SIGNS, LARVAL BURDENS, AND SEROLOGICAL RESPONSES OF DOGS EXPERIMENTALLY INFECTED WITH TRICHINELLA SPIRALIS OWEN, 1835

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Abstract. The effects of infections of Trichinella spiralis on 10 specific-pathogen-free Beagles were examined. Eight puppies received either 100, 500, 1,000, or 5,000 larvae, and 2 adult dogs received 1,000 larvae. Blood was drawn every 4 days, beginning 5 days before infection, for the determination of relative eosinophil numbers. Creatine kinase levels were monitored before infection, two weeks after infection, and one month after infection. The dogs were euthanized 1 month postinfection, and larvae were counted in muscle digests of 10 gram samples of diaphragm, pectoralis superficialis, masseter, biceps brachii, and vastus lateralis. The dogs displayed minor signs of gastrointestinal upset during the first week after infection. The dogs also developed a slight eosinophilia with a magnitude that was dependent on the number of larvae the dog received. All infected dogs, but one that had received 500 larvae, had a positive reaction with larval excretory-secretory products of T. spiralis; adult dogs had the greatest immunologic response. The creatine kinase levels were found not to be related to either the time postinfection or the magnitude of the larval dose. The number of larvae recovered from the muscles (maximum of 70 per gram) was dependent on the dosage of larvae received, but there was no significant predilection of the larvae for any of the five examined muscle groups.

Matoff (1935, 1936, 1937) published a series of papers on the infection of dogs with T. spiralis. The work by Matoff was performed, in part, because at this time other workers considered dogs to be difficult to infect with Trichinella (Seifert 1929). However, since the time of its publication, the work of Matoff has not been widely cited. Thus, although Matoff showed that as few as three muscle larvae were capable of producing infections in puppies, as recently as 1970, Beck stated that “The dog, in fact, is rather resistant to infection.”

The studies of Matoff dealt almost exclusively with whether or not dogs could be infected. There was no description of clinical signs in the infected animals, Matoff only reported whether or not larvae were present in the animals at necropsy. The present work was designed to examine the clinical course of disease in dogs receiving relatively low doses of infective-stage larvae.

MATERIALS AND METHODS

The dogs included 5 male and 3 female, 3-month-old, specific-pathogen-free Beagles (Hazelton-LRE Enterprises, Kalamazoo, MI), and 2, 12-month-old, female Beagles (Marshalls Inc., North Rose, NY). Six months previously, the two older dogs had received, per os, 150 infective eggs of Toxocara canis, but the infections had not become patent. The dogs were housed individually in AAALAC approved facilities, and given commercial dried dog food and water ad libitum. The cages were cleaned daily. The control sera used in the immunological procedures were from 5 male, 3-month-old, Beagles from Hazelton-LRE Enterprises that were housed under similar conditions throughout the study period.

The larvae of T. spiralis that were used to infect the dogs were of a strain that has been maintained by serial passage in rats for several years. At the time of infection, 2, month-old larvae were harvested from a rat using pepsin digestion (Appleton and McGregor 1987). The younger dogs were randomly assigned into pairs, and each was orally administered a dose of 100, 500, 1,000, or 5,000 larvae of T. spiralis. The two adult dogs were each given 1,000 infective larvae.
Fecal samples from each dog were examined daily for the first 16 days after infection using a zinc-sulfate, flotation method (Ash and Orlin 1987). Blood smears were made on each dog every 4 days beginning 5 days before infection and stained with Wright's Giemsa stain. Blood samples were taken from each dog for creatine kinase levels 5 days before the dogs were infected, 7 days p.i., and at necropsy. Ophthalmological exams were performed on each dog on days 7, 12, and 23 p.i. Physical exams were performed on each dog on days 1, 7, 12, and 15 p.i. The dogs were weighed 5 days before infection and 5, 6, 7, 11, and 15 days p.i. and at necropsy. Rectal temperatures were taken on days 1–7, 12, 15, and 21 p.i.

