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A COMPARATIVE STUDY OF PARASITOLOGICAL AND SEROLOGICAL TESTS FOR THE DETECTION OF TRICHINELLOSIS IN SWINE

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The sensitivity and reliability of parasitological (tongue and Mm. auriculares dorsales biopsies) and serological (ELISA) tests for the detection of swine trichinellosis in vivo were evaluated during the course of experimental Trichinella spiralis infection and in naturally infected swine detected by field epizootiology. Experimentally infected pigs receiving high doses of T. spiralis muscle larvae (5000 ML) were both serologically and biopsy positive as early as three weeks postinfection. In low dose (300 ML) infected animals, antibodies were detectable 5-6 weeks postinfection, but the muscle larvae were found in biopsy samples much more rarely. Field evaluation of the ELISA and biopsy methods supported, in general, the results obtained during the experimental T.spiralis infection. The results showed ELISA as a more sensitive method for the in vivo diagnosis of swine trichinellosis because it was capable of detecting infections at tongue and diaphragm larval densities as low as 0.05 larvae per gram. The biopsy procedures, although directly demonstrating the infection densities, could detect only infections with ≥ 2.5 larvae per gram.

Key words: Trichinella spiralis, diagnosis, ELISA, biopsy

INTRODUCTION

The frequency of human infection with *Trichinella spiralis* in the former constituent republics of SFR Yugoslavia is considerable, especially in Serbia and Croatia (Rapić et al., 1985; Čuperlović, 1991). Over the past decade, in Serbia, this endemic disease kept breaking out yearly and involved a varying number of cases. In 1985, for example, 1200 cases were registered in contrast to about 50 in 1989. By November 1994, about 280 subjects were reported with clinically developed trichinellosis. In the majority of cases, pork and its products (dried pork, sausages) originating from privately owned farms where slaughter escapes any veterinary inspection was the chief source of human infection.

A detailed and reliable study of the prevalence of *T. spiralis* infection in swine in Serbia, has been made od 377,574 slaughtered animals examined for *Trichinella* infection by both trichinoscopy and the enzymatic digestion method. Infections were detected in only 99 (0.026%) animals and almost all were light infections (Djordjević, 1989).

A mandatory veterinary and sanitary inspection of all pork intended for public consumption and a low prevalence of trichinellosis in such pork are in disagreement with the relatively high number of infected humans with clinically confirmed disease. Accurate evaluation of the level of endemicity of *T. spiralis* infection in swine is obviously limited owing to farmers' and their customers' ignorance of the risk from this parasite, but also by the low intensity of infection in pigs which can easily be missed by the standard parasitological procedures. This indirectly affects the evaluation of human trichinellosis, too. Namely, in the majority of cases, consumption of pork with a low number of *T. spiralis* muscle larvae (ML) will result in mild human infection, probably subclinical, which usually remains undiagnosed (Zimmermann, 1975; Murrell, 1983; Murrell and Nelson, 1984; Ivanoska et al., 1989).

Detection of the infection is an important parameter for research on the epidemiology and control of trichinellosis. The direct methods such as the pooled sample digestion method or trichinoscopy are postmortem techniques very reliable for heavier infections (Van Knapen et al., 1981). On the other hand, the serological ELISA test and a simple biopsy technique for direct demonstration of the parasite are applicable for antemortem detection of *Trichinella*.

This paper will concentrate on an analysis of the sensitivity and reliability of the parasitological (biopsy) and serological (ELISA) methods for the detection of low-and high-dose experimental infection in swine *in vivo*. The influence of a challenge infection on the parasitological and serological findings was studied in parallel. On the side, comparative evaluation of the applied techniques was made in farm-raised pigs. Slaughtered animals were also subject to parasitology by enzymatic digestion for confirmation of infection.

MATERIAL AND METHODS

A n i m a l s: For the experimental *T.spiralis* infection, twelve Yorkshire Landrace pigs were used. The pigs were of either sex, matched for age and weight (app. 30 kg at the beginning of the experiment) and were treated to be helminth-free. In 18 farm-raised pigs, serological testing and tongue biopsy were performed in parallel. These pigs originated from small individual farms located in the endemic regions of Serbia and were part of wider studies on the epizootiology and prevalence of trichinellosis in swine (Čuperlović et al., 1989). At the end of the study, the animals were sacrificed and their muscles were examined for

the presence of *T. spiralis* larvae by trichinoscopy and the enzymatic digestion method.

