The Relationship of Larval Recovery and Eosinophil of Mice Infected with Toxocara canis

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Abstract
A total of 48 Balb/c mice strain Mus musculus was experimentally infected with two doses (250 and 500) embryonated eggs of T. canis using stomach tube. The total number of larvae were recorded from different organs of infected mice 2, 4 and 6 days and 1, 2 and 3 weeks post-infection. A high percentage of larvae were recorded two days post-infection. No larvae recovered from brain, kidney, muscles and intestine. General increase in liver, spleen and body weights was found in infected mice as compared to control. Liver and spleen index were used as an indicator for liver and spleen response to the infection. High peak of eosinophil counts was recorded two weeks post-infection.

Keyword
Mus musculus, eosinophil counts, larvae of Toxocara canis, post-infection.

Introduction
The effects of T. canis infection in non-canine hosts offer a biological model for understanding the clinical findings of human toxocariasis (Basualdo-Farjat et al., 1995). The mice as an experimental model has been successfully used by many authors since the Toxocara larvae were identified as a cause of the disease in children. However, this murine model allows successfully pathological and immunological studies of toxocariasis in human since the course of infection in mice resembles most closely that one in man, several authors have noticed the ability of T. canis larvae to survive and continually migrate in the hosts' tissues inspite of the immune response (Schantz, 1989). In vitro observations have shown the eosinophiles attach to T. canis larvae in the presence of immune serum and complemet (Fattah et al., 1986; Badley et al., 1987). High eosinophil levels and a development of granulomas rich in eosinophiles in infected mice were reported by Piergili-Fioretti et al. (1989) and Buijjs et al. (1990). El-Shazly et al. (2002) found a hepatomegaly with an irregular liver surface and red spots with streaks in male mice infected orally with different doses of T. canis eggs, granulomas in infected mice appeared in liver four weeks post-infection. The aim of this study was to determine the distribution of T. canis larvae in different organs of mice orally infected with two doses of T. canis larvae, to clarify if the larvae reached to the brain and also to determine the eosinophil counts of infected and control mice at different periods post-infection.

Materials and Methods
Mice Infection
A total of 48 Balb/c mice with 1.5-2 months of age were divided to three groups. Groups (1 and 2) were inoculated orally with 250 and 500 embryonated eggs of T. canis by using of a stomach tube attached to an 18 gauge needle with a 1 ml syring (Sugane and Oshima, 1982), while group (3) was inoculated with one ml distilled water as a control. Groups (1 and 3) were killed after 1, 2 and 3 weeks post-infection, while groups 2 and 3 were killed after 2, 4 and 6 days post-infection. Recovery of T. canis Larvae from Infected...
Mice. The original procedure for recovery of larvae from tissues used by Sprent (1952) modified by Sugane and Oshima (1984) was applied in this study. A brief description of the procedure is: numbers of larvae in different organs (liver, spleen, lungs, muscles, heart, kidney and intestine) of the infected mice were counted as follows: The organ was crushed with a tissue homogenizer and digested with 0.1% pepsin at 37°C for four hours. The solution was passed through two layers of gauze, then, the suspension was centrifuged at 2000 rpm for 15 min. The sediment was put on a clean petridish and larvae were counted under a stereomicroscope (Biovision Bvs 320/Altay). The brain was passed between two pieces of transparent plate glass to determine the number of larvae under a stereomicroscope (Sugane and Oshima, 1982). No larvae were detected in eyes of infected mice. 

Liver and Spleen Index
The weight of liver, spleen and total body of infected and control mice were recorded by a balance (Metller E160/Germany). A procedure used by Simonsen (1962) to determine the index of the above organs as follows:

\[
\text{Liver and Spleen Index} = \frac{\text{Organ weight}}{\text{Body weight}} \quad \text{(grm.)} 
\]

Determination of Eosinophil Counts
A procedure of Sugane and Oshima (1984) was used for determination of eosinophil counts which briefly: one and half ml of blood were collected from killed infected and control mice (groups 1, 2 and 3) by heart puncture using 1 ml needle. The blood was diluted with Discomb’s solution (Discombe, 1946) prepared by:

- Aqueous Eosin: 0.1 grm.
- Acetone: 10 ml.
- Distilled water: 90 ml.

