Parasitic Females of *Strongyloides papillosus* as a Pathogenetic Stage for Sudden Cardiac Death in Infected Lambs

Yosio NAKAMURA*, Naotoshi TSUJI, Noriyuki Taira, and Hisashi HIROSE

National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba, Ibaraki 305, Japan

(Received 15 March 1994/Accepted 29 March 1994)

**Abstract.** The present study was aimed at elucidating the responsibility of parasitic females for sudden cardiac death following *Strongyloides papillosus* infection in calves. A preliminary experiment demonstrated a percutaneous infection with *S. papillosus* infective larvae to cause sudden cardiac death in lambs as in calves, indicating lambs could serve as a model to study fatal strongyloidiasis in calves. Parasitic females of *S. papillosus* were inoculated into the duodenum of lambs. Lambs given live worms developed continuous sinus tachycardia immediately after inoculation, and died of sudden cardiac arrest by ventricular fibrillation through a phase of the disease identical to the case of percutaneous larval infection. The lambs had high fecal egg counts at the time of death. Inoculation of homogenized worms did not produce fatal arrhythmias. These results demonstrate that live parasitic females of *S. papillosus* in the small intestine are responsible for cardiac dysfunctions regardless of the presence or absence of migratory larvae. —**Key words:** lamb, parasitic female, *Strongyloides papillosus*, strongyloidiasis, sudden cardiac death.

---


Infective larvae of *Strongyloides papillosus*, a parasitic nematode of ruminants and rabbits, invade a host by skin penetration and mature into parasitic females which produce eggs by parthenogenesis in the small intestine after migration [6].

It has been experimentally demonstrated that a heavy infection with *S. papillosus* causes sudden cardiac arrest by ventricular fibrillation which is preceded by continuous sinus tachycardia in calves [10, 11]. All cases of sudden death due to *S. papillosus* infection in calves occurred after the prepatent period of the parasitic when nearly all larvae had reached the small intestine and matured to lay eggs [10]. Parasitic females would thus appear to be the cause of sudden death. However, developmental stages of the parasitic responsible for fatal arrhythmias in infected animals, whether migratory larvae or parasitic females, have not yet been ascertained.

Fatal cases have been reported in lambs experimentally infected with *S. papillosus* [1, 4, 12]. Since none of the previous reports have described electrocardiogram (ECG) findings, pathogenic effects of *S. papillosus* infection on cardiac function in lambs remain unknown.

In the present study, pathogenicity of *S. papillosus* for lambs was investigated by percutaneous larval infection as a preliminary experiment. Following this, lambs were intraduodenally inoculated with live and homogenized *S. papillosus* parasitic females recovered from rabbit infections in order to determine whether or not parasitic females in the small intestine could cause cardiac dysfunction and death.

**Materials and Methods**

*Animals:* Nineteen helminth-free Suffolk lambs, from 4 to 9 months of age and weighing from 12 to 28 kg (mean 21 kg), were employed. Nine of them were restrained loosely by neck stanchions on a recording stand in an air-conditioned room for continuous recording of ECG and pneumogram measurements. They were accustomed to stanchions before the experiment. The other 10 lambs were maintained two each in small pens. All lambs were fed a diet of hay and concentrate twice a day. Water was provided *ad libitum*. Japanese-White rabbits weighing about 2 kg were used.

**Infective larvae and percutaneous infection:** Infective larvae of the Himeji strain of *S. papillosus* [10] were obtained by fecal culture after 51 to 57 serial passages in rabbits as previously described [9]. Thirteen lambs were percutaneously infected with 1,000 (2 lambs), 3,200 (3 including Lamb P-1), 10,000 (4 incl. Lamb P-2) and 32,000 (4 incl. Lamb P-3) infective larvae per kg of body weight as previously described [10].

