IMMUNODIAGNOSIS OF SWINE TRICHINELLOSIS

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Abstract. A rapid, sensitive and specific serologic test has been developed for the diagnosis of swine trichinellosis. The ELISA based test utilizes L1 stichosome antigens recovered as excretory-secretory (ES) products from *in vitro* cultivated muscle larvae. Field studies conducted with 20,000 commercial swine using crude ES antigen demonstrated that the test could detect 98% of the medically significant infections. The test had a false-positive rate of less than 3%. Because of difficulties in regulating the quality and quantity of ES antigen and the need to continually maintain infected laboratory animals for producing the diagnostic reagent, efforts have been made to clone and express the gene(s) encoding the immunodominant ES antigens. To date a cDNA sequence, designated TsA–12, which codes in part for a 53–kDa ES antigen, has been identified and expressed in bacteria. Results demonstrate that TsA–12 is recognized by immune sera and further suggest that the immunodominant 45–, 48– and 53–kDa ES proteins which share antigenic epitopes are distinct glycoproteins.

INTRODUCTION

The development of a specific, sensitive and rapid serology test for the inspection of swine at the slaughterhouse has been a major impetus for research on this disease. Numerous tests have been developed for the detection of trichinellosis in swine, the most recent of which is the enzyme-linked immunosorbent assay (ELISA) (Ruitenberg *et al*, 1974; Ruitenberg and Van Knapen, 1977). ELISA tests were initially performed using crude protein extracts from *T. spiralis* muscle larvae as the antigen; however, high false-positive rates rendered the crude protein an unacceptable reagent (Clinard, 1979; Taylor *et al*, 1980). A major breakthrough in developing a more specific antigen reagent which eliminated most false-positive reactions occurred when the crude parasite antigen was replaced with purified stichocyte antigen (Seawright *et al*, 1983) or with ES antigen purified from *in vitro*-cultivated muscle larvae (Gamble *et al*, 1983; Gamble and Graham, 1984; Murrell *et al*, 1986). Both the stichocyte antigen and ES antigen are composed predominantly of immunologically cross-reactive 45-,48- and 53-kDa proteins which collectively form the basis for the present ELISA test; however, only ES antigen has been field tested because of the ease with which it can be produced relative to stichocyte antigen (Murrell *et al*, 1986; Oliver *et al*, 1989).

At present, commercially available test kits which utilize *in vitro*-derived antigen are under evaluation by the Food Safety and Inspection Service of the US Department of Agriculture. Should the industry adopt the use of a slaughter house test for trichinellosis, a more rapid, reproducible and economical method for generating antigen will be required. Current research in this area has concentrated on the cloning and expression of the genes encoding the ES antigens to facilitate production and purification of large quantities of the diagnostic reagent.

This review will summarize results from recent field trials using ES antigen for parasite diagnosis and present current research on cloning the gene(s) encoding these diagnostic antigens and the potential application of the expressed gene products in the diagnosis of swine trichinellosis.

FIELD TRIALS

To date, two major field evaluations have been performed to assess the efficacy of *T. spiralis* ES antigen as a diagnostic reagent for swine trichinellosis. The first such test was conducted using sera obtained from 5 different