Blood with Discomb’s solution was sucked by a white cell pipette and mixed well with stain eosinophil. The Neubauer chamber (Superior/Germany) was used to eosinophil counts.

Calculation
Dillution factor = 20
Total area = 4 mm²
Depth = 1/10 mm
Count volum (Diluting) = 4 mm² \times \frac{1}{10} mm = 0.4 mm³
Total No. of eosinophiles = \frac{X}{0.4} \times 20
X = eosinophil number.

Results
The recovery of the larvae from Infected Mice
The recovery of the larvae was recorded in mice infected with 250 and 500 embryonated eggs of *T. canis* (plate1). Larvae were observed for the first time at two days post-infection, as shown in table (1). The high percentage of the recovery (100%) was found in liver of mice infected with (500) embryonated eggs at two days post-infection, while it was 66.6 and 62.5% at four and six days post-infection. The high percentage of recovered larvae was shown in liver (52.8%) in mice infected with (250) embryonated eggs two weeks post-infection, while it was 50 and 38.8% at one and three weeks post-infection (Figs. 1 and 2). Larvae appeared four days post-infection in lung of mice infected with 250 embryonated eggs of *T. canis*. Larvae were seen for the first time in spleen at first week post-infection with 250 larvae (Table 1). No larvae were recovered from brain, muscles, kidney, heart, and intestine. A statistical analysis
using Chi-Sq test showed a high significant difference between percentage of larvae recovered and doses of infection (P>0.05, $X^2=0.000$).

Plate (1): Larvae of *T. cains* recovered from lung (A), liver (B) and spleen (C) of mice infected with 250 and 500 embryonated eggs. (4X).
Table (1): The mean number of larvae recovered from different organs of mice infected with 250 and 500 embryonated eggs of *T. canis*.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Period</th>
<th>Mean number of larvae</th>
<th>% Recovery</th>
<th>% Recovery</th>
<th>% Recovery</th>
<th>% Recovery</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Lung</td>
<td>Spleen</td>
<td>Other</td>
<td>Total</td>
<td>liver</td>
</tr>
<tr>
<td>250</td>
<td>1 week</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>28</td>
<td>19</td>
<td>6</td>
<td>0</td>
<td>53</td>
<td>52.8</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>26</td>
<td>23</td>
<td>18</td>
<td>0</td>
<td>67</td>
<td>38.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>64</td>
<td>46</td>
<td>30</td>
<td>0</td>
<td>140</td>
<td>45.7</td>
</tr>
<tr>
<td>500</td>
<td>2 days</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>62.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>22</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>73.3</td>
</tr>
</tbody>
</table>

\[ P > 0.05, \quad \chi^2 = 0.000^* \]

* a = total number of larvae, b = dose (250, 500).

* % liver recovery = No. larvae in liver/ Total No. larvae × 100

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Figure (1): The mean number of larvae of *T. canis* recovered from different organs of mice infected with (250) embryonated eggs of *T. canis*. 
Figure (2): The mean number of larvae of *T. canis* recovered from different organs of mice infected with (500) embryonated eggs of *T. canis*.

**Liver, Spleen and Body Weights**

Table (2) shows the mean weights liver, spleen and total body of mice infected with 250 and 500 embryonated eggs of *T. canis*, both the liver and spleen weights were increased with increasing periods post-infection (Figs. 3; 4; 5 and 6) as compared to control, while, total body weight was increased gradually (Figs. 7 and 8). Analysis of variance (Balanced designs) shows that there was no significant difference between infection and weight of liver and spleen at two periods \( (P>0.05, F=0.123, 0.161) \). While, there was a significant difference between total body weight and infection doses \( (P>0.05, F= 0.028) \).

