Research Article

Development of Some Larval Nematodes in Experimental and Natural Animal Hosts: An Insight into Development of Pathological Lesions vis-a-vis Host-Parasite Interactions

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Infective third-stage larvae of three spiruroid nematodes, Ascarops strongylina and Physcocephalus sexalatus of pigs and Spirocerca lupi of dogs, were recovered from 14 species of coprophagous beetles belonging to 4 different genera. These larvae were fed to rabbits and/or guinea pigs to study their development in these experimental hosts. Larvae of A. strongylina reached the adult stage in all rabbits and one guinea pig. The adult worms recovered in these hosts were 40% and 4%, respectively, and became diminutive in comparison to their natural hosts. The larvae of P. sexalatus became reencysted in the gastric wall of rabbits inducing marked pathological changes. The infective larvae of S. lupi became reencapsulated in the stomach wall of the rabbit and also showed development in the aortic wall. Adults of Toxocara canis of dog, collected from 5 different regions of the Indian subcontinent, varied significantly in size. The mouse passage of infective larvae of one of these types led to the recovery of the adults from the experimental dogs that were smaller in size and caused severe pathology in natural experimental hosts. Developmental effects shown in experimental hosts and host specificity are of value in understanding the evolution of nematode parasitism.

1. Introduction

Host-parasite interactions by parasitism remained unexplained till 20th century. Graham Bell and Austin Bert from Canada hypothesized the term, “a recombination, favoured by antagonistic” coevolution between the host and the parasite [1]. It was further defined as the resultant product of ecological, sociological, and physiological causes [2]. The phenomenon not only increased the parasite’s reproductive capacity but also enhanced its virulence and/or pathogenicity. Host specificity, on the other hand, is the result of accommodation between the two, that is, the host and the parasite. The nature of the parasite, ambient conditions, and the host infected are the key factors determining the outcome of such a relationship in terms of virulence, pathogenicity, reproductive potential, maturity of the parasite, and finally the response of the host. In this communication, experimental results on development of some larval nematodes in natural and experimental animal hosts have been discussed in connection to host-parasite relationship.

2. Materials and Methods

The infective third-stage larvae (L₃) of the spiruroid nematodes (Table 1) were obtained from 14 species of naturally-infected coprophagous beetles belonging to 4 different genera. The larvae were studied under coverslip preparation and identified as described by Alicata [3], Porter [4], Watanabe [5], and Ryzhikov and Nazarova [6]. In all experiments, the infective larvae were administered with the help of a stomach tube to the experimental and natural hosts, namely, rabbit,
<table>
<thead>
<tr>
<th>Experimental animal</th>
<th>Infective dose ($L_n$)</th>
<th>Mode of oral infection</th>
<th>Necropsy (DPI)</th>
<th>Results</th>
<th>Recovery percentage</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. strongylii</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R$_1$</td>
<td>75</td>
<td>Single dose</td>
<td>22</td>
<td>M = 5 (+YA 3) F = 17 (+YA 2)</td>
<td>36.0</td>
<td>Several worms (M or F) were under the 4th ecdisys</td>
</tr>
<tr>
<td>R$_2$</td>
<td>121</td>
<td>Single dose</td>
<td>48</td>
<td>M = 24 (+YA 2) F = 24</td>
<td>41.5</td>
<td></td>
</tr>
<tr>
<td>R$_3$</td>
<td>100</td>
<td>2 successive days (30 + 70)</td>
<td>39</td>
<td>M = 16 (+YA 1) F = 19 (+YA 4)</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>R$_4$</td>
<td>128</td>
<td>3 different days (Mx. = 55; Mi. = 21)</td>
<td>31</td>
<td>M = 17 (+YA 8) F = 28 (+YA 5)</td>
<td>45.3</td>
<td></td>
</tr>
<tr>
<td>GP$_1$</td>
<td>130</td>
<td>Single dose</td>
<td>108</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GP$_2$</td>
<td>50</td>
<td>3 different days (Mx. = 28; Mi. = 8)</td>
<td>41</td>
<td>M = 2 F = Nil</td>
<td>4.0</td>
<td>No lesion noted in the mucosa</td>
</tr>
<tr>
<td><strong>P. sexalatus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R$_1$</td>
<td>279</td>
<td>9 different days (Mx. = 80; Mi. = 6)</td>
<td>45</td>
<td>Two $L_3$ recovered from multiple nodules on the gastric wall</td>
<td>—</td>
<td>Many nodular lesions showed cut sections of larvae. Several encapsulated larvae detected in the cut sections of gastric wall.</td>
</tr>
<tr>
<td>R$_2$</td>
<td>87</td>
<td>10 different days (Mx. = 2; Mi. = 3)</td>
<td>48</td>
<td>One $L_1$ recovered from the omentum close to ventral lobe of liver</td>
<td>—</td>
<td>Several encapsulated larvae found in the cut sections of stomach wall.</td>
</tr>
<tr>
<td>R$_3$</td>
<td>223</td>
<td>11 different days (Mx. = 72; Mi. = 50)</td>
<td>53</td>
<td>No larvae recovered</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><strong>S. lupi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R$_1$</td>
<td>123</td>
<td>12 different days (Mx. = 52; Mi. = 1)</td>
<td>59</td>
<td>—</td>
<td>—</td>
<td>Encystment on the gastric wall; 4 nodular lesions on the intima 4–8 mm from the bulbous aorta. Internal haemorrhage detected on postmortem suspected either from rupture of minute vessels or wall of aorta.</td>
</tr>
<tr>
<td>R$_2$</td>
<td>90</td>
<td>4 different days (Mx. = 54; Mi. = 7)</td>
<td>37 (died on the previous night)</td>
<td>14 YA recovered from the intima; may cut in the lesioned patches in stomach wall ($L_n$) or aorta ($L_n$)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

