Differential biological behaviour of *Trichinella pseudospiralis* and *T. spiralis* in birds (*Gallus domesticus*)

**Gómez-Barro, A.; Armas-Serra, C. DE; Bolas-Fernández, F.**


**Summary**

White Leghorn chickens were orally infected with *Trichinella pseudospiralis* or *T. spiralis*; their digestive tracts were examined for adult worms at days 5, 10, 14, 19, 24 and 28 post-infection (p.i.) and their muscles examined for larvae at days 24, 34, 41 and 53 p.i. *T. pseudospiralis* was shown to complete its biological cycle in the birds, the muscle larvae recovered from chickens were viable in mice. "In vitro" release of newborn larvae by female worms recovered on several days, from day 5 to 26 p.i., was relatively high. By contrast, in birds infected with *T. spiralis* only 1% of the larvae administered were able to develop to adults in the intestine. None of the birds harboured muscle larvae.

**Key Words:** *Trichinella pseudospiralis*, *T. spiralis*, birds, infectivity.

**Resumen**

Se determina el asentamiento intestinal y muscular de *Trichinella pseudospiralis* y *T. spiralis* en pollos de un híbrido comercial tipo Leghorn, mediante la recuperación de vermes adultos durante los días 5, 10, 14, 19, 24 y 28 postinfección (p.i.) y de larvas musculares durante los días 24, 34, 41 y 53 p.i. *T. pseudospiralis* completó su ciclo biológico en las aves, recuperándose larvas musculares viables. La larvición "in vitro", determinada sobre hembras aisladas desde el día 5 al 26 p.i., fue relativamente alta. Por el contrario, sólo el 1% de las larvas de *T. spiralis* alcanzaron el estado adulto en las aves, sin encontrarse en ninguno de los animales larvas musculares.

**Palabras Clave:** *Trichinella pseudospiralis*, *T. spiralis*, pollos, capacidad infectante.

**Introduction**

The circulation of *Trichinella pseudospiralis* among different species of wild birds in nature seems to be confirmed after this helminth has been recently isolated from two *Corvus frugilegus* in a Soviet survey of 744 birds of the Falconiformes and Corvidae, from a buzzard (*Buteo buteo*) examined in Spain, and from a young male Cooper hawk (*Accipiter cooperi*) captured in northern California.

Although the participation of birds in the natural cycle of trichinellosis is poorly understood, the facts mentioned above clearly indicate that attention should be paid to the epizootological and ecological role played by birds as possible links in the epidemiological cycle of trichinellosis. It is consequently necessary to know the susceptibility of such avian hosts to *Trichinella* infection. The present study is an attempt to elucidate the infectivity of *T. pseudospiralis* and *T. spiralis* to *Gallus domesticus*, at their intestinal and muscular phases, as well as the infectivity in mammals of muscle larvae recovered from birds.
Materials and Methods

Experiments were performed in 2 days old White Leghorn chickens and 30 days old NMRI mice. The strain of *T. pseudospiralis* used was the original Garkavi isolate in 1932, kindly provided by Dr. Z.S. Pawlowsky of the University of Poznan (Poland). The strain of *T. spiralis* was isolated from the musculature of a wild cat captured in Asturias (Spain) in 1963 and subsequently maintained by serial passages through rats and mice.

The experimental design was divided into three parts; the first aimed to study the intestinal and muscular infection of *T. pseudospiralis* and *T. spiralis* in chickens, the second to know whether the larvae recovered from the muscles of chickens were or not infective in mice, and the third, to determine the "in vitro" release of newborn larvae from female worms.

**Experiment 1**

*T. pseudospiralis* and *T. spiralis* larvae were obtained from the carcasses of infected mice by artificial digestion in a fluid containing 0.5% pepsin and 0.7% HCl at 37°C on an incubator shaker for 45 minutes and 1 hour, respectively. The larvae were counted in McMaster counting chambers and administered by tube into the crop of birds in doses of 2000 larvae per bird.

Six birds of each group were killed by ether asphyxias on days 5, 10, 14, 19, 24 and 28 p.i.; the digestive tracts from the gizzard to the anus were removed and examined for worms by autolysis of intestinal mucosa after incubation in saline solution at 37°C for 4 hours.

After day 24 p.i., in which the microscopic examination of thick sections of muscles revealed larvae within the muscle fibers of *T. pseudospiralis* infected birds, six new birds of each group were killed on days 34, 41 and 53 p.i.; pieces of musculature 10 g each from the thigh and breast were obtained from each bird and digested in the pepsin-HCl solution, as described above, and the *Trichinella* larvae recovered were counted.

**Experiment 2**

Muscle larvae recovered from birds on day 34 p.i., as mentioned above, were used to infect two groups of mice with 500 larvae/mouse; seven mice were killed by cervical dislocation on day 7 p.i. and their small intestines examined for worms; the other group of mice were killed on day 30 p.i. and their carcasses examined for the presence of muscle larvae.

**Experiment 3**

Mature female worms were recovered from the intestine of *T. pseudospiralis* infected chickens at days 5, 14, 20, 25 and 26 p.i. using a modified Baereman apparatus with physiological saline solution. Each female worm was placed individually in NUNCIN plates containing 1 ml of Hanks solution with 1% MEM (Minimal Essential Medium) and 1% Glutamine. Incubation was carried out for 24 hours at 37°C and the number of newborn larvae released during this period of time was determined by direct counting under an inverted microscope at a magnification of x40.