**Parasitic females and intraduodenal inoculation:** Rabbits were percutaneously infected with 500,000 infective larvae each as previously described [9], fasted on Day 9 and killed on Day 10 after infection. The small intestine was removed, slit longitudinally and incubated on a 16 mesh sieve in sterile Hanks' solution (pH 7.4, Nissui Pharmaceutical Co., Tokyo, Japan) containing 200 units/ml penicillin and 1 mg/ml streptomycin for 3 hr at 37°C in a humidified 5% CO2 incubator. Parasitic females of *S. papillosus* which passed through the sieve were collected and washed with Hanks' solution by natural sedimentation and aspirating the supernatant. At the time of collection, more than 95% of the worms were alive and motile. For live worm inoculation, they were kept in fresh Hanks' solution at 37°C. For homogenized worm inoculation, they were disrupted in Hanks' solution containing 1 mM phenylmethylsulfonylfluoride (PMSF) with a glass homogenizer on ice. The resultant suspension (not filtered for sterilization) was added to 24 volumes of Hanks'
solution without PMSF, and kept at 37°C. Both live and homogenized worms were inoculated into the duodenum of lambs as described below within 1 hr of preparation.

Intraduodenal inoculation was performed as follows. Six lambs were fasted the day before and on the day of surgery. They were anesthetized with xylazine (0.2 mg/kg) and placed in left lateral recumbency. An incision 10 cm long and 4 cm away from the last rib was made on the right abdominal wall of the lambs under a local anesthesia with procaine (0.2 g/animal). Using a glass syringe with a 1.5 mm inside diameter needle, 2,500, 10,000 and 22,500 live worms per kg of body weight (6, 17 and 27×10⁴ worms/animal, respectively) in Hanks’ solution were gently inoculated into the duodenum 3 cm posterior to the pylorus of three lambs (Lambs I-2 to I-4). The inoculation volumes were 60 to 150 ml, and were easily injectable without clogging the needle with worms. Two lambs (Lambs I-5 and I-6) were inoculated with the worm homogenate (30×10⁴ worms/animal equivalent) in 60 ml of Hanks’ solution, and one lamb (Lamb I-1) served as a control was injected with 150 ml of Hanks’ solution only into the duodenum by the same method used for live worm inoculation. The surgery was completed by suturing the abdominal wall and administering intramuscularly tetracyclines (5 ml/animal). The lambs regained consciousness within 30 min of the surgery.

ECG and pneumogram measurements: Stainless steel wire electrodes for ECG recordings in an A-B lead were attached to nine lambs at appropriate skin sites 7 days before the experiment [7]. Continuous ECG and pneumogram recordings were carried out as previously described [11] on three out of the 13 lambs for percutaneous infection (Lambs P-1 to P-3) and all six of the lambs for intraduodenal inoculation from 3 days before the experiment. Heart and respiration rates were counted at least every hour when a lamb was at rest and the recording baseline was stable, excluding a period of 1 hr after the start of feeding. Their normal heart and respiration rates were 50-70/min and 12-23/min, respectively. A heart rate of more than 80/min was considered tachycardia [2]. Daily fecal weights and rectal temperatures were monitored for the nine lambs. Their normal temperature was 38.5-39.5°C.

Fecal egg counts: Fecal examination of rectal samples was carried out daily by a modified McMaster technique for determining eggs per gram of feces (EPG). At necropsy, fecal egg counts were also determined with samples of cecum contents.

Plasma endotoxin level: Jugular blood samples were collected into heparinized tubes weekly and also within a few minutes of death in the case of lambs which died. Plasma endotoxin level was determined using a colorimetric assay (Endotoxin Test D, Seikagaku Kogyo, Tokyo, Japan).

Necropsy: Lambs which died were necropsied within 3 hr of death, and surviving lambs were killed on Day 42 after percutaneous infection, or on Day 14 or 21 after intraduodenal inoculation by bleeding under an anesthesia with xylazine. The animals were weighed, and organs were examined for gross lesions. Worms in the small intestine were recovered and counted as previously described [13].

RESULTS

Percutaneous infection of infective larvae: The prepatent period was 9 to 11 days. The two lambs given the lowest larval dose showed no clinical abnormalities for 42 days after infection. Eleven of the lambs infected with 3,200 or more larvae/kg died on Days 11 to 20 after infection (Table 1). They had high EPG values but did not show diarrhea. Rectal temperature was 0.5 to 1.0°C higher than usual from Days 6-10 after infection until death, and anorexia and a decrease in daily fecal weight were observed the day before death in Lambs P-1, P-2 and P-3 (which died on Days 13, 14 and 11 after infection, respectively). Plasma endotoxin levels remained below 20 pg/ml (considered normal in the present assay) in all 13 of the lambs.

