# Comparative Assessment of a Double Antibody Enzyme Immunoassay Test Kit and a Triple Antibody Enzyme Immunoassay for the Diagnosis of *Trichinella spiralis spiralis* and *Trichinella spiralis nativa* Infections in Swine

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## ABSTRACT

**Enzyme immunoassays using the triple antibody enzyme linked immunosorbent assay (ELISA) with both *Trichinella spiralis spiralis* and *T. spiralis nativa* excretory-secretory (ES) antigens and a commercial *Trichinella spiralis* enzyme immunoassay test kit were carried out on sera from pigs that were infected with light, moderate and high doses of infective *T. spiralis spiralis* and *T. spiralis nativa* respectively.**

**Seroconversion occurred in all pigs given infective *Trichinella* larvae although no trichinae were recovered from pigs given *T. spiralis nativa* larvae and examined between days 92 and 99 postinfection by pepsin digestion. Anti-*Trichinella* antibodies were detected in pigs infected with *T. spiralis spiralis* and *T. spiralis nativa* by ELISA using either the homologous or heterologous ES antigen. The commercial *Trichinella spiralis* enzyme immunoassay test kit also detected anti-*Trichinella* antibodies in both the *T. spiralis spiralis* and *T. spiralis nativa* infected pigs. The commercial test kit did not appear to be as sensitive as the triple antibody ELISA since it usually took two to three days longer for seroconversion to be detected by the former procedure. Finally seroconversion occurred more rapidly in swine infected with *T. spiralis spiralis* than with pigs receiving comparable doses of *T. spiralis nativa.***

## RÉSUMÉ

**Cette expérience portait sur le sérum de porcs infectés avec un nombre restreint, modéré ou élevé de larves de *Trichinella spiralis spiralis* et *T. spiralis nativa;* elle consistait à y rechercher des anticorps contre *Trichinella,* des deux façons suivantes: 1- par l'épreuve ELISA à triple anticorps, avec des antigènes composés d'excrétions et de sécrétions de *T. spiralis spiralis* et *T. spiralis nativa*; 2- à l'aide d'une trousse commerciale d'ELISA, pour *Trichinella spiralis.***

**Une séroconversion se produisit chez tous les porcs qui avaient reçu les larves précitées, en dépit du fait que la digestion pepsique ne révéla aucune trichine, au bout de 92 et 99 jours, chez les sujets auxquels on avait administré des larves de *T. spiralis nativa.* La technique ELISA dans laquelle on utilise un antigène homologue ou hétérologue, composé d'excrétions et de sécrétions de trichines, permit de détecter des anticorps contre *Trichinella,* chez les porcs infectés avec les deux sous-espèces précitées. La trousse commerciale pour l'épreuve immuno-enzymatique de *T. spiralis* donna de résultats identiques; elle ne sembla toutefois pas aussi sensible que l'ELISA à triple anticorps, parce qu'elle ne détecta les anticorps que deux ou trois jours plus tard. Finalement, la séroconversion se produisit plus rapidement chez les porcs infectés avec *T. spiralis spiralis* que chez ceux auxquels on avait administré une dose comparable de *T. spiralis nativa.***


## INTRODUCTION

The enzyme linked immunosorbent assay (ELISA) using excretory-secretory (ES) antigens has been shown to be a sensitive and specific test to detect anti-*Trichinella* antibodies (1-5). Smith and Snowdon (5) showed that the ELISA using a *Trichinella spiralis spiralis* ES antigen detected anti-*Trichinella* antibodies in swine vaccinated with high doses of *Trichinella spiralis nativa* larvae. To investigate further the serological diagnosis of *T. spiralis nativa* infections in swine, a study was undertaken to carry out comparative ELISA on sera from swine infected with low, moderate and heavy doses of *Trichinella spiralis spiralis* and *T. spiralis nativa* using both homologous and heterlogous antigens respectively. The sera from all swine were also tested using a commercially available enzyme immunoassay test kit.