* R: rabbit; GP: guinea pig; DPI: days post infection; M: male, F: female, A: adult; YA: young adult; $L_3$/$L_4$: third- or fourth-stage larvae, Mx: maximum; Mi: minimum; —: not counted/no recovery.
Table 2: *Toxocara canis:* host related effect between large, medium, and small types.

<table>
<thead>
<tr>
<th>Type</th>
<th>Length</th>
<th>Diameter</th>
<th>Volume/area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large (n = 2)</td>
<td>17.45 ± 0.65**</td>
<td>2.2 ± 0.00**</td>
<td>38.39 ± 1.43**</td>
</tr>
<tr>
<td>Medium (n = 4)</td>
<td>11.30 ± 0.49***</td>
<td>1.85 ± 0.12***</td>
<td>20.93 ± 1.73***</td>
</tr>
<tr>
<td>Small (n = 8)</td>
<td>7.01 ± 0.77***</td>
<td>1.10 ± 0.07***</td>
<td>7.80 ± 1.06***</td>
</tr>
<tr>
<td>Overall (n = 14)</td>
<td>9.73 ± 1.11</td>
<td>1.10 ± 0.07</td>
<td>15.92 ± 3.09</td>
</tr>
</tbody>
</table>

All specimens in Tables 2 and 3 are gravid females; length expressed in cms and diameter in mm. Means within a column with different superscript differing significantly (**P < 0.01**).

Table 3: *Toxocara canis*: Host related effect between local (Ludhiana) versus passage (Assam type).

<table>
<thead>
<tr>
<th>Type</th>
<th>Length</th>
<th>Diameter</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ludhiana n = 2</td>
<td>7.65 ± 0.25</td>
<td>1.50 ± 0.00</td>
<td>11.47 ± 0.38</td>
</tr>
<tr>
<td>Passage n = 2</td>
<td>6.70 ± 0.40</td>
<td>0.90 ± 0.10</td>
<td>6.07 ± 1.03</td>
</tr>
<tr>
<td>F value</td>
<td>4.056</td>
<td>36.00*</td>
<td>24.31*</td>
</tr>
<tr>
<td>Overall n = 4</td>
<td>7.12 ± 0.34</td>
<td>1.20 ± 0.18</td>
<td>8.77 ± 1.62</td>
</tr>
</tbody>
</table>

Significance level P < 0.05.