ECG findings observed in Lambs P-1 to P-3 are summarized in Table 2. In the three lambs, continuous sinus tachycardia of which maximum rate was 100-140/min started at Days 6 to 9 after infection, and finally terminated in loss of cardiac function by ventricular fibrillation (Fig. 1). Atrialventricular block (the second degree), ventricular premature beat (R on T) and/or paroxysmal ventricular tachycardia appeared after the development of sinus tachycardia. No significant changes were seen in respiration rate until the onset of ventricular fibrillation at which time accelerated respiration (45-74/

<table>
<thead>
<tr>
<th>Dose (×10⁴/kg)</th>
<th>n</th>
<th>Survival time (days)</th>
<th>Maximum EPG (×10³)</th>
<th>Worms recoveredb¹ (×10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>2</td>
<td>42⁴</td>
<td>11-20</td>
<td>0-1</td>
</tr>
<tr>
<td>3.2</td>
<td>3</td>
<td>13-20</td>
<td>49-96</td>
<td>3-16</td>
</tr>
<tr>
<td>10.0</td>
<td>4</td>
<td>13-18</td>
<td>43-127</td>
<td>27-92</td>
</tr>
<tr>
<td>50.0</td>
<td>4</td>
<td>11-15</td>
<td>47-88</td>
<td>44-159</td>
</tr>
</tbody>
</table>

a) From the small intestine at necropsy.
b) Killed.
Table 2. ECG findings in lambs infected or inoculated with *S. papillosus*

<table>
<thead>
<tr>
<th>Lamb No.</th>
<th>Starting time of abnormal findings</th>
<th>Final pattern</th>
<th>Survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sinus tachycardia</td>
<td>Other abnormalities</td>
<td></td>
</tr>
<tr>
<td>P-1</td>
<td>8 days pi-</td>
<td>VT (7 hr bd-)</td>
<td>VT→VF</td>
</tr>
<tr>
<td>P-2</td>
<td>9 days pi-</td>
<td>VPB and VT (6 hr bd-)</td>
<td>VPB→VF</td>
</tr>
<tr>
<td>P-3</td>
<td>6 days pi-</td>
<td>ST depression (9 hr bd-)</td>
<td>VPB→VF</td>
</tr>
<tr>
<td>I-1</td>
<td>9–33 min pi only</td>
<td>None</td>
<td>—</td>
</tr>
<tr>
<td>I-2</td>
<td>1 hr pi-</td>
<td>VT (5 days pi-)</td>
<td>VT→VF</td>
</tr>
<tr>
<td>I-3</td>
<td>6 min pi-</td>
<td>AVB (9 hr pi-), VPB (20 hr pi-)</td>
<td>VT→VF</td>
</tr>
<tr>
<td>I-4</td>
<td>5 min pi-</td>
<td>ST depression (18 hr bd-), VT (16 hr bd-), AVB (15 hr bd-)</td>
<td>VPB→VF</td>
</tr>
<tr>
<td>I-5</td>
<td>6–16 min pi only</td>
<td>None</td>
<td>—</td>
</tr>
<tr>
<td>I-6</td>
<td>8–25 min pi only</td>
<td>None</td>
<td>—</td>
</tr>
</tbody>
</table>

a) Lambs P-1 to P-3 were percutaneously infected with infective larvae (infection doses were 3,200, 10,000 and 32,000/kg of body weight, respectively). Lambs I-1, I-2 to I-4, and I-5 to I-6 were intraduodenally inoculated with Hanks' solution only (control), live parasitic females, and homogenized parasitic females, respectively (see Table 3 for inoculation doses).
b) pi: post infection or post inoculation, bd: before death, AVB: atrioventricular block, VF: ventricular fibrillation, VPB: ventricular premature beat, VT: ventricular tachycardia.
c) Killed.