swine herds containing naturally-infected animals as determined by pepsin:HCl digestion of 5–10g tongue or diaphragm tissues during epizootiologic field investigations (Murrell *et al*, 1986). The sera in this study were obtained from 305 pigs of which 162 were verified as being *T. spiralis* infected. Samples were classified as positive in ELISA testing by two criteria: those sera displaying optical density (OD) values > 5 (criterion I), and those where OD values > 4 (criterion II) relative to normal background controls. After examining sera from both infected and uninfected animals, the overall ELISA results indicated that between 93% and 96% of infected animals could be detected by ELISA using larval ES antigen (Table 1); the infected swine not detected had infection rates of less than 1 larvae per gram of muscle. These results supported previous conclusions derived from similar tests performed on experimentally-infected animals (Gamble *et al*, 1983). Both the sensitivity and specificity of the ELISA test exceeded 92% regardless of the criterion category used (Murrell *et al*, 1986) and correlated well with the epizootiologic studies. Although the putative number of false-positive reactions was low and within an acceptable range, the question has been raised whether or not these sera came from animals harboring low level infections of *T. spiralis* that went undetected during the epizootiologic field investigations. Since numerous studies have shown that the ELISA is sensitive to diaphragm larval densities as low as 0.1-1 pg, the digestion of 10 g of tongue tissue, which generally shows lower worm burdens than diaphragm (Kazacos *et al*, 1986), could easily have missed low levels of infection that were subsequently detected by ELISA. If such were the case, the false-positive rate for the ELISA could be significantly less than reported.

Although the rate of detection in this field trial was greater than 92%, controversy exists over the number of false-negative reactions that can be tolerated in a commercially available test. The determination of the lowest level of swine infection that will generate clinically significant human disease has contributed substantially to this controversy. Failure to detect 6 of the infected pigs in this study was attributed in part to the overall low level of infection observed within these animals (< 5 larvae per gram) and to the variability that occurs among hosts in their ability to mount an immune response to parasitic infections. Furthermore, the nutritional state of the animal and the potential suppressive effect of concurrent infections on the immune responses to *T. spiralis* were also suggested as contributing factors to false-negative reactions. Unfortunately, these problems will present difficulties for any test which relies upon the host to generate a detectable immune response to infection. Nevertheless, the promising results obtained using ES antigen in the small scale field trial described here was a major impetus for the initiation of a large scale field trial using 20,000 commercial swine.

A commercially prepared ELISA test utilizing the ES antigen reagent (AVID/Porcine™ from Idetek, San Bruno, California) was evaluated in a high volume abattoir; the total number of market swine tested was 20,978 (Oliver *et al*, 1989). Matched sera samples and diaphragm muscle samples were analyzed to determine the sensitivity and specificity of the test. The results are shown in Table 2. Serological analysis on these animals showed an average of –4.0% EIA. There were 288 animals with a result of 6.5% EIA or greater. Individual digestions using 2 to 147 grams of diaphragm tissue were performed on 157 of 288 serologically "positive" animals and two hogs were found to be lightly infected with *T. spiralis* (both had 0.2 larvae per gram). Therefore, at an ELISA threshold of 2.8%, 98% of medically significant infections would be detected; at this level, the false-positive rate would be 2.8%. Such false-reactions could be retested by the digestion method to assure they were safe for human consumption.

CLONING AND EXPRESSION OF GENE(S) ENCODING ES ANTIGEN

Evidence has been advanced above indicating that *in vitro*-derived ES antigen is an excellent immunodiagnostic reagent for swine trichinellosis; however, the production of antigen is not without its problems. The cost to generate the culture-derived reagent using rodents for parasite propagation, combined with incon-

Table 1

ELISA results using culture-derived excretory-secretory antigen to analyze sera from naturally-infected swine.

Source	Infected pigs* (positive/total infected)		Negative pigs* (positive/total negative)	
	Criterion I	Criterion II	Criterion I	Criterion II
Illinois	4/5	4/5	1/47	1/47
Indiana	29/31	29/31	3/32	3/32
New Jersey	116/123	120/123	5/51	7/51
Maine	2/3	3/3	2/13	2/13
Total	151/162	156/162	11/143	13/143
(%)	(93)	(96)	(8)	(9)

* Infections were determined by pepsin:HCl digestion of tongue or diaphragm tissue.

