Distribution of Trichinella spiralis Larvae in Selected Muscles and Organs of Experimentally Infected Swine

A. W. Kotula, K. D. Murrell, L. Acosta-Stein and L. Lamb

US Department of Agriculture, Beltsville, MD 20705

Summary
Thirty-two Hampshire-Yorkshire pigs (6 to 8 wk old) were inoculated with the Beltsville strain of Trichinella spiralis at a level of about 880 larvae/kg of body weight (about 15 kg). At about 100 kg, the pigs were slaughtered and 10-g samples of muscle and other tissues were digested in pepsin-HCl and examined microscopically for T. spiralis larvae. The mean number of larvae recovered/gram was: tongue, 452; diaphragm, 391; obliquus abdominis internus, 130; serratius ventralis, 116; psoas major, 105; triceps brachii, 100; biceps femoris, 83; semitendinosus, 74; intercostal, 60; semimembranosus, 58 and longissimus dorsi, 37. The liver and spleen samples contained none. Larvae were found in one sample each of the blood, brain and kidney, in two samples of the heart, and in four samples of lymph tissue. Each of these samples was from a different pig except the positive samples of brain and heart, which were from the same pig. The larvae found in the blood, brain, kidney, heart and lymph were first stage larvae and, therefore, do not indicate migration of newborn larvae from the gut. The presence of these larvae in nonstriated muscle tissue may have been due to contamination of the organs from infected skeletal muscle. These data confirmed previous reports of the distribution of T. spiralis larvae among individual muscles of the carcass. Further, the data suggest that cross-contamination of organ tissue is possible during evisceration and, therefore, organ meat from infected swine cannot be assured to be free of T. spiralis larvae.

Key Words: Trichinella Spiralis, Parasite, Larvae, Infection, Pork, Meat.

Introduction
The distribution of Trichinella spiralis larvae in pork skeletal muscles packaged for retail sale has not been adequately characterized. Hill (1957, 1968), Zimmermann and Schwarte (1961) and Olsen et al. (1964), reported the distribution of T. spiralis larvae in skeletal muscles of 4.5 to 9 mo old pigs. The muscles selected for evaluation by these investigators did not represent muscles of greatest retail value. Zimmermann (1970) evaluated the destruction of the larvae in muscle groups of retail cuts from only three pigs. In his study, each retail cut was digested completely and, therefore, data were not obtained in individual skeletal muscles from the pork carcass. More detailed data on the distribution of T. spiralis larvae in muscles of retail pork cuts are considered necessary.

The importance of the occasional finding of the larvae in tissues other than skeletal muscle has been questioned (Matoff and Komandarev, 1963). Mauss and Otto (1942) reported an occurrence of one larva in organs and tissue other than striated muscle or lysed blood when mice had an average infection rate of about 10,000 larvae/mouse in the striated muscles. Organ meats (liver, heart, kidney, et cetera) that do not contain striated skeletal muscle have been thought to be free of T. spiralis (Matoff and Komandarev, 1963), however, Hill (1957, 1968) found larvae in testes, stomach wall, liver, brain, lungs, small intestine wall, pancreas, aorta, bladder, spinal cord and heart in some of the 55 pigs he examined; the incidence ranged from 2 to 18% for the various tissues evaluated. Zimmermann and Schwarte (1961) also reported that swine tissues devoid of skeletal muscles were infected with T. spiralis larvae. Matoff and Komandarev (1963)
TABLE 1. DISTRIBUTION OF THRICHELLA SPIRALIS LARVAE IN TISSUES OF EXPERIMENTALLY INFECTED SWINE

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean number larvae/g</th>
<th>Infection rate, % of diaphragm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>452 ± 51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>391 ± 38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>Obliquus abdominis internus (bacon)</td>
<td>130 ± 13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33</td>
</tr>
<tr>
<td>Serratus ventralis (Boston)</td>
<td>116 ± 15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td>Psoas major (center loin)</td>
<td>105 ± 13&lt;sup&gt;de&lt;/sup&gt;</td>
<td>27</td>
</tr>
<tr>
<td>Triceps brachii (Boston)</td>
<td>100 ± 18&lt;sup&gt;de&lt;/sup&gt;</td>
<td>26</td>
</tr>
<tr>
<td>Biceps femoris (ham)</td>
<td>83 ± 10&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>21</td>
</tr>
<tr>
<td>Semitendinosus (ham)</td>
<td>74 ± 9&lt;sup&gt;def&lt;/sup&gt;</td>
<td>19</td>
</tr>
<tr>
<td>Intercostal (rib)</td>
<td>60 ± 7&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>15</td>
</tr>
<tr>
<td>Semimembranosus (ham)</td>
<td>58 ± 8&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>15</td>
</tr>
<tr>
<td>Longissimus muscle (center loin)</td>
<td>37 ± 5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a,b,c,d,e,f</sup>Means (n = 31) with different superscripts differ (P<0.05) by the mean separation analysis (Waller and Duncan, 1969).

