Evaluation of the ELISA for the Serological Diagnosis of Trichinosis in Canadian Swine

H.J. Smith*

ABSTRACT

An ELISA using a *Trichinella spiralis spiralis* excretory-secretory antigen was evaluated as a procedure for the diagnosis of trichinosis in swine in Canada. Field and experimental trials were carried out using both indirect serological (ELISA) and direct parasitological (pepsin-digestion) methods concurrently on serum and musculature, respectively, from each animal.

The ELISA is a sensitive and specific test for the detection of *Trichinella* antibodies in porcine sera when present. The development of *Trichinella* antibodies appears to be dependent on the magnitude of the infection established, age of the infection when the animal is tested and the immunocompetence or response to infection of individual animals. False negative reactions were recorded in both field and experimental trials. In the field study, five of the 1009 swine examined were parasitologically positive with light infections ranging from 0.01 to 0.046 larvae per gram (la/g) of musculature yet all were serologically negative. Experimentally it was shown that *Trichinella* antibodies develop slowly, at least two to three months postinfection, in pigs with very light infections. Even in pigs which developed infections of 33 to 55 la/g of musculature, seroconversion occurred >23 and <30 days postinfection. The immunocompetence or response to infection of individual pigs was variable as illustrated by one pig inoculated with 3000 infective larvae which had consistently lower titers compared to others in the same group despite the establishment of a muscle infection of 8.5 la/g of musculature. One false positive reaction was recorded in the experimental trial in an animal which had received 100 larvae and seroconverted at about three months postinfection. Larvae were not recovered despite pepsin-digestion of 1200 g of musculature.

Based on the results of these trials, the ELISA cannot be recommended as a procedure to certify or ensure the safety of pork or pork products from infection with trichinosis, although it should prove a useful procedure in epidemiological studies of trichinosis within herds or defined foci of infection.

Key words: *Trichinella spiralis spiralis*, swine, ELISA, pepsin-digestion, diagnosis.

RÉSUMÉ

Cette expérience consistait à évaluer la technique ELISA qui fait appel à un antigène composé d'excrétions et de sécrétions de *Trichinella spiralis spiralis*, comme moyen de diagnostiquer la trichinose porcine, au Canada. L'auteur réalisa à cette fin des études cliniques et expérimentales, à l'aide de la méthode ELISA indirecte et de la digestion à la pepsine qu'il utilisa respectivement sur le sérum et les muscles de chaque porc.

La technique ELISA est un test sensible et spécifique pour détecter les anticorps contre *Trichinella*, dans le sérum de porcs qui en contient. Le développement de ces anticorps semble dépendre de la gravité de l'infection, de son âge au moment du test, ainsi que de l'immunocompétence individuelle des porcs. L'auteur enregistra de fausses réactions négatives, dans ses études tant cliniques qu'expérimentales. Dans les premières, cinq des 1009 porcs impliqués affichaient une légère infection qui se traduisait par la présence de 0,01 à 0,046 larve par gramme de tissu musculaire, bien que tous donnèrent des résultats sérologiques négatifs. Dans les secondes, il s'avéra que les anticorps contre *Trichinella* se développent lentement, en au moins deux à trois mois après l'infection, chez les porcs qui n'en présentent qu'une très légère. Même chez ceux qui recelaient de 33 à 55 larves par gramme de tissu musculaire, les anticorps sériques n'apparurent qu'entre 23 et 30 jours après l'infection. L'immunocompétence individuelle des porcs varia, comme le démontra l'un d'eux, après une infection avec 3000 larves; il afficha constamment un faible titre d'anticorps, par rapport à d'autres sujets de son groupe, en dépit d'une infection de 8,5 larves par gramme de tissu musculaire. Un porc qui avait reçu 100 larves donna une fausse réaction positive, au bout d'environ trois mois, puisque la digestion à la pepsine de 1200 g de son tissu musculaire n'y démontra aucune larve.

D'après les résultats précités, on ne peut recommander la technique ELISA comme moyen de certifier l'absence de trichines dans la viande de porcs, ou dans ses sous-produits. Elle devrait cependant s'avérer utile dans des études épizootiologiques de la trichinose, dans des troupeaux ou des foyers définis d'infection.