## MATERIALS AND METHODS

### ANIMALS

York x Landrace piglets weighing approximately 12 kg were purchased

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from a commercial swine producer. The pigs were maintained on commercially prepared hog grower. The Canadian Council on Animal Care guidelines outlined in the "Guide to the Care and Use of Experimental Animals, Volume I" were followed.

### EXPERIMENTAL DESIGN

Twenty-one piglets were divided into seven groups of three each. Pigs in groups 1, 2 and 3 were infected by drenching with 1000, 8000 and 64,000 infective *Trichinella spiralis spiralis* larvae respectively while the pigs in groups 4, 5 and 6 were infected with comparable doses of *T. spiralis nativa* larvae. The pigs in group 7 were noninfected controls.

Sera were collected from each pig postinfection on days 0, 7, 14, 16, 18, 20, 22, 24, 26, 28, 30, 33, 35, 37, 42, 63, 70, 77 and at slaughter and examined serologically for anti-*Trichinella* antibodies.

Pigs were killed between days 92 and 99 postinfection. Two hundred and ten grams of musculature (35 g each of tongue, masseter, diaphragm, intercostal, psoas and rectus abdominis) from each pig were examined by the pepsin digestion procedure for the presence of infective *Trichinella* larvae.

### LABORATORY PROCEDURES

Pepsin-digestion of muscle was carried out in a 1% pepsin-1% HCl digestion mixture as previously outlined (3).

Examination of sera for the presence of anti-*Trichinella* antibodies was carried out in duplicate using the ELISA triple antibody procedure of Gamble *et al* (1) but modified as previously described (3) with *T. spiralis spiralis* and *T. spiralis nativa* ES antigens prepared as described by Gamble *et al* (1). An optical density (OD) reading at 414 nm of $\geq 5$ x the mean of three negative porcine sera was considered to be positive. Sera from both *T. spiralis spiralis* and *T. spiralis nativa* infected pigs were also examined using a commercial *Trichinella spiralis* enzyme immunoassay test kit (Idetek, Inc., San Bruno, California 94066).

## RESULTS

### PARASITOLOGICAL FINDINGS

*Trichinella* infections were established in all pigs given infective *T. spiralis spiralis* larvae but not in pigs administered *T. spiralis nativa* (Table I) or in the controls. For the most part the infections of *T. spiralis spiralis* established in the pigs tended to be dose related.

### SEROLOGICAL FINDINGS

Seroconversion occurred in all pigs infected with *T. spiralis spiralis* and *T. spiralis nativa* while no *Trichinella* antibodies developed in the three control pigs. Anti-*Trichinella* antibodies were detected in the sera of pigs infected with *T. spiralis spiralis* and *T. spiralis nativa* by ELISA using both the homologous and heterologous antigens (Table I). The *T. spiralis nativa* antigen tended to give slightly higher OD readings. In those instances where the *T. spiralis nativa* antigen gave a positive OD reading before the *T. spiralis spiralis* antigen, the OD reading of the latter was consistently elevated.

The *T. spiralis* enzyme immunoassay test kit also detected anti-*Trichinella* antibodies in both the *T. spiralis spiralis* and *T. spiralis nativa* infected pigs. The enzyme immunoassay test kit was not as sensitive as the triple antibody ELISA usually taking two to three days longer to detect seroconversion.

Seroconversion occurred more rapidly in pigs infected with *T. spiralis spiralis* than in pigs receiving comparable doses of *T. spiralis nativa.* Once seroconversion occurred, anti-*Trichinella* antibodies were detected in all pigs up to the time of slaughter 92 to 99 days postinfection.