Table 4: Development of *Toxocara canis* in pups/dogs (mouse passage of L_2).

<table>
<thead>
<tr>
<th>Age in months and sex</th>
<th>Necropsy (DPI)</th>
<th>Recovery of adults</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>3 female</td>
<td>58</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>2.5 female</td>
<td>58</td>
<td>03</td>
<td>07</td>
</tr>
<tr>
<td>3 female</td>
<td>57</td>
<td>46</td>
<td>42</td>
</tr>
<tr>
<td>2.5 female</td>
<td>89</td>
<td>02</td>
<td>10</td>
</tr>
<tr>
<td>2 male</td>
<td>26 (died)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3 male</td>
<td>45</td>
<td>37</td>
<td>47</td>
</tr>
<tr>
<td>3 male</td>
<td>58</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2.5 female</td>
<td>47</td>
<td>07</td>
<td>09</td>
</tr>
</tbody>
</table>

Mean 17.7

guinea pig, mice, or pups. During this study, unidentified larvae recovered from the beetle hosts were fed to 2 rabbits.

The differently-developed juveniles/larvae collected from viscera of the animals were studied alive and from the preserved specimens after appropriate clearing. The tissues showing conspicuous gross lesions were fixed in 10% formalin and serially-cut into 5-6 μm thick paraffin sections and stained with haematoxylin and eosin for histopathological evaluation.

The female specimens of *Toxocara canis* collected either in physiological saline or 10% formalin (Tables 2 and 3) were obtained either locally from pups/dogs or were received by us from 4 different cities in Assam, Gujrat, M.P., and U.P. located at a distance of 800–2,500 kms from Ludhiana, Punjab, India.

For mouse passage, 12 albino mice were administered 100 second stage larvae (Table 4) at fortnightly interval, for a period of 1.5 months. The deskinned mice cut into pieces, were fed to 8 pups/dogs (one mouse each) no earlier than two months postinfection. The animals were maintained as per Institutional Animal Ethics Committee Guidelines. The data were analysed using SPSS 16.0 version by one-way analysis of variance and the means were compared by Tukey’s b and Duncan’s Multiple Range Test.

3. Results

The distinctive development patterns of the three spiruroid nematodes including two stomach worms of pig, that is, *Ascarops strongylina* and *Physocyclus sexalatus* and the third, the oesophageal worm of dog, that is, *Spirocerca lupi* are depicted in Table 1. The experimental infection of *A. strongylina* in rabbit and guinea pigs showed a marked difference in development and recovery of the parasite. Microscopically, the pathological lesions in *A. strongylina* consisted of damage by the adults and juveniles to the lining.
of mucosa and destruction of the underlying glands by the young adults that reached nearly the base of the mucosa (Figures 1 and 2).

In contrast to *A. strongylina*, *P. sexalatus* showed poor development and recovery in infected rabbits. In one rabbit, a third-stage larva was found that had migrated to the omentum (Table 1), whereas in two other animals, *L₃* remained arrested mostly in the gastric wall and encapsulated in different layers without undergoing further development. The encapsulated juveniles were found in the mucosal, submucosal, and muscular coats (Figures 3 and 5) of the gastric wall. The nodular lesions revealed the parasites surrounded by necrotic debris, neutrophils, and activated macrophages. At the periphery of such lesions, the inflammatory reaction consisted of lymphocytes, macrophages, and eosinophils, while in the areas adjacent to the mucosa, there were aggregates of macrophages and lymphocytes (Figure 4).

The unidentified larvae did not develop in any of the rabbits, rather they became reencapsulated in the submucosa (Figure 6). Adjacent to the sections of this parasite, chronic inflammatory response consisted of necrosis and dystrophic calcification, surrounded by extensive fibroplasia and eosinophils.