The dogs were euthanized by an overdose of barbiturate 26 or 28 days postinfection on each day, one dog from each pair of the younger dogs and one adult dog were euthanized. At necropsy, approximately 10 grams of tissue from the diaphragm, pectoralis superficialis, masseter, biceps brachii, and vastus lateralis muscles were collected for the determination of the number of *Toxocara spiralis* larvae present. Prior to digestion, the tissue was weighed to the nearest hundredth of a gram, dried, and digested using the same methodology as had been used for the rat tissue. Larvae were counted in pepsin digests of the tissue. To detect the presence of adult worms in the small intestine, the mucosa was scraped and diluted with saline. The resulting sediment was examined using a stereomicroscope. From each dog, portions of the esophagus, diaphragm, and tongue were fixed for histological examination. For the 2 dogs that received the highest doses, additional tissues were fixed for sectioning from all major organs.

The Western Blot analysis used to examine the dog sera for the presence of antibodies to *T. spiralis* was a modification of a technique previously used for rat serum (Appleton et al. 1988). Excretory-secretory antigen from muscle larvae was electrophoresed under reducing conditions in a 10% polyacrylamide gel electrophoresis system. Transfer and blots were performed as described previously (Appleton et al. 1988). The serum from the infected and control dogs was applied to the nitrocellulose strips at a dilution of 1 to 50 in Dulbecco’s phosphate buffered saline with 0.1% gelatine and 0.05% Tween 20. The peroxidase conjugated rabbit anti-canine IgG (Cappel Laboratories) was diluted in the same solution and applied to the strips at a dilution of 1 to 2,000. Blots were developed with the substrate, 4-chloro-naphthol (Kirkegaard & Perry, Gaithersburg, MD).

The change in the percentage of eosinophils in peripheral blood, body weight, rectal temperature, and levels of creatine kinase in the serum were examined using analyses of variance for repeated measures. For the purpose of normalization for statistical analyses, the percent eosinophils in the peripheral blood was expressed as square roots and the levels of creatine kinase were expressed as Napierian logarithms. For the graphical representation of the change in the square roots of the percent eosinophils before and after infection, 95% confidence intervals for the mean percent eosinophils for the different groups of dogs were calculated using the pooled standard deviation produced from a one-way analysis of variance that was performed on each dosage group. The mean number of larvae per gram, normalized for the purposes of comparison using a fourth-root transformation, recovered from the five muscle groups of each dog and the dose of muscle larvae that the dogs received were compared using a two-way analysis of variance. After it was shown that the number of larvae per gram recovered from each muscle type was not significantly related to the dosage of larvae the dogs received, a one-way analysis of variance was used to compare the larval recoveries at the different dosages; Tukey’s multiple comparison test was used to determine the significant differences associated with each dosage group.

**RESULTS**

After infection, the dogs showed very few signs of clinical disease. Neither of the dogs that had received 100 larvae had any signs of gastrointestinal distress; also, 2 dogs that received 1,000 larvae also showed no signs of gastrointestinal upset (Table 1). Brief episodes of mild diarrhea were seen in 4 of the dogs, and vomiting was seen in 3 of the dogs (Table 1). Vomiting and diarrhea were seen only in one dog that had received 500 larvae.

There were no larvae or adults of *T. spiralis* found in any of the fecal samples examined. The body temperatures of the dogs did not change significantly during the course of the study period. There were no abnormalities noted during the ophthalmological examinations of the dogs on days 7, 15, and 21 p.i. The creatine kinase levels (the means [and ranges] for days —5 and 7 p.i. and necropsy, days 26 or 28 p.i. were 385 [194 to 655], 563 [171 to 808], and 497 [235—1,555] u/l, respectively) were not
Table 1. Age, weight, larval dose, and gastrointestinal signs observed in dogs after infection with *Trichinella spiralis*