![ECG changes](image)

Fig. 1. ECG changes in Lamb P-2 percutaneously infected with *S. papillosus* infective larvae which died on Day 14 after infection. (a) Normal rhythm before infection; (b) atrioventricular block in the presence of sinus tachycardia 4 hr before death; (c) ventricular premature beat and paroxysmal ventricular tachycardia 4 hr before death; (d) onset of ventricular fibrillation (the upper strip) and pneumogram changes (the lower strip) at the time of death.
min) appeared (Fig. 1d). Respiration completely ceased within 3 min after ventricular fibrillation began in the three lambs.

At necropsy, seven of the lambs which died lost 4 to 14% (mean 8%) of the initial weight, and others did not have body weight loss. Parasitic females were recovered from the small intestine and ranged in numbers from 3,000 to 159,000 (Table 1). The mucous membranes of the duodenum and jejunum were congested in five of the lambs which died. Petechial hemorrhages were sporadically observed in the lungs of 10 lambs (including one lamb which survived). A few petechial hemorrhages were found beneath the epicardium of four lambs which died. No gross lesions were observed in the other organs.

Intraluminal inoculation of parasitic females: Lamb I-1, the control injected with Hanks’ solution, and the two lambs (Lambs I-5 and I-6) with homogenized parasitic females inoculated into the duodenum, did not show any abnormalities for 21 and 14 days, respectively, except for a decrease in daily fecal weight on the day of surgery and transient sinus tachycardia (maximum 96–150/min) just after inoculation (Table 2). Plasma endotoxin levels remained below 19 pg/ml in the three lambs. At necropsy, the lambs did not have body weight loss. No gross lesions were observed.

The three lambs (Lambs I-2 to I-4) with live parasitic females inoculated into the duodenum died on Days 2 to 9 after inoculation, having high EPG values (Table 3). Clinical signs observed up to time of death were as follows: a rectal temperature increase up to 1.0°C in Lamb I-2 continuously from Day 2 until death and in Lamb I-3 transiently on Days 1 to 3 after inoculation; anorexia in Lamb I-4 on the day of death, and a decrease in daily fecal weight in Lambs I-2 and I-4 on the day of death (and in all three lambs on the day of surgery). No diarrhea was observed and plasma endotoxin levels remained below 17 pg/ml in the three lambs.

The sequences of ECG and respiratory changes seen in Lambs I-2 to I-4 were identical to those observed in the cases of percutaneous larval infection. The three lambs developed continuous sinus tachycardia (maximum 120–160/min) immediately after inoculation up to terminal ventricular fibrillation preceded by various arrhythmias (Table 2, Fig. 2).

At necropsy, no inflammatory changes were observed on or near the surgery site, and the three lambs did not have body weight loss. Parasitic females were recovered from the small intestine and ranged in numbers from 19,000 to 82,000 (Table 3). In Lambs I-2 and I-4, the mucous membranes of the duodenum and jejunum were congested and a few petechial hemorrhages were found beneath the epicardium. No gross lesions were observed in the other organs. Infective larvae, which were cultured from the eggs in the cecum contents of Lamb I-3, produced a patent infection in rabbits infected percutaneously.

**DISCUSSION**

The lambs percutaneously given high larval doses of the Himeji strain of *S. papillosus* died suddenly without significant premonitory symptoms and gross lesions. The relationship between larval infection doses and survival time of the lambs was similar to results of previous reports on infected calves and lambs [1, 10, 12]. Continuous ECG recordings on three of the lambs demonstrated a percu-