Mots clés: *Trichinella spiralis spiralis*, porcs, ELISA, digestion à la pepsine, diagnostic.

INTRODUCTION

The enzyme linked immunosorbent assay (ELISA) using a crude saline extract of muscle larvae as antigen was first used for the serological diagnosis of trichinosis in man in 1974 (1). In 1975, Ruitenberg *et al* (2) introduced the micro-ELISA for the serodiagnosis of *Trichinella* infection. Van Knapen *et al* (3,4,5,6) have carried out extensive studies on the application of an ELISA to diagnose trichinosis using a crude worm extract (CWE) antigen.

*Agriculture Canada, Animal Pathology Laboratory, P.O. Box 1410, Sackville, New Brunswick, Canada E0A 3C0.

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Major drawbacks in the development of ELISA were found to be poor discrimination between seropositive and seronegative individuals due to high background levels (absorbance value of the negative pool), false positive reactions due to nonspecific serum fractions and false negative reactions in immunologically low responding animals (7). Recently, high background levels have been reduced with improved antigens (8,9). In 1983, Gamble *et al* (10) described a triple antibody sandwich ELISA using an excretory-secretory (ES) antigen which, when compared to the CWE antigen, eliminated false positive reactions in sera from farm raised pigs and gave good sensitivity in naturally infected pigs with low parasitic burdens.

In order to critically evaluate the ELISA as a potential procedure for the diagnosis of trichinosis in swine in Canada, experimental and field trials were carried out in 1985 using the ELISA and an ES antigen.

MATERIALS AND METHODS

EXPERIMENTAL PROCEDURES

All swine in the experimental and field trials were examined by both direct parasitological (pepsin-digestion of musculature) and indirect serological (ELISA) methods.

Pepsin-digestion of musculature was carried out in a 1% pepsin-1% HCl digestion mixture at 37°C with constant agitation using magnetic stirrers. Larvae were recovered following washing (several times) with water, allowing to settle, and discarding the supernatant, until the mixture became clear.

The ELISA used was the triple antibody procedure of Gamble *et al*, and a *Trichinella spiralis spiralis* ES antigen (10). A sheep anti-rabbit IgG antiserum conjugated with horseradish peroxidase (HRPO) (CooperBiomedical Inc., Malvern, PA 19355, USA) was used instead of goat anti-rabbit IgG conjugate and the same enzyme. ABTS (2,2 — azino-di 3-ethylbenzothiazoline — 6 — sulfonic acid) was used instead of 5-ASA (5-amino-salicylic acid) as substrate. An optical density (OD) reading ≥ 5 X the mean of three negative swine sera was considered to be positive.

EXPERIMENTAL ANIMALS

Pigs were purchased as weanlings from a commercial swine producer. Wistar laboratory rats for propagation of *Trichinella* larvae were purchased from a commercial animal breeder. Pigs and rats were maintained on commercially prepared feed. The Canadian Council on Animal Care Guidelines outlined in a "Guide to the Care and Use of Experimental Animals, Volume I" were followed.

EXPERIMENTAL TRIAL

Eighteen pigs were divided into six groups of three each. Groups 1, 2, 3, 4, 5 and 6 were infected with 0, 100, 500, 1500, 3000 and 10,000 *Trichinella spiralis spiralis* larvae by gavage. Actual numbers of larvae were given to groups 2 and 3 while estimated numbers were given to groups 4, 5 and 6. The *T. spiralis spiralis* isolate was originally recovered from a pig in Nova Scotia and had been maintained in Wistar rats since 1974. All pigs were bled at 0, 9, 16, 23, 30, 37, 44, 51, 58, 65, 72, 79 and 86 days postinfection and the sera were examined by ELISA for *T. spiralis spiralis* antibodies. Each serum sample was examined by ELISA on three different occasions. The pigs were killed at the termination of the trial and musculature was examined by the pepsin-digestion procedure. Four hundred grams of musculature, including tongue, masseter, diaphragm, intercostals, psoas and rectus abdominis, were examined from each pig in groups 1, 4, 5 and 6. In Groups 2 and 3, 800 g of muscle tissue from each animal were examined with 1200 g from pig 2 of group 2.