Experimental protocol: *T. spiralis*, a strain isolated from infected swine muscles in a Belgrade slaughterhouse in 1976 (Lalić, 1989), was maintained by passage in Wistar rats. Infective ML for the inoculum were prepared from rat carcasses after digestion in 1% pepsin - 1% HCl solution. Inoculation was made by oesophageal intubation.

The experimental pigs, four per group, were subject to the following treatments: oral infection with 300 ML on day 0 (group A), oral infection with 5000 ML on day 42 (group C) and both the primary infection (300 ML) and the challenge dose (5000 ML) (group B). Each animal was bled at the beginning of the experiment (day 0) and then on days 21, 36, 42, 63 and 84 for serology. On days 21, 42, 63 and 84 of the experiment, biopsies were performed on the tongue and Mm. auriculares dorsales. Pigs were sacrificed on days 101 postinfection (group A), 118 (group B) and 125 (group C) and their tongues and diaphragms excised for evaluation of muscle larvae burden.

Biopsy: The biopsies were performed on tongue muscles and Mm. auriculares dorsales. The tongue biopsy was made as follows: the mouth was opened wide with a plastic speculum and the tongue was fixed and extended using steady traction. The biopsies (0.1 g each) were obtained by a 5 mm spherical dental punch under local anesthesia. Usually, three specimens (0.2-0.4 g total) were taken from each animal and were stored on ice until parasitological examination.

Mm. auriculares dorsales biopsy was carried out by a simple surgical procedure which included preoperative preparation of the site of biopsy, local anesthesia, skin incision, surgical removal of the muscle, sutures. The specimens weighed 0.2-0.8 g depending on animal size.

Entire biopsies were examined parasitologically in a stereomicroscope using the compression method.

Enzymatic digestion: Muscle larvae were recovered by enzymatic digestion of the tongue and diaphragm of each pig using the magnetic stirrer technique. Each muscle was weighed and then ground and mixed thoroughly, and 20 g were taken for digestion in a 1% pepsin - 1% HCl solution. Muscle larvae were recovered, counted and expressed as larvae per gram of muscle (LPG) or as total ML for the total tongue or diaphragm weight.

ELISA (a double antibody indirect method): Serum samples taken from each pig were assayed for antibody reactivity with *T. spiralis* ML excretory-secretory (ES) antigens by the ELISA (Gamble et al., 1983; Čuperlović et al., 1987). Briefly, microtitre plates were coated with ES antigens obtained after the *in vitro* cultivation of *T. spiralis* ML (Gamble, 1985). Swine antibody from the test sera (1:10 and 1:100 dilution) which bound to antigen was indicated by reaction with a horseradish peroxidase conjugate of sheep anti-swine IgG (INEP, Zemun) followed by

the substrate ABTS. The reaction was read at 410 nm 10 minutes later and then was related to positive or negative reference standards.

RESULTS

During the course of the infection no clinical signs were noticed in any of the 12 experimentally infected pigs, not even in animals infected with 5000 *T. spiralis* ML in whom, following previous experience, a transient inappetence could have been expected.

Tongue and Mm. auriculares dorsales biopsies performed under local anesthesia produced no harmful effects on any pig's health and both tissues healed well following the procedure. Tongue biopsies averaged 0.3 g (0.2-0.4 g) in mass and pigs resumed feeding immediately after the treatment. Mm. auriculares biopsies had greater muscle yield (0.2-0.8 g) depending on animal size.

The results of parasitological findings after final ML recoveries from the diaphragm and tongue are given in Table 1. The recovery of developed larvae from these muscles was correlated to the infection dose. In low-dose infection (300 ML), worm burdens ranged from 0.05-0.2 LPG in tongue and diaphragm tissues of primary infected pigs, while high-dose infection yielded 40-500 - fold final burdens. Primary infection with 300 ML provoked high resistance against a challenge dose of 5000 ML, with the level of 96% protection.

