Transmammary Transmission of *Strongyloides venezuelensis* (Nematoda) in Rats

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**ABSTRACT:** Sprague-Dawley-derived dams were inoculated subcutaneously with 4,000 infective filariform larvae suspended in water. The inoculations were given once from 10 hours to 9 days postpartum. Nurslings were killed at intervals after the inoculations and their tissues were squashed between glass slides for microscopic examination. Larvae were first found in the nurslings 3 days after the inoculation. There was no extraintestinal migration in these animals but suckling rats inoculated orally had the usual heart-lung phase of the life cycle. A few larvae were recovered from mammary tissue of nursing dams. About 50 times as many worms were recovered from the nurslings as from their dams.

Several species of parasitic nematodes can be transmitted by the transmammary route (Stone and Smith, 1973). Among the members of the genus *Strongyloides*, infection of the newborn by this means has been demonstrated for *S. ransomi* in pigs (Moncol and Batte, 1966; Stewart et al., 1969, 1976), *S. westeri* in equines (Lyons et al., 1973, 1977) and *S. ratti* in rats (Katz, 1969; Zamirdin and Wilson, 1974; Wilson et al., 1976a, b, 1978a). Larvae of *S. papillosus* have been recovered from the milk of goats and sheep (Moncol and Grice, 1974), sheep and cattle (Lyons et al., 1970), and buffaloes (Chauhan et al., 1974), and those of *S. fuelleborni* from human milk (Brown and Girardeau, 1977).

The objective of this study was to investigate the possibility of transmammary transmission of *Strongyloides venezuelensis* and, if it does occur, to elucidate both the migratory pathway and the biological significance.

**Materials and Methods**

The strain of *S. venezuelensis* used in this study was isolated from a rat trapped in Tel Aviv by Dr. Guta Wertheim of the Hebrew University, Jerusalem, Israel, and has been maintained serially by the author since 1964.

Charcoal-fecal cultures were made from infected stock animals and served as the source of the filariform larvae for the experimental work. Cultures were baermannized and the larvae in 0.075 ml (Stoll pipet) samples of the suspension were counted. The volumes of the larvae in water were adjusted to obtain the desired inoculum size.

Using a 1-ml tuberculin syringe with a 25 gauge needle, mother rats were inoculated subcutaneously in the interscapular region with 4,000 filariform larvae in 0.05 ml. After the injection, the rats were kept separate from their litters for 30 min to allow any worms which might have seeped from the injection site time to penetrate the skin. Cages were cleaned daily or every other day to prevent contamination of the young. Water and Purina Rodent Laboratory Chow® were provided ad libitum to all animals.

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Table 1. *Strongyloides venezuelensis* recovered from nurslings transferred from infected dams to uninfected dams at various times (hours) after inoculation.

<table>
<thead>
<tr>
<th>Time of transfer</th>
<th>Neg.</th>
<th>Pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–75</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>76</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>77</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>81–120</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Not transferred, remained with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>infected dams</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>uninfected dams</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

In experiments 1 through 4, the rats were Charles River CD strain while in experiment 5, the rats were CAMM SD/BR strain. The matings and litters were the first for these animals.

Nurslings transferred from one mother rat to another were marked by cutting off one or more toes.

**Results**

**Experiment 1: Time of onset of transmammary transmission**

One dam of each of three pairs of time-matched dams was inoculated 24 hr after parturition. Before returning the infected dam to her litter, one baby was transferred to an uninfected dam. After restoration of the infected dam to her litter, another baby was removed and transferred periodically up to 120 hr. At each transfer, one of the recipient's babies was removed to maintain litter size. Seven days after the inoculation of the dams, the baby rats were killed and their duodenums squashed between microscope slides and examined for worms.

No worms were found in any of the 17 nurslings killed at 17 different times up to 75 hr postinoculation whereas those killed at eight different times from 81 to 120 hr were all infected (Table 1). The three nurslings which had remained continuously with the infected dams and the six which had remained with the uninfected mothers served as controls.

**Experiment 2: Duration of transmammary transmission**

The offspring of an uninfected dam were transferred at intervals to an inoculated one. Eight days postinoculation, the transferred animals and the controls were examined.

Newborn rats were found infected at five intervals prior to but not at four intervals beginning 117.5 hr postinoculation of their foster mother (Table 2).

**Experiment 3: Route of migration in the offspring**

Litters were used which ranged in age from 10 hr to 9 days at the time of the inoculation of their dams. Suckling rats were killed at various times from 14 to 223 hr after the dam had been inoculated and their stomachs, duodenums, and lungs and, on occasion, liver, kidneys, spleen, and blood were examined by squashes on slides. These organs as well as mammary glands of dams were also minced and placed in a modified Baermann apparatus utilizing a pilsner glass. Some of the worms recovered were measured with an ocular micrometer after
Table 2. *Strongyloides venezuelensis* recovered from nurslings transferred from an uninoculated to an inoculated dam at various times (hours) after inoculation and autopsied 8 days postinoculation of the foster mother.

<table>
<thead>
<tr>
<th>Time of transfer</th>
<th>Neg.</th>
<th>Pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>24, 46, 72, 93, 103</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>117.5, 118.5, 142, 167</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Not transferred, remained with infected dam</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>uninfected dam</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

being stained with iodine solution. Several larvae from mammary tissue were inoculated subcutaneously into an adult female rat and one larva was inoculated into a nursling. These animals were later examined by charcoal culture of feces and direct examination of feces and tissues.