<table>
<thead>
<tr>
<th>Dog number</th>
<th>Sex</th>
<th>Age in months at infection</th>
<th>Weight (kg) at infection</th>
<th>Larval dose</th>
<th>Days after infection when diarrhea noted</th>
<th>vomitting noted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>3</td>
<td>4.0</td>
<td>100</td>
<td>None seen</td>
<td>None seen</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>3</td>
<td>3.4</td>
<td>100</td>
<td>None seen</td>
<td>None seen</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>3</td>
<td>3.0</td>
<td>500</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>3</td>
<td>3.8</td>
<td>500</td>
<td>7</td>
<td>None seen</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>3</td>
<td>4.4</td>
<td>1,000</td>
<td>1 and 6</td>
<td>None seen</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>3</td>
<td>3.2</td>
<td>1,000</td>
<td>3 and 9</td>
<td>None seen</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>3</td>
<td>4.0</td>
<td>5,000</td>
<td>6, 7 and 8</td>
<td>None seen</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>12</td>
<td>5.3</td>
<td>5,000</td>
<td>8</td>
<td>None seen</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>12</td>
<td>9.0</td>
<td>1,000</td>
<td></td>
<td>None seen</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>12</td>
<td>9.4</td>
<td>1,000</td>
<td></td>
<td>None seen</td>
</tr>
</tbody>
</table>

Table 2. Number of *Trichinella spiralis* larvae recovered from each dog at necropsy and summary of the Western blot analyses

<table>
<thead>
<tr>
<th>Dog number</th>
<th>Larval Dose</th>
<th>Diaphragm</th>
<th>Pectoralis superficialis</th>
<th>Masseter</th>
<th>Biceps brachii</th>
<th>Vastus lateralis</th>
<th>Mean</th>
<th>Reaction in Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>3.14</td>
<td>4.24</td>
<td>2.02</td>
<td>4.31</td>
<td>2.88</td>
<td>3.47</td>
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<tr>
<td>2</td>
<td>100</td>
<td>4.22</td>
<td>5.16</td>
<td>2.85</td>
<td>6.60</td>
<td>4.17</td>
<td>4.73</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>28.45</td>
<td>19.03</td>
<td>5.63</td>
<td>9.08</td>
<td>14.13</td>
<td>16.36</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>35.80</td>
<td>16.92</td>
<td>21.80</td>
<td>33.63</td>
<td>23.01</td>
<td>26.69</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1,000</td>
<td>9.34</td>
<td>2.63</td>
<td>4.80</td>
<td>1.00</td>
<td>4.82</td>
<td>4.00</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>1,000</td>
<td>32.72</td>
<td>11.06</td>
<td>0.87</td>
<td>10.93</td>
<td>3.34</td>
<td>11.40</td>
<td></td>
</tr>
<tr>
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<td>70.36</td>
<td>39.15</td>
<td>40.94</td>
<td>17.97</td>
<td>34.08</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5,000</td>
<td>55.11</td>
<td>63.61</td>
<td>48.92</td>
<td>47.08</td>
<td>41.02</td>
<td>51.20</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1,000</td>
<td>14.18</td>
<td>6.40</td>
<td>8.64</td>
<td>8.30</td>
<td>7.10</td>
<td>8.79</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>1,000</td>
<td>16.43</td>
<td>10.87</td>
<td>7.54</td>
<td>11.06</td>
<td>9.49</td>
<td>13.14</td>
<td>+ + +</td>
</tr>
</tbody>
</table>
Fig. 1. Percent eosinophils in peripheral blood of dogs infected with *Trichinella spiralis*. 

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found to change significantly for any of the dosage groups over the course of the study; although the older dogs did have lower values throughout the study period (range 171 to 280).

The adult dogs lost about one half kilogram of weight during the course of the study (Table 1). The younger dogs all grew, but had a depression in weight gain during the first week postinfection. None of these changes, however, were statistically significant.

The dogs did develop an eosinophilia; the maximum value was 16% (range from 0% to 16%). An eosinophilia developed in the younger dogs that received the two larger doses of larvae (Fig. 1); the maximum eosinophilia in the dogs receiving the highest dose was 16%, but was recorded at this level on only a single occasion. The increase in eosinophils was significantly related to the dosage of larvae that the dogs received (p < 0.0256) and significantly increased over the study period (p < 0.0001). The two older dogs had increased numbers of eosinophils in their blood at the beginning of the study period, and no significant increase occurred during the study (Fig. 1).

![Figure 2](image-url)

**Fig. 2.** Number of larvae (expressed as the fourth root) per gram of muscle in pairs of dogs infected with *Trichinella spiralis*. Dogs 1 and 2 received 100 larvae, dogs 3 and 4 received 500 larvae, dogs 5 and 6 received 1,000 larvae, dogs 7 and 8 received 5,000 larvae, and dogs 9 and 10 received 1,000 larvae. The boxes represent 95% confidence intervals about the mean that is based on a pooled standard deviation from a one-way analysis of variance.