Table (2): weight of Liver, spleen and total body of mice infected with *T. canis* larvae.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Period</th>
<th>weight Infected Mice (grm.)</th>
<th>Weight Control (grm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Spleen</td>
</tr>
<tr>
<td>250</td>
<td>1 week</td>
<td>1.27</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>2.61</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>2.85</td>
<td>1.25</td>
</tr>
<tr>
<td>500</td>
<td>2 days</td>
<td>1.21</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>1.29</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
<td>1.56</td>
<td>0.26</td>
</tr>
</tbody>
</table>

\( P > 0.05, \ F= 0.123, 0.161 \)
\( P > 0.05, \ F= 0.028^* \)
Figure (3): The relationship between infection with (250) embryonated eggs of *T. canis* and mean of liver weight.

Figure (4): The infection with (500) embryonated eggs of *T. canis* and mean of liver weight.

Figure (5): The relationship between infection with (250) embryonated eggs of *T. canis* and mean of spleen weight.

Figure (6): The infection with (500) embryonated eggs of *T. canis* and mean of spleen weight.

Figure (7): The relationship between infection with (250) embryonated eggs of *T. canis* and mean of body weight.

Figure (8): The infection with (500) embryonated eggs of *T. canis* and mean of body weight.
Liver and Spleen Indices

The liver and spleen indices were shown in table (3). The value of liver index at two days and one week post-infection was less than one, while, in four and six days post-infection the value was more than one with a high value shown at three weeks post-infection. The value of spleen index was increased stationary at both doses and a high value was 5.07 at three weeks post-infection (Fig. 9; 10).

Table (3): Liver and Spleen indices of mice infected with 250 and 500 embryonated eggs of *T. canis*.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Period</th>
<th>Liver Index</th>
<th>Spleen Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>1 week</td>
<td>0.96</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>1.91</td>
<td>2.36</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>2.03</td>
<td>5.07</td>
</tr>
<tr>
<td>500</td>
<td>2 days</td>
<td>0.99</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>1.03</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
<td>1.26</td>
<td>1.24</td>
</tr>
</tbody>
</table>

P > 0.05, $X^2 = 0.000*$

Figure (9): Spleen and liver index of mice infected with (250) embryonated eggs of *T. canis*.

Figure (10): Spleen and liver index of mice infected with (500) embryonated eggs of *T. canis*.
**Eosinophil Counts**

The mean number of eosinophil counts in mice infected with 250 and 500 embryonated eggs of *T. canis* at different periods was shown in table (4). In group one there were stationary increases at one and two weeks post-infection (1250 and 3500) then decreased at three weeks post-infection (1050), while in group two, a high peak was observed six days post-infection (1150) and a low (950) at two days post-infection (Figs. 11 and 12). In control group there was no rapid increase in eosinophil counts. A statistic analysis using Chi-Sq test showed that there were significant differences among doses, periods and number of eosinophil count (P>0.05, X²=0.000).

Table (4): The mean number of eosinophil in mice infected with 250 and 500 embryonated eggs of *T. canis* and control.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Period</th>
<th>Mean No. Eosinophil Infected</th>
<th>Mean No. Eosinophil Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>1 week</td>
<td>1250</td>
<td>112.5</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>3500</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>1050</td>
<td>112.5</td>
</tr>
<tr>
<td>500</td>
<td>2 days</td>
<td>950</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>1050</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
<td>1150</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>8950</td>
<td>587.5</td>
</tr>
</tbody>
</table>

P > 0.05, X²= 0.000*

![Graph showing eosinophil counts](image)

**Figure (11):** The mean number of eosinophil in mice infected with (250) embryonated eggs of *T. canis* and control.
Figure (12): The mean number of eosinophil in mice infected with (500) embryonated eggs of *T. canis* and control.

