving

![Fig. 1 Changes of fecal EPG counts in rats of Groups A, B, C and D that infected with Strongyloides venezuelensis.](image)

Table 1. Experimental design of infection, fecal egg counts and necropsy of rats infected with Strongyloides venezuelensis

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Body weight</th>
<th>Infection</th>
<th>Fecal egg counts</th>
<th>Necropsy</th>
<th>SVZ worms in the intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of larvae per animal</td>
<td>Prepatent period DAI</td>
<td>Maximum value</td>
<td>DA1</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>142</td>
<td>100</td>
<td>7</td>
<td>2,200</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>145</td>
<td>100</td>
<td>8</td>
<td>800</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>154</td>
<td>1,000</td>
<td>6</td>
<td>12,200</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>156</td>
<td>1,000</td>
<td>7</td>
<td>18,400</td>
<td>18</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>148</td>
<td>10,000</td>
<td>6</td>
<td>85,400</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>150</td>
<td>10,000</td>
<td>6</td>
<td>106,600</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>16</td>
<td>140</td>
<td>100,000</td>
<td>6</td>
<td>134,000</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>142</td>
<td>100,000</td>
<td>7</td>
<td>346,000</td>
<td>12</td>
</tr>
<tr>
<td>E</td>
<td>18</td>
<td>146</td>
<td>1,000,000</td>
<td>Died</td>
<td>0</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>146</td>
<td>1,000,000</td>
<td>Died</td>
<td>0</td>
<td>.</td>
</tr>
</tbody>
</table>

Mean 146.9 6.6 11.5

DA1: Days after infection.

a) Infection dose per kg of body weight was equivalent to 714,000.
b) Infection dose per kg of body weight was equivalent to 704,000.
c) Infection dose per kg of body weight was equivalent to 6,849,000.
d) Died in moribund state with hemorrhage from nasal cavity and eye orbit.
The LD$_{90}$ per kg of body weight was estimated to be 2,244,000 (log 6.35).
rats of Groups A-D had no gross lesions, however, the two rats that died (Nos. 18, 19 in Group E) had serious hemorrhagic lesions in the lungs. In the migratory larval counts of the rats that died, many larvae were found in the lungs, hind-limbs, trunk muscles, fore-limbs, diaphragm and mandibular muscles (Table 2). In the small intestinal examination, a few SVZ worms were detected in most of the rats (Table 1).

DISCUSSION

In the present experiment was designed with a minimum size using only 2 rats in each groups, however, the major objection of this experiment might be achieved. The highest EPG were observed in the rats exposed to largest doses of larvae. The maximum SVZ-EPG (85,400–346,000 in rats Nos. 14, 15, 16) were very similar to maximum SPL-EPG value in calves that died suddenly [8, 10] and rabbits that died in a moribund and emaciated state [3, 7, 9]. However, rats in the present experiment did not show any clinical signs other than transient itching.

The two rats (Nos. 18, 19) exposed to 1,000,000 larvae died before the patent period, and showed severe hemorrhagic pneumonia induced by the migrating larvae. On the other hand, sudden death in calves infected with SPL occurred in the patent period [8]. In SPL-infected lambs [2], the worm developmental stage associated with pathogenicity and death was the parasitic females and not migrating larvae.

In the present experiment, 2 rats (Nos. 16, 17) infected with 100,000 SVZ larvae per animal survived. This dose was equivalent to 714,000 and 704,000 larvae per kg of body weight (kg/BW), respectively. Two rats (Nos. 18, 19) infected with 1,000,000 larvae died. This dose was equivalent to 6,849,000 larvae/kg/BW (Table 1). These data are not sufficient for obtaining a accurate LD₉₀, because too long proportional distance as 10 times (log 1.0) with only 2 rats in each group. However, the LD₉₀ of SVZ in rats was estimated to 2,244,000 (log 6.35) larvae/kg/BW.

On the other hand, in SPL infected rabbits, one died and two others survived among three rabbits (2.78–2.92 kg of BW) infected with 50,000 larvae [7, 9]. This dose was equivalent to approximately 17,000 (log 4.2) larvae/kg/BW. Moreover, the LD₉₀ in SPL infected rabbits was estimated to be more than 100,000 (log 5.0) larvae /kg/BW [3]. Rabbits infected with massive doses of SPL did not died suddenly, but only after protracted time of anorexia and emaciation. In SPL infected calves, 2 of 2 survived at a larvae dose of 1,000 larvae/kg/BW, 3 of 5 died at a dose of 3,200 larvae, and all calves died suddenly following a dose of 10,000 larvae, respectively [8]. Therefore the LD₉₀ of SPL in calves was 2,610 (log 3.42) larvae/kg/BW. In the experimental infected lambs, 3 animals died suddenly that infected with more than 3,200 (log 3.5) larvae/kg/BW [2].

The LD₉₀ (2,244,000) of SVZ in rats was clearly higher than the LD₉₀ (17,000) of SPL in rabbits and the LD₉₀ (2,610) of SPL in calves. The sudden death demonstrated in calves infected with SPL was not observed in rats infected with SVZ.

ACKNOWLEDGEMENT. We are grateful to Dr. James C. Williams of Louisiana State University for critical review of the manuscript.
REFERENCES