No gross lesions were noted at necropsy. The histopathological sections of esophagus, tongue, and diaphragm contained rare to many typically small discrete foci of inflammation composed of a centre of moderately dense accumulation of macrophages. These foci were also often surrounded by a variably thick mantle of lymphocytes, plasma cells, and eosinophils. Larvae were occasionally present in the centres of these foci and were immediately surrounded by thick, dense, lightly eosinophilic capsule. Most larvae were seen to be within muscle fibers and in the early stages of encapsulation, i.e., there was hydropic change in the muscle cell and an increase
number of muscle cell nuclei that had become enlarged and vesicular. There were no lesions present in the brain, liver, lung, adrenal gland, small intestine, colon, stomach, pancreas, kidney, bladder, or heart in any of the examined sections.

The number of larvae recovered from the different muscles of each dog were not found to differ significantly; the number of larvae recovered ranged from 0.87 to 70.35 larvae per gram of muscle (Table 2). The mean number of larvae was significantly related to the number of larvae that the dogs initially received (p < 0.0001).

Fig. 3. Western blot analysis of sera of dogs collected 26 or 28 days after infection with *Trichinella spiralis* (10 and 9, sera from older dogs receiving 1,000 larvae; 8 and 7, sera from dogs receiving 5,000 larvae; 6 and 5, sera from dogs receiving 1,000 larvae; 4 and 3, sera from dogs receiving 500 larvae; 2 and 1, sera from dogs receiving 50 larvae; A through E, sera from uninfected dogs; N, no serum). The antigen used was excretory-secretory antigen from cultures of muscle larvae. Molecular weights (×10^3), as determined by prestained molecular weight indicators, are shown on the left.

The greatest number of larvae was recovered from the dogs that received 5,000 larvae (Fig. 2). The dogs that received 500 larvae had significantly (p < 0.01) fewer larvae than the dogs that received 5,000 larvae and significantly (p < 0.01) more larvae than the dogs that received 100 larvae. There was no significant difference (p > 0.10) detected in the mean number of larvae recovered from the older and younger dogs that received 1,000 larvae, nor was there any significant difference between the number of larvae recovered from the dogs that received 1,000 larvae and the dogs that received 100 larvae.

The serum of all dogs, except one that had received 500 larvae, had a positive reaction with larval excretory-secretory products in Western Blot analysis (Table 2 and Fig. 3). The adult dogs showed the greatest immunologic response. None of the uninfected animals showed any reaction to the antigen.
DISCUSSION

Although all the dogs in the study developed infections with *T. spiralis*, the infections caused very little clinical disease. The slight signs of gastrointestinal upset seen during the first few days of the infection could easily have been overlooked by a pet owner. Matoff (1936) gives no indication that dogs receiving 1,000 or fewer muscle larvae had any signs of gastrointestinal upset. Only 2 of the 29 dogs that Matoff (1937) gave large numbers of larvae had diarrhea; one on day 1 and one on day 2 postinfection.

The lack of fever seen in these dogs is similar to what was found by Martinez-Gomez et al. (1965) in dogs that were given large numbers of *Trichinella*, 6,500 larvae per kilogram. These authors found that the rectal temperature of the dogs remained at a constant near normal temperature throughout the course of the 30 days of the study; the maximum temperature that was observed was 40.5 °C. Thus, it may be that dogs, like humans (Beaver et al. 1984), develop fevers proportional to the severity of the infection with *T. spiralis*, and that the small infectious doses used in this study produced insufficient somatic larvae to cause marked fevers.

The dogs did develop a slight eosinophilia as a result of the infection, but these values were usually within the normal levels of 2% to 10% reported for dogs (Coles 1986). Overall the levels seen in this study were lower than the range of 11% to 38% reported by Beaum and Jorgensen (1941) for a group of 10 adult dogs during the second week after infection with a range of 90,000 to 480,000 larvae. The 2 older dogs in the current study had a slightly elevated eosinophil count at the beginning possibly due to their prior inoculation with eggs of *Toxocara canis*. The secondary infection with *T. spiralis* did not increase the number of eosinophils present in these dogs significantly during the course of the infection; this might have been expected based on similar work in rats with larval ascarids and *T. spiralis* (Fgallová and Prokopič 1989).