During the trial a fecal examination was carried out on each pig for common gastrointestinal helminths using the simple flotation technique and a saturated sodium nitrate solution.

FIELD TRIAL

Between March and September, 1985, musculature and sera were collected from 1009 swine (market hogs, sows and stags) sent to slaughter at an abattoir under Federal Inspection. All swine originated in Nova Scotia. When available, samples were collected from swine in an area which has been a known focus of trichinosis infection (11). From 40 to 210 g of diaphragm from each pig were examined by the pepsin digestion procedure. Mean sample size was 136 g of muscle tissue with only 5.8% of the samples having < 100 g.

RESULTS

EXPERIMENTAL TRIAL

Parasitological and serological findings are given in Table I. Larvae were detected in the musculature of only one of three pigs given 100 larvae. All pigs given ≥ 500 larvae developed infections. Muscle infections established correlated positively with doses of infective larvae. All pigs were negative for gastrointestinal helminths based on results of fecal examination.

None of the pigs had positive ELISA readings ≤ 23 days postinfection. All pigs given 10,000 larvae and one pig which had received 1500 larvae reacted positively on day 30 postinfection. None of the pigs given lower doses (i.e. 100 or 500 larvae) seroconverted until at least 65 days postinfection. One infected pig that had received 500 larvae had not seroconverted at the conclusion of the trial. Group 5 pigs which were given 3000 infective larvae took from 37 to 58 days postinfection to seroconvert. Pig 2 in group 5 that had received 3000 infective larvae consistently had lower antibody levels than other pigs which had received ≥ 1500 larvae. The readings obtained for this pig consistently approximated the 5 × OD of normal sera criterion used so that it gave either a high negative or low positive reading depending on the cut-off point established for that particular test. There was usually an increase in titer (OD reading) for one and occasionally two weeks prior to an animal reacting positively.

FIELD TRIAL

Trichinella larvae were found in five pigs giving a prevalence of 0.496% in the 1009 pigs examined. Infections were light, ranging from 0.01 to 0.046 larvae per gram (la/g) of musculature.

All sera from the 1009 pigs were negative for *Trichinella spiralis spiralis* antibodies by ELISA.

TABLE I. Parasitological and Serological Findings in Pigs given from 0 to 10,000 *Trichinella spiralis spiralis* Larvae and held for 86 Days Postinfection

Group	Pig	Dose of *T. spiralis spiralis*	Infection Estab. (la/g)	ELISA Findings[a] Days Postinfection 0	9	16	23	30	37	44	51	58	65	72	79	86
1	1	0	0	---	---	---	---	---	---	---	---	---	---	---	---	---
	2	0	0	---	---	---	---	---	---	---	---	---	---	---	---	---
	3	0	0	---	---	---	---	---	---	---	---	---	---	---	---	---
2	1	103	0	---	---	---	---	---	---	---	---	---	---	---	---	---
	2	100	0	---	---	---	---	---	---	---	---	---	---	---	---	+++
	3	101	0.01	---	---	---	---	---	---	---	---	---	---	---	++-	---
3	1	500	0.0075	---	---	---	---	---	---	---	---	---	---	---	---	---
	2	505	0.7575	---	---	---	---	---	---	---	---	---	---	+++	+++	+++
	3	510	0.0075	---	---	---	---	---	---	---	---	---	--+	++-	+++	+++
4	1	1500	3.5	---	---	---	---	---	+++	+++	+++	+++	+++	+++	+++	+++
	2	1500	2.3	---	---	---	---	+++	+++	+++	+++	+++	+++	+++	+++	+++
	3	1500	3.3	---	---	---	---	---	+++	+++	+++	+++	+++	+++	+++	+++
5	1	3000	5.4	---	---	---	---	---	---	---	---	++-	+++	+++	+++	+++
	2	3000	8.5	---	---	---	---	---	---	+++	+-+	--+	--+	+--	---	+--
	3	3000	28.0	---	---	---	---	---	+++	+++	+++	+++	+++	+++	+++	+++
6	1	10000	55.7	---	---	---	---	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	10000	33.3	---	---	---	---	+++	+++	+++	+++	+++	+++	+++	+++	+++
	3	10000	35.5	---	---	---	---	+++	+++	+++	+++	+++	+++	+++	+++	+++

[a] Results of three tests on each serum sample

DISCUSSION

The results of this study indicate that the ELISA using an ES antigen readily detects *Trichinella* antibodies in porcine sera. Only one false positive reaction was recorded. This animal had been given 100 infective larvae and seroconverted approximately three months postinfection. No muscle larvae were recovered despite pepsin digestion of 1200 g of musculature.