Table 1. *Trichinella spiralis* muscle larvae (ML) recovery from swine after low-dose, high-dose and challenge infections

Group/Treatment	TONGUE			DIAPHRAGM		
	Weight (g)	LPG	Total ML	Weight (g)	LPG	Total ML
A. 300 ML	293.0 ± 15.5	0.075 ± 0.02	22.3 ± 4.9	350.3 ± 14.5	0.16 ± 0.03	61.8 ± 7.9
B. 300 + 5000 ML	296.8 ± 33.5	1.49 ± 0.6	430.7 ± 139.6	349.0 ± 47.5	1.21 ± 0.3	444.1 ± 112.8
C. 5000 ML	276.0 ± 18.9	20.15 ± 11.1	5980.2 ± 3463.6	348.0 ± 23.8	35.2 ± 16.3	11889.7 ± 4962.6

Experimental pigs received on oral inoculation of either 300 *T. spiralis* ML on day O (group A) or 5000 *T. spiralis* ML on day 42 of the experiment (group C) or both (group B), and were sacrificed on days 101, 125 or 118 postinfection, respectively. LPG - larvae per gram tissue. Data are presented as group mean ± SEM.

Larval recoveries obtained in biopsy tissues (Figure 1) were also correlated to the infection dose as shown in high-dose infections in which muscle larvae were found in three out of four animals on day 21 postinfection and in all four infected pigs on day 42 postinfection. In the case of low-dose infection or that

followed by challenge, larval findings in biopsy tissues were rare and possible to detect only 42 days postinfection.

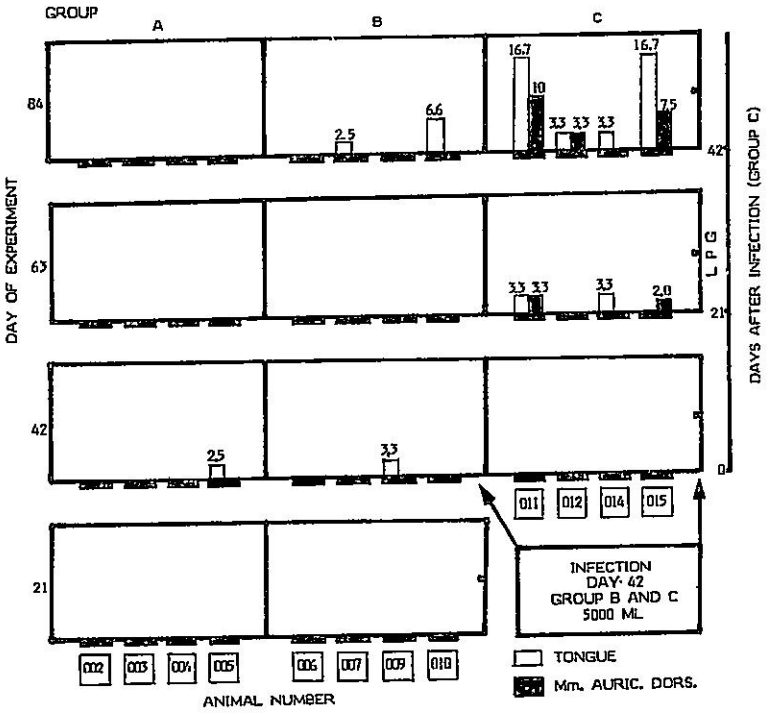

Figure 1. Assessment of *Trichinella spiralis* larval densities (LPG) in swine biopsy tissues following low-dose, high-dose and challenge infections. Tongue (open bars) and Mm. auriculares dorsales (solid bars) biopsies were performed at three week intervals postinoculation. Group A - 300 *T. spiralis* ML on day 0; group B - 300 ML on day 0 plus 5000 ML on day 42; group C - 5000 ML on day 42 of the experiment.

Temporal appearance of antibodies to *T. spiralis* ML ES products in the sera of experimental pigs is shown in Figure 2. The results of the ELISA revealed that these antibodies can be detected in the circulation as early as 21 days postinfection. However, the antibody findings were positive in all four high-dose infected pigs (group C), but in only one out of eight low-dose infected pigs. On days 36 and 42 postinfection, six and seven out of the eight low-dose infected pigs, respectively, had antibodies in circulation. The challenge infection with 5000 ML in pigs from group B did not influence antibody detection nor even their titres (data not shown), except for one pig where antibodies were detected only after challenge infection.

No relationship existed between worm burdens (both after biopsy and final ML recoveries) and ELISA OD values in either group.