In two experimental rabbits, the *L₃* of *S. lupi* were found, not only reencapsulated in the gastric wall, but some of the infective larvae were seen to have migrated to the aorta causing typical lesion of "aortic spirocercosis." The recovered larvae from the gastric wall of the rabbits measured: 2.18 mm
Figure 6: Sections of stomach showing an old parasitic granuloma of an unidentified larva and chronic inflammatory changes along with necrosis, fibrosis, and dystrophic calcification surrounded by a distinct fibrous encapsulation (H. E. original magnification × 100X).

Figure 7: A section of stomach rabbit showing chronic granuloma around *S. lupi* in the submucosa with extensive fibrosis around the parasite and a peripheral zone of chronic inflammatory cells (H. E. original magnification × 100X).

× 0.093 mm–2.52 mm × 0.097, which falls in the range of *L.3* recovered from the dung-beetles (2.19 mm × 0.085–2.48 mm × 0.12 mm). Interestingly, the young adults recovered from the aorta measured: 2.58 mm × 0.17–5.29 mm × 0.21 mm.

The histopathology of the lesions from the stomach wall revealed oedema of the gastric mucosa and a zone of necrosis, surrounded by extensive inflammation, consisting predominantly of macrophages, lymphocytes, and some eosinophils. Early fibroblastic proliferation was also evident. Around the parasite in the submucosa, activated macrophages were found surrounded by lymphocytes, eosinophils, and mature collagen fibres (Figure 7).

In the aorta, the three layers depicted varying grades of changes. The intima was greatly thickened owing to proliferation of fibro-cellular tissue (Figure 9). The endothelial lining was disrupted and internal elastic lamina was fragmented (Figure 8). In the parasitic tunnels, a hyalinised necrosed thin layer was surrounded by connective tissue proliferation and inflammatory response, predominantly consisting of lymphocytes and eosinophils, a few plasma cells, and macrophages. In the media, there was a marked infiltration of eosinophils, many plasma cells, some lymphocytes, and a few macrophages (Figure 10). In the outer media, serofibrinous exudation was observed, in addition to necrotic foci having

Figure 8: Section of aorta of a rabbit with a longitudinally cut section of *S. lupi* in media. Note the extensive tissue damage, necrotic debris in the parasitic tunnels/cavities, fragmented tunica elastica interna, and marked infiltration of inflammatory cells. Arrow indicates intima (H. E. original magnification × 100X).

Figure 9: A section of chronically-infected aorta with *S. lupi* in the intimal region showing parasite remnants in dystrophic calcification surrounded by fibroblastic reaction within tunica intima (H. E. original magnification × 400X).

Figure 10: A section of aorta with longitudinally cut section of *S. lupi* in media. Note the extensive tissue damage, necrotic debris in the parasitic tunnels/cavities, fragmented tunica elastica interna, and marked infiltration of inflammatory cells. Arrow indicates intima (H. E. original magnification × 100X).
dystrophic calcification particularly inside the tracts left by the juveniles (Figure 9). In the tunica adventitia, the changes were similar but relatively milder.

The adult gravid females of *T. canis* collected from 5 different regions of the Indian subcontinent were significantly different in length, diameter, and area (Tables 2 and 3). The experimental pups/dogs, which were fed L₂ of *T. canis* via mouse passage (Table 4), exhibited a series of clinical signs, including diarrhea and debility (Figure 11).

On postmortem examination (26–89 DPI), the intestine revealed congestion, oedema, and consolidation in lungs. Microscopically, the liver showed portal hepatitis and vacuolar degeneration of hepatocytes, while the lung depicted interstitial pneumonia (Figure 12). The intestine revealed necrotic enteritis with marked infiltration of lymphocytes and plasma cells in the lamina propria leading to thickening and blunting of villi. There was exfoliation of the lining epithelium of the tips of villi (Figure 13). The crypts of Liberkhuhn showed goblet cells hyperplasia.