<table>
<thead>
<tr>
<th>Lamb No.</th>
<th>Body weight (kg)</th>
<th>Inoculation$^a$</th>
<th>Dose /kg</th>
<th>(×10³) /animal</th>
<th>Survival time (days)</th>
<th>Maximum EPG (×10⁶)</th>
<th>Worms recovered (×10⁷)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>23</td>
<td>control</td>
<td>0</td>
<td>0</td>
<td>21$^{(a)}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I-2</td>
<td>24</td>
<td>LPF</td>
<td>2.5</td>
<td>60</td>
<td>9</td>
<td>42</td>
<td>19</td>
</tr>
<tr>
<td>I-3</td>
<td>17</td>
<td>LPF</td>
<td>10.0</td>
<td>170</td>
<td>7</td>
<td>58$^{(a)}$</td>
<td>22</td>
</tr>
<tr>
<td>I-4</td>
<td>12</td>
<td>LPF</td>
<td>22.5</td>
<td>270</td>
<td>2</td>
<td>155$^{(a)}$</td>
<td>82</td>
</tr>
<tr>
<td>I-5</td>
<td>23</td>
<td>HPF</td>
<td>13.0</td>
<td>300</td>
<td>14$^{(a)}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I-6</td>
<td>17</td>
<td>HPF</td>
<td>17.6</td>
<td>300</td>
<td>14$^{(a)}$</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ control: Hanks’ solution only, LPF: live parasitic females, HPF: homogenized parasitic females.

$^b$ From the small intestine at necropsy.

$^c$ Killed.

$^d$ EPG value of cecum contents at necropsy.
taneous heavy infection with *S. papillosus* to cause sudden cardiac death also in lambs. The sequence of arrhythmias which occurred in the lambs was identical to our earlier findings in infected calves [11]. It consisted of continuous sinus tachycardia and various ventricular arrhythmias including terminal ventricular fibrillation. Without restoration to regular heart beats after ventricular fibrillation, the sudden onset of attack was directly linked to death in infected lambs as in infected calves. Lambs can serve as a model to study fatal strongyloidiasis in calves.

Autoinfection is impossible in *S. papillosus*, which has only eggs and no larvae in feces inside the intestines of hosts [8]. By intraduodenal inoculation of live parasitic females, three lambs in which no migratory larvae existed died of sudden cardiac death identical to the cases of percutaneous larval infection. It is strongly suggested that the death which occurred in the lambs had no relation to secondary microbial infections through worm inoculation, since two lambs intraduodenally inoculated with homogenized worms (not sterilized) could survive having no abnormalities except for transient sinus tachycardia. The transient sinus tachycardia just after inoculation of homogenized worms might be induced by a reflex against the volume of Hanks' solution injected into the duodenum, since it was observed even in the control Lamb I-1.

Additionally, in sheep, the lungs represent the primary target organ and respiratory arrest, often unrelated to circulatory arrest, occurs in acute endotoxemia [5]. The toxic or shock-like states cause severe interstitial pneumonitis which can lead to acute respiratory distress [3]. The sudden death could be certainly distinguished from endotoxic shock, by judging from normal respiration until cardiac arrest and the complete absence of lesions in the lungs of the lambs intraduodenally inoculated with live worms.

Thus, the present results demonstrate that parasitic females in the small intestine can cause sudden cardiac death regardless of the presence or absence of migratory larvae. Somatic components of parasitic females themselves could be excluded from causative substance(s) for the death, since inoculation of homogenized worms failed to produce cardiac arrest in lambs. The following are potential factors which could be responsible for cardiac dysfunctions in *S. papillosus* infection: (1) mechanical stimuli by parasitic females which induce intestinal reflexes having some effects on the cardiovascular system; (2) products of parasitic females which act directly or indirectly upon cardiodynamics including regulatory systems.

Sinus tachycardia began before worms started laying eggs in the cases of percutaneous larval infection in two calves (from Day 5 after infection) [11], and in Lamb P-3 (from Day 6 after infection) in the present study. Migratory larvae are first detected in the small intestine of infected lambs at 88 hr after infection, and thereafter the number increases [13]. Thus, migratory larvae in somatic tissues and/or immature worms in the small intestine still can be suspects of cardiac dysfunction in infected calves and lambs. However, research targets can be focused on parasitic females to elucidate the developmental mechanism of fatal strongyloidiasis in calves and lambs.

**ACKNOWLEDGEMENTS.** We are grateful to Dr. J. C. Williams of Louisiana State University for critical review of the manuscript. This study was supported by Grant-in-Aid of Preceded Research Program (PRP 92-7-1) provided by the Ministry of Agriculture, Forestry and Fisheries, Japan.

**REFERENCES**