In light infections, detectable antibodies develop slowly with false negative reactions frequently recorded based on the results of this study. At least 65 days elapsed before pigs given ≤ 500 larvae developed positive titers. One pig receiving 500 larvae had not seroconverted by three months postinfection. Even swine that received 3000 infective larvae took from 37 to 58 days postinfection to seroconvert. It is also evident that there is considerable variation in the immunological response to infection of different pigs. For example, pig 2 of group 5 which had received 3000 infective larvae had consistently lower titers, (with false negative reactions in some tests) compared to others of the same group despite the fact that a muscle infection of 8.5 la/g was established in the animal. In pigs given large doses (10,000) of infective larvae, there may be a period of up to five days or longer when the larvae in the musculature are infective yet false negative ELISA reactions may occur. The age of the muscle larvae, when they first become infective for a new host, is variously stated to be between 17 and 21 days after infection (12). All swine in this study were negative when bled on day 23 postinfection, but those infected with large doses of larvae had seroconverted when bled on day 30.

Gamble *et al* (10) reported that pigs receiving the high dose (10,000 larvae/pig) infections were serologically positive as early as day 15 postinoculation, while in low dose (500 larvae/pig) infections, antibodies were detected much later. It should be noted that they were using a strain of *Trichinella* that apparently gave much higher infections in swine. The strain of *Trichinella spiralis spiralis* used in this study had been maintained in rats since 1974. The infectivity of isolates or species of *Trichinella* is influenced by host and several workers have reported that a change of host has reduced the infectivity of *Trichinella* isolates in the initial passage (13).

The infections in rats used as a source of larvae in this study had been established for about six months. It is not known if the age of the infection affected the infectivity of the larvae. Nevertheless, the fact is that pigs which had 33 to 55 la/g of musculature at slaughter gave false negative ELISA reactions until some point >23 and <30 days postinfection.

Based on both the field and experimental trials reported in this study, a number of false negative ELISA reactions were recorded with most of these occurring in swine with <5 la/g of musculature. However, false negative results were recorded in one animal with an infection of 8.5 la/g of musculature. Murrell *et al* (14) in the United States report that the ELISA detects 93% of infected swine when a criterion of 5 × OD of normal sera is used and that the majority of false negative reactions are in swine with <5 la/g of musculature. The development or rate of development of *Trichinella* antibodies would appear to be related to the magnitude of the infection established, the age of the infection when the animal is tested and the immunological response to infection of individual animals.

The exact cause of the one false positive serological reaction recorded is not known. Presumably the light infection stimulated the pig to generate an antibody response but failed to establish an infection which could be detected by the pepsin digestion technique about three months postinfection. Cross reactions with other worms is unlikely as suggested by the negative fecal examinations.

The ELISA cannot be recommended as a procedure to certify or ensure the safety of pork or pork products from infection with trichinosis because of false negative reactions related to the magnitude and/or the age of the infection. On the other hand, the ELISA should prove to be a useful procedure in epidemiological studies of trichinosis within herds or defined foci of infection. Similar conclusions have also been made by Murrell *et al* (14) during field evaluation of the ELISA for swine trichinosis in the United States.

The parasitological results of the field trial carried out on 1009 swine originating in Nova Scotia gave a prevalence of infection of 0.496%, which is comparable to that of 0.4% reported by Frank (15) from the same

region in 1952 also using a pepsin digestion procedure. This is in marked contrast to the findings of Faubert *et al* (16) who reported a 2.5% prevalence of trichinosis in Quebec in 2046 sows aged between two and three years using an ELISA and a CWE antigen. Faubert *et al* (16) were not able to substantiate their serological findings by pepsin digestion and trichinoscopic parasitological examinations on the same animals.

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