suggested that Hill's observations of larvae in tissue devoid of striated muscles were due to a) incomplete removal of striated muscle from the organ, b) the presence of degenerated striated muscle or c) the presence of newly born T. spiralis larvae circulating in the blood stream. Matoff and Komandarev (1963) mentioned that T. spiralis larvae may lie in degenerated striated muscle. They defined such muscle as having undergone fatty degeneration wherein the muscle fiber structure is effaced by the deposition of fat during the maturation of the host. Visual observation of larvae, encysted in tissue, is of particular interest when applied to organ tissue to ensure that the organs are actually infected. The present study was conducted to characterize the distribution of T. spiralis larvae among selected muscles of experimentally infected swine. Tissue from nonmuscle organs were also examined for the presence of T. spiralis larvae.

No unusual efforts beyond normal procedures were made to prevent the possibility of cross-contamination.

Approximately 200-g samples from various striated skeletal muscles were excised from the pork carcass after it had been chilled to 2 C. The numbers of T. spiralis larvae in 10 g of each tissue were counted after digestion. Approximately 200-g samples of liver, kidney, tongue, heart, spleen, brain, diaphragm and selected striated muscles were placed in double-lined plastic bags for evaluation for the presence of T. spiralis larvae. Samples, 10 g each, were taken from the internal portions of the 200-g samples of liver, kidney, tongue, heart, spleen, brain and diaphragm to prevent the possibility of skeletal muscle being present in the sample. Both the medulla and the cortex of the kidneys were sampled. The 10-g sample from the tongue was obtained by combining portions from the tip, middle and back. Ten grams of lymph tissue and 10 ml blood samples obtained during bleeding were also collected and evaluated. The presence of newborn larvae in either the lymphatic or the circulatory system, indicative of adult worms harboring in the pig, would not be detected because newborn larvae do not survive the digestion procedure (Mauss and Otto, 1942). The presence of any first-stage larvae in the tissue of the organs would indicate that T. spiralis larvae were present in tissue other than striated skeletal muscle either as a contaminant during evisceration or as encysted larvae.

**Materials and Methods**

*Infected Pork Samples.* Thirty-two Hampshire-Yorkshire weanling pigs (6 to 8 wk old) were inoculated, using an esophageal tube, with the Beltsville strain of T. spiralis at a level of about 880 larvae/kg of body weight (about 15 kg). When the pigs reached about 100 kg, they were immobilized electrically, then exsanguinated and the tissues listed in table 1 were removed from the carcass with standard evisceration procedures used in the meat industry.
Each 10-g sample was placed in a Mason jar, together with 57 g of tap water warmed to 40°C and blended (Osterizer Galaxie Blender4) for 5 to 15 s to produce a homogeneous slurry. Two-hundred milliliters of a pepsin5 solution (10 g/liter pepsin) were added to the Mason jar and stirred; 2.4 ml of 1 N HCl was added and stirred. The sample in digestion solution was incubated at 37°C for 18 to 22 h to allow for the digestion of the sample and release of the larvae.

The digested sample and pepsin-HCl were filtered through a 60-mesh screen into a pilsner glass. The larvae were allowed to settle for 20 min before washing twice with tap water at 40°C with 20-min settling times. The larvae were transferred to a 50 ml conical tube during the final rinse. After settling, the tap water was aspirated to 1 ml, allowing the larvae to remain in the bottom of the tube. Nine milliliters of a nutrient broth-12% gelatin solution (ratio of 4 to 1) was warmed to 40°C and added to raise the volume to 10 ml. A blunted, 18-gauge needle attached to a 1-ml syringe was adjusted to .7 of the depth of the solution to withdraw sequential 1-ml samples. The sample was vortexed 15 s before filling and emptying the syringe six times in rapid succession. On the seventh filling, the syringe was adjusted to retain a 1-ml sample. Samples were removed sequentially from the 10 ml of nutrient broth-gelatin containing the larvae. One-milliliter samples representing the second, fourth and eighth aliquots were removed from the tube to the suspended larvae.