**Discussion**

The larval migration to the liver and lungs showed a recovery peak 2-4 days post-infection. This observation suggested that *T. canis* larvae after hatching in intestine migrated by a hepatopulmonary route in the mice. Furthermore, the differences between inoculated eggs and larvae recovered from infected mice may be due to the number of inoculated eggs passing out through the intestine without hatching. Larval recovery from mice organs in this study is evidence that larvae are not only trapped but survive within these organs. The absence of the larvae from the brain, muscles, heart, and kidney may be due to the low doses and short period which make larvae trapped only in main organs associated with immune response of infected mice (Kayes and Adams, 1980). A gradual decrease in number of recovery larvae was observed first week post-infection, while the number was increased two and three weeks post-infection in liver, lungs and spleen. The second recovery peak may be related to the new redistribution of the larvae in mice. Piergili –Fioretti et al. (1989) reported that the larvae of *T. canis* in mice experimentally infected required 2-3 days to reach liver and lung respectively. Parsons and Grieve (1990) pointed out that the larval trapping in mice infected with *T. canis* larvae occurred within the liver at five days post-infection, and the larvae recovered from different organs of mice infected with 125 eggs of *T. canis* at 4, 8, 12 and 16 weeks post-infection. Furthermore, they showed that liver larval trapping did not protect the eyes or brain from larval migration. Oshima (1983) isolated larvae from brain, skeletal muscles and other organs of mice infected with 500 eggs of *T. canis* 2, 4 and 6 weeks post-infection with higher number at six weeks post-infection. Oshima (1961) demonstrated that approximately 98% of larvae were concentrated in the liver and lungs of mice infected with *T. canis* larvae at 44 hours post-infection. A similar result was obtained in this study when 100% of larvae were obtained at two days post-infection. Oshima (1983) pointed out that the percentage of larvae recovered from the liver was increased as a period of infection increase in Balb/c mice infected with 500 eggs of *T. canis*, and this increase
was higher in secondary than primary infection. In the present study, there was an increase in liver, spleen and body weight of infected mice as compared to control. This might be related to the number of larvae found in these organs and due to the histopathological changes which took place in these organs like fibrosis, necrosis and infiltration of the blood cell as a result of hepatic and spleenic responses. Oshima (1961) studied the effect of body weight of mice infected with *T. canis* larvae and he showed that the number of larvae recovered from large mice was greater than small mice. The liver index was increased at six days and three weeks post-infection throughout the current study. The high doses inoculum might be corugouse hepatic response. Similar result was found in case of spleen index that returned to its normal value with short period after infection. Kayes *et al.* (1985) and Piergili-Fioretti *et al.* (1989) showed that liver and spleen indices increased between 11-14 days and continued till 21 days post-infection, also, they observed that with a small inoculum the spleen index returned to its normal value which was quicker than high dose inoculum. It was found that the eosinophil count in this study was increased till 6th day post-infection in group (1), while, in group (2) a rapid increase in the count was found two weeks post-infection, with low peak at three weeks post-infection. This increase in eosinophil level in massive infections was sygronized with a strong immune response in spleen. The increase of eosinophil levels might result from parasitic infection and some eosinophil cells entered the immune system by attacked larvae. Oshima (1983) reported that a high peak of eosinophil levels was quite clear between 10-14 days post-infection of mice infected with *T. canis* then decreased till 40 days post-infection. Parsons *et al.* (1989) pointed out that circulating eosinophile levels increased in cats infected experimentally with *T. canis* larvae with a peak value 15790 and 1050 at 25 and 32 days post-infection respectively. On the other hand, Kayes and Adams (1980) and Piergili-Fioretti *et al.* (1989) found that eosinophilic high levels occurred at the 14th day post-infection. Sugane and Oshima (1980) demonstrated that eosinophile counts in peripheral blood of infected mice with *T. canis* eggs reached the highest level on the 2nd day post-infection and there- after decreased abruptly, then, it was increased again after 20 days post-infection. Oshima (1983) observed that eosinophil adherence on the cuticle of *T. canis* larvae in mice infected with 500 embryonated eggs after 72 hours. The larvae remained active and no structural damages obseved till the 6th week post-infection when eosinophilic granuloma was surrounded the larvae in the muscles of the mice. larvae in brain were not encapsulated and did not induce immunological reaction by the host (Overgaauw, 1997b). Cox and Holland (2001) showed that many factors may affect the infection of mice with *T. canis* larvae such as strain of mice, dose and larval intensity and period post-infection.

**References**


Sprent, J. F. (1952). On the migratory behaviour of the larvae of various...