**Results**

Tables 1 and 2 show the results of the examination of intestinal and muscular stages of *T. pseudospiralis* and *T. spiralis* infection in *G. domesticus*. The infectivity of *T. pseudospiralis* muscle larvae recovered from chickens in indicated in Table 3.

"In vitro" release of newborn larvae from *T. pseudospiralis* female worms is shown on Table 4.

**Table 1**

<table>
<thead>
<tr>
<th>Day post-inoculation</th>
<th>N.° of birds</th>
<th>N.° of adult recovered*</th>
<th>% recovery of the inoculum</th>
<th>N.° of adult recovered*</th>
<th>% recovery of the inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6</td>
<td>80.3 ± 13.0</td>
<td>4.2</td>
<td>210 ± 6.2</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>140.0 ± 81.1</td>
<td>7.2</td>
<td>125 ± 5.4</td>
<td>0.6</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>73.0 ± 23.3</td>
<td>3.7</td>
<td>67 ± 4.2</td>
<td>0.3</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>81.7 ± 13.6</td>
<td>4.1</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>72.5 ± 13.3</td>
<td>3.6</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>28</td>
<td>6</td>
<td>10.0 ± 8.6</td>
<td>0.5</td>
<td>0</td>
<td>--</td>
</tr>
</tbody>
</table>

(*) Data expressed as mean values ± standard error

**Table 2**

<table>
<thead>
<tr>
<th>N.° of larvae per gram of muscle*</th>
<th>24 h in vitro</th>
<th>2,000 larvae/bird</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day post-inoculation</td>
<td>N.° of birds</td>
<td>T. pseudospiralis</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>79.9 ± 10.5</td>
</tr>
<tr>
<td>34</td>
<td>6</td>
<td>75.9 ± 15.6</td>
</tr>
<tr>
<td>41</td>
<td>6</td>
<td>65.8 ± 23.9</td>
</tr>
<tr>
<td>53</td>
<td>6</td>
<td>165.4 ± 37.0</td>
</tr>
</tbody>
</table>

(*) Data expressed as mean values ± standard error

**Table 3**

<table>
<thead>
<tr>
<th>N.° of larvae recovered</th>
<th>N.° of adult recovered</th>
<th>N.° of larvae recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day post-inoculation</td>
<td>7</td>
<td>217.9 ± 37.4</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>3210.1 ± 9858.2</td>
</tr>
</tbody>
</table>

(*) Mean values ± standard error

**Discussion**

Although it is widely known that *T. pseudospiralis* can complete its life cycle in an avian host, controversial results regarding the susceptibility of birds to *Trichinella* infection have been reported by various researchers. The strain or species of the parasite as well as the host used in these experiments may be critical for the analysis of the results. So, we attempted...
the comparative study of the susceptibility of
G. domesticus to T. pseudospiralis and T. spiralis infection.

Birds infected with T. pseudospiralis developed a moderate but prolonged in-
testinal phase, intestinal worms being re-
covered even 28 days after infection; this
fact, as Bober and Dick\(^1\) reported, clearly
shows a better adaptation of T. pseudospira-
alis to the gut of birds, as compared with
mammals.

The recovery of muscle larvae from
birds killed on days 24-53 p.i. was in
agreement with values obtained by Mees-
rodtch et al.\(^2\) from Falco sparverius, alt-
ough these authors observed a progress-
ive increase in the number of muscle lar-
va from days 2D to 40 p.i. The overlap-
ning between the presence of adult
worms in the intestine and larvae in the
musculature (day 24 p.i.) is a pheno-
menon also remarked by these authors.
Bo-
ber and Dick\(^1\) reported the infectivity of T.
pseudospiralis for Coturnix spp. and Larus
sp., obtaining a great variability that was
related to the species of host used.

In the experimental group of birds in-
fected with T. spiralis, only 1% of the lar-
vae administered could establish in the
gut, ording the intestinal phase on day 14
p.i. The recovery rate as well as the per-
manence of worms in the gut were less
than values known previously\(^3\) in chickens.
The destruction of worms by the mecha-
nical grinding of the gizzard, the rapid re-
jection of the intestinal worms, the se-
quential digestive effect of the stomach
and intestinal secretions and the high bo-
ty temperature of birds can explain the
resistance of these birds to T. spiralis in-
fection. Ooi et al.\(^4\) have explained how im-
possible it is for this strain to grow and de-
velop into the muscle fibers of an avian
host because of the incapacity of embryos
to transform the infected muscle cells into
the so-called "nurse-cell". However, in our
experiments, we never saw embryos or
larvae in the muscle tissue of G. domes-
ticus.

"In vitro" release of newborn larvae
from T. pseudospiralis female worms reco-
ered from birds confirmed that, although
a great number of larvae do not succeed in
establishing themselves in the gut, those
that do it grow to fertile adults, releasing
embryos that are able to develop in the
muscle fibers of birds to larvae that are
perfectly viable in mammals. This fact lead
us to consider the birds as possible links
in the epidemiological chain of T. pseudos-
piralis, mainly because of the intraspecies
food relations among the birds them-
seves and between birds and mammals,
which may allow their participation in the
maintenance and distribution of trichine-
losis in nature, as has been remarked by Tomasevicova and Novokov\(^5\).

References

4. Ooi, H.K.; Oku, Y.; Kamiya, M. and Oh-
5. Shikinov, B.- Spontaneous infection of birds with Trichinella pseudospiralis Garka-