Statistical Analysis. The number of trichinae in each tissue was evaluated in 31 infected swine. The statistical evaluation of the data involved determining the mean number of larvae in the striated muscles, the standard deviation and the standard error of the means. Differences (P<.0001) in the numbers of larvae among the muscles. Based upon previous reports describing the distribution of the larvae by blood circulation (Matoff and Komandarev, 1963), one might expect larvae in the striated muscles evaluated in this study to be randomly distributed. Previous research by Olsen et al. (1964) and Zimmermann (1970) also described variability in numbers of encysted larvae in certain muscle groups. In our study, the largest number of larvae was found in the tongue, with the next largest number in the diaphragm. Our results agree with the findings of others, (Zimmerman and Schwarte, 1961; Matoff and Komandarev, 1963; Olsen et al., 1964; Scholtens et al., 1966; Hill, 1968; Zimmermann, 1970). Matoff and Komandarev (1963), Olsen et al. (1964) and Scholtens et al. (1966), however, indicated that the diaphragm had more larvae than the tongue, while Zimmermann (1961) and Zimmermann and Schwarte (1961), reported that the tongue sometimes

4Mention of brand names does not imply endorsement by the United States Government.
5Fisher Scientific, Silver Spring, MD.
had more larvae; Hill (1968) reported the tongue exhibited the greatest numbers.

Mauss and Otto (1942) suggested that young larvae are transported throughout the body in the blood and attempt to penetrate all organs and tissues, but are only able to develop in striated muscle. Matoff and Komandarev (1963) proposed that larvae that penetrate nonstriated tissue do not develop to the infective first stage, but rather degenerate and perish. In our study, the liver and spleen samples did not contain larvae. Larvae were found, however, in one sample each of the blood, brain and kidney, in two samples of the heart and in four samples of lymph tissue. Each of these samples was from a different pig except the positive samples of brain and heart, which were from the same pig. The larvae found in these organs were first stage larvae and therefore migration of newborn larvae from the gut was not indicated. Contamination of the organs with larvae from infected skeletal muscle is a possible explanation for our observations. This has also been described by Matoff and Komandarev (1963) and Gibson (1957). Because the incidence of T. spiralis larvae in the organ tissue was so low, the adherence of infected striated muscle to the knife during evisceration may have contaminated the organs. Organ meat from infected swine, however, cannot be assumed free of T. spiralis larvae as long as the possibility of such cross-contamination exists in commercial establishments.

The mean numbers of larvae in the three muscles of the ham ranged from 58 to 83/g, with the semimembranosus (58/g) lower (P > .05) than the semitendinosus (74/g) and the biceps femoris (83/g). Zimmermann (1970) hypothesized that the number of larvae in these ham muscles would not differ and reported his data on the basis of larvae per gram (LPG) of a homogenate of the ham muscles. The level of larvae in the homogenate of the ham, expressed as a percentage of the number in the diaphragm, was 13, 26 and 32 for the three hams he evaluated. The LPG in the three muscles of the 31 hams we analyzed, as a percentage of the number in the diaphragm was 15, 19 and 21% for the semimembranosus, semitendinosus and biceps femoris muscles, respectively.

The number of LPG in the two muscles of the center loin did differ (P < .05): the psoas major had a mean of 105/g and the longissimus...
muscle had 37/g, the lowest of all the muscles we evaluated. The serratus ventralis and the triceps brachii of the Boston shoulder had 116 and 100/g, respectively, and those mean values were different (P<.05). The LPG of the two muscles in closest proximity to the ribs, diaphragm and intercostal muscles were different (P<.05); the intercostal had only 15% of the larvae present in the diaphragm. The obliquus abdominis internus muscle of the bacon contained about 30% as many larvae as the diaphragm and the difference was significant.

These data confirm previous reports and provide additional information on the distribution of larvae in specific muscles of experimentally infected swine. The levels of infection among the striated muscles were highly correlated at the 1% level except for the relationship between the diaphragm and the triceps brachii or the semimembranosus, where the significance was at the 5% level (table 2). The highest correlation (r = .95) was between the psoas major and the longissimus muscle, which comprise the two main muscles of the center loin. Surprisingly, the number of T. spiralis larvae in these two muscles was found to be different (P<.05). The distinct differences in LPG among the muscles suggests the larvae encapsulate differentially on the basis of muscle physiological characteristics. Additional research is needed to define the migration of the newly born larvae.

**Literature Cited**