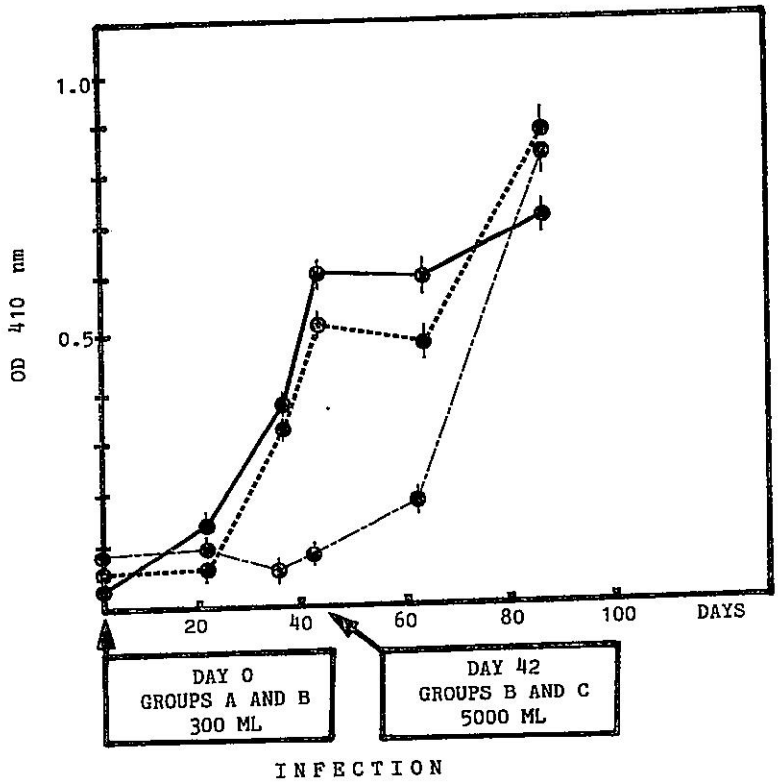

Figure 2 Development of antibody responses in swine following low-dose, high-dose and challenge infections. Graphs indicate ELISA optical densities (OD) for 1:10 diluted serum samples; values are the mean ± SEM (● – ●) Group A - 300 *T. spiralis* ML on day 0; (● – – – ●) Group B-300 mL on day 0 plus 5000 ML on day 42; (● – · – · – ·) Group C - 5000 MLon day 42 of the experiment.

A comparative evaluation of the applied methods for *in vivo* detection of *T. spiralis* was made on 18 farm-raised pigs. Ten of the 18 slaughtered pigs were parasitologically positive, as determined by *T. spiralis* larvae findings in diaphragm muscles on trichinoscopy. The antibodies were detected by the ELISA in 10 animals, while ML were recovered in biopsy tissues of only seven of these pigs, with worm burdens ranging from 3.3 to 683.6 LPG. In eight pigs with negative final ML burden, seven were both ELISA and biopsy negative. However, although no larvae were recovered after biopsy and slaughter, the ELISA detected antibodies in one pig at both 1:10 and 1:100 serum dilutions.Again, no relationship was seen between the ML findings in biopsy tissues and ELISA OD values.

DISCUSSION

The methods used for the detection of *T. spiralis* infection either *in vivo* (biopsy, serological tests) or postmortem (enzymatic digestion of pork) proved

reliable and sensitive in cases of high-dose infection at an almost parallel level. In low-dose infections, however, even after the challenge, only postmortem enzymatic digestions of tongue and diaphragm muscles (20 g) and/or serology were able to detect a very low level of infection. During the course of this experimental *T. spiralis* infection, pigs receiving 5000 ML became serologyically and biopsy positive as early as three weeks postinoculation. In low-dose infections, antibodies in circulation and, rarely, muscle larvae in biopsies (at least 2.5 LPG) were detectable much later, i. e. 5-6 weeks postinfection.

Field evaluation of the ELISA and biopsy methods yielded results which were in agreement, in general, with experimental infection testing. The results of the ELISA in naturally infected pigs strongly support the high sensitivity and reliability of this assay as all 10 serologically positive animals were confirmed to be infected since *T. spiralis* larvae were found in their diaphragm muscles. The tongue biopsy procedure detected only infections ≥ 3 LPG. A single ELISA positive antibody finding in a parasitologically negative pig might be a false-positive result or the reflection of low-intensity infection. Namely, as the pig originated from an endemic region, it could have been sufficiently exposed to *T. spiralis* to generate an antibody response but fail to develop recoverable muscle larvae.