### 4. Discussion

Watanabe [5] was the first researcher who succeeded in infecting three rabbits with third-stage larvae of *A. strongylina* and the recovery percentages were 35%, 23.3%, and 20%, respectively. The adult worms were recovered between 40 and 112 DPI. In the present experiments in 5 rabbits, the recovery was from 36.0 to 45.3 percent, which was much higher in comparison to the earlier study [5]. The recovery of adults from one guinea pig in this study, however, was very low (4%) and the other guinea pig was negative (Table 1). The prepatent period of *A. strongylina* was found to be 41 days in rabbits. The adult worms recovered from the animals were diminutive in size than the natural host, the pig. From this experiment, it appeared that the rabbit was a good experimental model for *A. strongylina* infection. Previously, mature adults of *A. strongylina* were collected from wild rodents as second record as a definitive host of this nematode [7].

During experiment with *P. sexalatus* in three rabbits, the L₃ of this parasite did not develop to adulthood but became reencapsuled in the gastric wall inducing severe pathological changes, though both parasites, that is, *A. strongylina* and *P. sexalatus* are known to reside in the stomach of the same host.
natural host, that is, the pig. The recovery of an L₃ from
the omentum could simply be due to erratic migration or
due to the effect of biological “incompatibility” between the
parasite and its host to avoid host's immune response or due
to a more primitive lifestyle it had pursued before it had
coaccommodated in its natural host, the pig. Undoubtedly,
physiology/ecology and other factors in the stomach of rabbit
and pig are unlikely to be similar.

The experiments with the L₃ of *S. lupi* revealed that in
both rabbits, the larvae not only reenccysts in the gastric
wall, but also followed the migratory route to the aorta as
in natural host that is, dog. In a recent study, ten beagle
dogs were experimentally challenged with 40 infectious *S.
lupi* larvae orally [8]. These dogs died due to rupture of an
aortic aneurism. Seven dogs became infected and presented
with esophageal nodules and worm eggs in their feces. One
dog did not become infected [8].

The pathological changes induced by the larvae of *P.
sexalatus* were rather chronic in nature in the gastric wall than
those in the aorta. In the aortic wall the change was more
acute, particularly in the tunica media. The development of
*S. lupi* both in the gastric wall and its migration to the
aortic wall are, therefore, interesting for both “parasitism” and
“specificity” of the parasite-host system.

In Russia, ruminants, particularly sheep and cattle may
act as normal definitive hosts of the two species, *A. strogylina*
and *P. sexalatus*, whereas, in other parts of the world, these
two species are found only in pigs [9]. Phylogenetically rabbit
is a herbivore in contrast to guinea pig, which is akin to
carnivorous host. Differential development of the two species
of these helminth parasites in different regions of the world
demonstrates that not only genetic characterization and the
variability in their evolution affect their development, but also
climatic and ecological factors of the region are responsible
for the host specificity. The same holds largely true for *S. lupi*,
which is the typical parasite of canines, namely, dog, fox, and
jackal, although in the present experimental study, it showed
partial development in a herbivore like rabbit.

We presume that significant differences in length, diam-
eter, and area of adult gravid females of *T. canis* collected
from 5 different regions of the Indian subcontinent might
be related to the production of more eggs in *T. canis*. Sonin and Rykovoskii [9] also reported that the longer
worms, Ascaris lumbricoides, produced more eggs. However,
passage through mice of infective eggs from medium-sized
worms (Assam type) and the eventually recovered worms
from pups/dogs were found to be reduced in length, which
were comparable to small form of local type (Table 3). The
altered pathological response in experimental host may
due to abnormal localization and/or poor adaptation to the
tissue microenvironment of experimental/new hosts [13].

5. Conclusion

The present study employing several species of nematodes
in natural and experimental hosts demonstrated significant
variations in the development of the parasite, pathologi-
ical responses, and host-parasite specificity, which may be
of value in understanding the ecology and evolution of
parasitism, particularly those having zoonotic importance,
namely, *T. canis* and *S. lupi*. The study also throws light on
the development of pathological lesions vis-a-vis host-parasite
interactions.

Conflict of Interests

The authors declare that there is no conflict of interests
regarding the publication of this paper.

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