None of the suckling rats examined at 12 intervals prior to 75 hr postinoculation of the dams had worms but after that 28 of 45 rats examined at 22 intervals had worms (Table 3). The larvae in the milk were swallowed, passed through the stomach and took up residence in the duodenum. Worms were not found in the lungs, kidneys, spleen, blood, or liver. Worms found in the stomach were always larvae. In the duodenum up to 94 hr postinoculation only larvae were observed; from 94 to 117 hr there were larvae and nonovigerous adults, 117 to 162 hr nonovigerous adults, and from 200 to 223 hr ovigerous adults.

Larvae recovered from the stomach and from mammary glands appeared basically third-stage-like. However, measurements indicated some growth had taken place; filariform larvae from cultures had a mean length of $568 \pm 3.4 \mu m$ while those from mammary tissues and the stomach had mean lengths of $607 \pm 7.1$ and $603 \pm 17.6 \mu m$, respectively. Adults from the duodenum measured on the average $3.00 \pm 0.075$ mm.

Larvae were recovered from the mammary glands of two rats 4 days after inoculation but none were found in two others on the 7th day. These larvae failed to produce patent infections in either an adult or suckling rat.

**Experiment 4: Need for extraintestinal migration**

Using a feeding needle, an inoculum of 125 coproculture filariform larvae in 0.05 ml was placed in the pharyngeal region of each of 10 members of a suckling litter. The baby rats were kept separate from the mother for 10 min. At 15 min postinoculation and at various intervals thereafter, nurslings were killed and their lungs, stomachs, and duodenums examined by tissue squashes.

Table 3. *Strongyloides venezuelensis* recovered from nurslings at various times (hours) after inoculation of their mothers.

<table>
<thead>
<tr>
<th>Time</th>
<th>No. examined</th>
<th>No. with worms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stomach only</td>
</tr>
<tr>
<td>14–74</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>76–109</td>
<td>35</td>
<td>1</td>
</tr>
<tr>
<td>117–223</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

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Table 4. *Strongyloides venezuelensis* recovered from nurslings at various times (hours) after their oral inoculation with larvae from cultures.

<table>
<thead>
<tr>
<th>Time</th>
<th>Lungs</th>
<th>Stomach</th>
<th>Duodenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3.5–68.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>75.5</td>
<td>Larvae</td>
<td>—</td>
<td>Nonovigerous adults</td>
</tr>
<tr>
<td>92.0</td>
<td>—</td>
<td>—</td>
<td>Nonovigerous adults</td>
</tr>
</tbody>
</table>

Worms were found in the lungs and the duodenum 75.5 hr postinoculation but not at seven intervals prior to that (Table 4).

**Experiment 5: Importance of the transmammary route**

Two virgin females and four dams 3 to 6 days postpartum were inoculated with filariform larvae. Seven days postinoculation the adults and the nurslings were killed and the total number of worms in their duodenums was determined by direct count in tissue squashes.

Of the initial inoculum, 43.2% ended up in the litters while only 0.87% of the worms were found in the dams. The duodenums of the control rats yielded 3.16% of the inoculum. The male and female babies harbored the same numbers of worms.

**Discussion and Conclusions**

The experiments reported here show that transmammary transmission of *Strongyloides venezuelensis* can take place when the lactating dam is inoculated with filariform larvae 10 to 216 hr postpartum. The passage to the nurslings starts approximately 72 hr postinoculation and lasts only 24 to 48 hr.

Various factors may influence transmammary transmission (Wilson et al., 1978a, b). The fact that transmammary transmission starts later in *S. venezuelensis* than in *S. ratti* (Katz, 1969; Zamirdin and Wilson, 1974) is consistent with biological differences of these two species (Petriello and Katz, 1966; Wertheim, 1970). The patent infections from the oral inoculations of filariform larvae probably resulted from larvae penetrating the buccal mucosa. Because larvae were found in the stomach 15 min after an oral inoculation but did not take up residence in the duodenum until 75 hr, it appears that the larvae which were swallowed before making a tissue migration could not establish themselves in the duodenum. This is not surprising since the literature (Michel, 1974) on prenatal and transmammary transmission indicates that while *Strongyloides* species in general require a tissue migration, larvae which have had a sojourn in tissue, as would those passed in milk, need not have another extraintestinal migration.

Under the conditions used in experiment 5, the transmammary route seems to be favored over the normal migratory pathway in the lactating female rat. Wilson et al. (1976a) reported for *S. ratti* that 24.4% of the initial inoculum ended up in the litter while only 2% was present in the dam. It appears that the controls in experiment 5 had a worm burden lower than what one might expect. However, the yield from the controls was still greater than that of the dams.

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Wilson (1977) has proposed for *S. ratti* that a switch in the larvae’s migration to the mammary gland, instead of the intestine, occurs in the lungs. This switch would take place at the time the larvae first enter the lungs; instead of going into the alveoli, the larvae would remain in the blood and eventually be carried to the mammary gland. Because *S. venezuelensis* larvae in the mammary gland are about the same size as those in the lungs, it would seem that Wilson’s theory may also hold for *S. venezuelensis*.

**Literature Cited**