**TABLE I. Infections Established and Serological Findings in Swine Experimentally Infected with Low, Moderate and High Dose of Infective *Trichinella spiralis spiralis* and *T. spiralis nativa* Larvae**

| Larval Species | Larval Dose (k) | Pig | Infection Established (l/g) | Seroconversion (dpi[a]) *T.s. spiralis* antigen | Seroconversion (dpi[a]) *T.s. nativa* antigen | Seroconversion (dpi[a]) IAT[b] |
|---|---|---|---|---|---|---|
| | 1 | 1 | 0.32 | 28 | 28 | 30 |
| | | 2 | 0.08 | 28 | 28 | 28 |
| | | 3 | 110.7 | 20 | 20 | 24 |
| | | $\bar{X}$ | | 25.3 | 25.3 | 27.3 |
| *T. spiralis spiralis* | 8 | 4 | 12.1 | 22 | 22 | 22 |
| | | 5 | 81.4 | 22 | 20 | 22 |
| | | 6 | 50.7 | 22 | 20 | 24 |
| | | $\bar{X}$ | | 22 | 20.6 | 22.6 |
| | 64 | 7 | 221.4 | 22 | 20 | 24 |
| | | 8 | 370.2 | 18 | 18 | 18 |
| | | 9 | 466.6 | 20 | 20 | 28 |
| | | $\bar{X}$ | | 20 | 19.3 | 23.3 |
| | 1 | 10 | 0 | 35 | 35 | 35 |
| | | 11 | 0 | 35 | 30 | 37 |
| | | 12 | 0 | 28 | 33 | 33 |
| | | $\bar{X}$ | | 33.3 | 31 | 35 |
| | 8 | 13 | 0 | 28 | 28 | 28 |
| | | 14 | 0 | 24 | 24 | 24 |
| | | 15 | 0 | 24 | 24 | 26 |
| *T. spiralis nativa* | | $\bar{X}$ | | 25.3 | 25.3 | 26 |
| | 64 | 17 | 0 | 26 | 24 | 30 |
| | | 18 | 0 | 26 | 24 | 26 |
| | | 19 | 0 | 24 | 24 | 26 |
| | | $\bar{X}$ | | 25.3 | 24 | 27.3 |

[a]Days postinfection
[b]Immunoassay test kit

## DISCUSSION

Swine given comparable doses of infective larvae seroconverted more rapidly to *T. spiralis spiralis* than to *T. spiralis nativa.* As shown previously (2,3) the rate of development of anti-*Trichinella* antibodies and seroconversion occurred more rapidly in pigs receiving the larger doses of infective larvae. All pigs given various dose levels of *T. spiralis nativa* infective larvae seroconverted even though no trichinae were recovered by pepsin digestion of musculature at the time of slaughter.

Excretory-secretory antigens prepared from *T. spiralis spiralis* and *T. spiralis nativa* larvae, and used with the triple antibody ELISA appeared to be equally effective in diagnosing infections with either species in swine. These findings are consistent with previous studies which demonstrated the close antigenic relationship between *T. spiralis spiralis* and *T. spiralis nativa* (4,5).

The *Trichinella spiralis* enzyme immunoassay test kit also detected anti-*Trichinella* antibodies in swine infected with either *Trichinella* subspecies although seroconversions on the average were detected two to three days later by the test kit procedure.

These studies again demonstrate that there may be a period of several days between the presence of infective larvae in the musculature of pigs at 18 days postinfection (6) and the serological detection of anti-*Trichinella* antibodies.

There is little doubt that the triple antibody ELISA using ES antigen and the *Trichinella spiralis* enzyme immunoassay test kit developed for the rapid diagnosis of trichinosis in swine at slaughter and in the field detect the presence of anti-*Trichinella* antibodies if present in sera. However, because of the lag that usually occurs between the presence of infective larvae in the musculature and detectable anti-*Trichinella* antibodies in the sera (even in heavy infections), enzyme immunoassays with currently available antigens cannot be used with impunity to certify pork products "trichinae-free" even though this has been an objective and expectation of the pork-producing industry (7-9).

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