The largest number of *T. spiralis* larvae, regardless of the infection dose, occurs in diaphragm and/or tongue musculature (Kotula et al., 1984; Đorđević, 1989), an important fact considering the applicability of tongue and Mm. auriculares dorsales biopsy techniques. Infection levels ≤ 3 LPG can easily be missed by the tongue biopsy procedure because a muscle tissue portion of only 0.3 g is obtainable with no harm to animal health. Of course, ML distribution throughout the tongue is anything but uniform and is, therefore, likely to yield occasional biopsy-positive results even in cases of low-intensity infections. Mm. auriculares dorsales biopsy, even with a 0.8 g specimen, is limited by the lower *T. spiralis* ML densities in these muscles. Thus, tongue and especially Mm. auriculares dorsales biopsies are slightly less sensitive methods for *T. spiralis* larvae detection than trichinoscopy at slaughter when 0.25 g of diaphragm muscle is tested. The biopsy technique is undoubtedly important in the diagnosis of swine infection as well as in other swine trichinellosis studies, but is limited by the infection intensity and sample size (Kazacos et al., 1986). Since a negative biopsy finding does not rule out the possibility of infection, a confirmatory test is required.

The ELISA test for immunodiagnosis of *T. spiralis* infection in swine proved sensitive and specific, and demonstrated great efficiency in detecting the positive animals and early seroconversions (Gamble et al., 1983; Murrell et al., 1986; Čuperlović et. al., 1987; Sofronić-Milosavljević et al., 1988). In our experimentally infected pigs, the ELISA was able to detect infections with tongue or diaphragm larval densities of 0.05-0.1 LPG. However, as previous studies (Gamble et al., 1983; Sofronić-Milosavljević et al., 1988) showed that the antibody findings and their appearance in the circulation were not in direct correlation with worm burdens but only with infection dose, it cannot be adopted as a rule that the ELISA would detect serum antibodies of all swine with infection densities less than 1 LPG. The high sensitivity of the ELISA is greatly promising for the control of swine

trichinellosis, e. g. in monitoring swine herds for outbreaks of the disease. In addition, it was proposed that this assay can be the foundation of an on-line laboratory-based inspection system (Oliver et al., 1989).

Due to the importance of swine trichinellosis for human health, the possible presence of *T. spiralis* muscle larvae in pork must be double-checked by the enzymatic digestion method to assure its safety for human consumption. However, 1 g muscle specimens as recommended under state regulations seem to be too small, especially when the intensity of infection is less than 1 LPG. Therefore, it is necessary to raise the quantity of examined samples at least in the high-risk, endemic areas.

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UPOREDNO OTKRIVANJE TRIHINELOZE SVINJA PRIMENOM PARAZITOLOŠKIH I SEROLOŠKIH TESTOVA

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SADRŽAJ

Osetljivost i primenljivost parazitoloških (biopsija jezika i Mm. auriculares dorsales) i serloških (ELISA) testova za otkrivanje trihineloze svinja *in vivo* određivana je tokom eksperimentalne infekcije sa *Trichinella spiralis* i/ili kod prirodno inficiranih životinja.

Svinje inficirane sa visokom dozom larvi *T. spiralis* (5000 ML) bile su serološki i biopsijom pozitivne već od treće nedelje posle infekcije. Kod svinja inficiranih sa niskom dozom larvi (300 ML) antitela u cirkulaciji su otkrivana tek od pete do šeste nedelje nakon infekcije, a parazitološki nalaz u bioptičkom materijalu bio je retko pozitivan. Ispitivanja izvršena na terenu u osnovu su se slagala sa rezultatima dobijenim nakon arteficijelne infekcije.

Kod svih ovih ispitivanja utvrđeno je da je ELISA znatno osetljiviji metod za *in vivo* otkrivanje trihineloze, s obzirom da je na ovaj način bilo moguće ustanoviti i infekcije gde je broj razvijenih mišićnih larvi po gramu tkiva dijafragme i/ili jezika bio 0,05. S druge strane, biopsijom je bilo moguće otkriti trihinelozu svinja samo ako je po jednom gramu mišićnog tkiva bilo ≥ 2,5 larvi.