The lack of significant change in the level of creatine kinase during the course of the infection is very similar to what has been reported previously in humans (Hennekeuser et al. 1968, Wiśniewska 1970). Similarly, the lack of association between the number of larvae present in the tissues and the level of creatine kinase is also similar to that previously reported for rats and humans (Wiśniewska 1970).

The weight of the dogs was apparently affected by these small larval dosages. However, due to the lack of similar measurements made on uninfected controls, the small sample sizes, and the compounding factor of mixed sexes with in males growing much more rapidly than females, it is hard to determine if there was any significant relationship between the dose of larvae given to each dog and the amount of weight lost by each animal. Similar work in swine has shown that much larger dosages of larvae are required to produce weight loss in pigs (Scholtens et al. 1966).

The number of larvae recovered from the dogs reached levels as high as 70 per gram of muscle. This is similar to the numbers reported by Matoff (1936) for dogs given 500 to 1,000 larvae; he recovered 64 larvae per gram of muscle in a dog that had been given 500 muscle larvae. When Matoff (1937) gave dogs large doses of muscle larvae (greater than 100,000 per dog), he recovered very large numbers of larvae, up to 1,504 larvae per muscle press, from the muscles of many of the younger dogs (Based on his previous work (1936), there were usually about 60 tissue presses per gram of muscle examined.). These numbers were much higher than those reported for older dogs (1937) from which only about 0 to 6 larvae were recovered from each tissue press. Schanbacher et al. (1978)-infected adult dogs with doses of 10,000 to 30,000 muscle larvae per kilogram (approximately 160,000 to 630,000 per dog)
and recovered a maximum of only 2.26 larvae per gram of muscle (a composite sample of tongue, gastrocnemius, diaphragm, deltoid, and masseter muscles) at 6 weeks after infection. Thus, as previously stated by Matoff (1937), it would appear that there may be an age related resistance to infections of _T. spiralis_ in dogs.

The lower rate of infection with 1,000 larvae than with 500 or 5,000 larvae is difficult to explain. The two younger dogs may have developed fewer worms by change, and the two older dogs may have developed similar infection levels due to age resistance. Another possibility is that if the adult worms are as fecund in young dogs as they are in adult dogs, the older dogs might have harboured fewer muscle larvae because their dose of larvae per kilogram was reduced in comparison to total muscle mass. This has been postulated to occur with trichinosis in swine (Olsen et al. 1964, Kotula et al. 1988). Matoff (1936), however, used large and small breeds of dogs and found no apparent difference in the number of larvae recovered from each.

The lack of any significant difference between the number of larvae recovered from the various muscle types may have been due to the small sample sizes (n = 2) of the groups that were being compared. Statistical differences have been reported for the distribution of _T. spiralis_ larvae within the muscles of swine (Kotula et al. 1984). Samples of other muscles might have revealed differences; Kotula et al. (1984) found the greatest number of larvae in the tongue, an organ that was only examined in histological sections in this study.

The results of the Western Blot analyses are complicated by the prior attempt to infect the adult dogs with _Toxocara canis_. It is known that there is cross reaction between the phosphorylcholine antigens of the larvae of _T. spiralis_ and _T. canis_ (Sugane and Oshima 1983), and the increased antibody response, like the elevated eosinophilia seen in these 2 dogs at the beginning of the study, may have been due to their prior infection with _Toxocara_. The young dogs were from a supplier that historically has no _T. canis_ in its colony (Glickman et al. 1981). The responses of all these young dogs were similar. However, it is difficult to explain why one of the dogs given 100 larvae had one of the greatest responses, and why one of the dogs receiving 500 larvae had no antibody as detected by this assay. Overall, the Western Blot analysis was capable of detecting antibody in all but one of the dogs by the end of the fourth week of infection. Before this or a similar assay could be used in field surveys, however, it would be necessary to determine what level of false positive reactions would be caused by prior or concomitant infections with _Toxocara canis_ or perhaps _Toxascaris leonina_.

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