Table 2

Results of swine trichinellosis testing using both ELISA and muscle digestion procedures.*

ELISA results	Tissue digestion	
	Positive	Negative
Positive	2	286
Negative	0	20,690
	Total	20,976

* 20,978 Market hogs were tested. A minimum of 5 g muscle (diaphragm) samples were taken for digestion.

sistencies in the quality and amount of ES antigen obtained by these culture procedures, have imposed additional drawbacks to the application of ELISA testing of swine sera. To obviate these and other problems, efforts have been made to clone and express the gene(s) which encodes the immunodiagnostic ES antigens to assess whether a recombinant protein can reliably and adequately function as a substitute for the naturally-derived antigen.

Several groups have successfully cloned and expressed antigens from *T. spiralis*. Sugane and Matsuura (1990) identified and characterized a cDNA sequence encoding a *T. spiralis* 46-kDa antigen that is also present within ES products; however, this particular clone was detected using polyclonal mouse infection sera. As such, it is unclear whether this gene encodes one of the immunodominant ES antigens that form the basis of the ELISA test.

Zarlenga and Gamble (1990) identified one clone, designated TsA–12, by screening a cDNA expression library synthesized from *T. spiralis* L1 messenger RNA with polyclonal sera against monoclonal antibody-purified ES antigen and with moderate dose infection sera from experi-

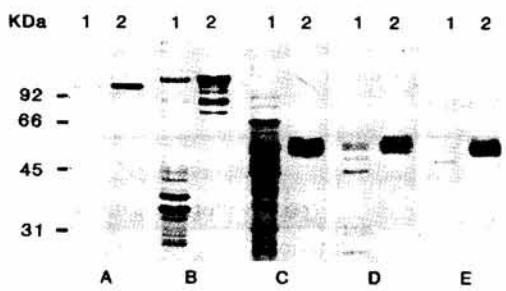

Fig 1 – Western blot analysis of purified TsA-12 recombinant antigen and *T. spiralis* naturally-derived antigens. Separated proteins were either visualized by Coomassie Blue staining (lanes 1) or blotted to nitrocellulose and screened with rabbit anti-TsA-12 serum (lanes 2). Separated proteins are as follows: A, purified beta-galactosidase; B, TsA-12 fusion protein; C, *T. spiralis* muscle larvae crude worm extract; D, *T. spiralis* muscle larvae ES protein, and; E, monoclonal antibody affinity-purified 49- and 53-kDa ES antigens.

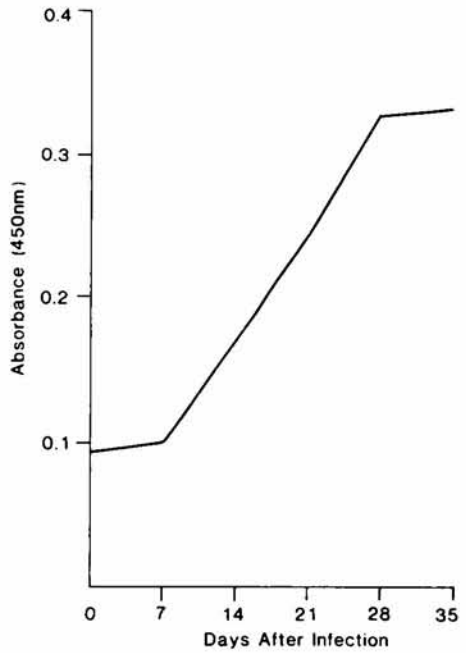

Fig 2 – Detection of anti TsA-12 antibodies in mice inoculated with 150 muscle larvae. Five mice were bled at each time interval defined above and the resulting ELISA values averaged.

mentally infected pigs. TsA-12 reacted positively with both sera and was specific for *T. spiralis* since no cross-reactivity was observed with sera from animals infected with *Ascaris suum* or *Trichuris suis*. However, when Western blots containing ES antigen were screened with rabbit antibodies to purified TsA-12 to identify the homologous native protein, hybridization was only observed with the 53-kDa component of ES antigen (Fig 1). Since antibody was generated against the primary structure of TsA-12 only, the results suggested that the antigenically cross-reactive 43-, 48-, and 53-kDa immunodominant ES antigens were distinct glycoproteins and not derived from a common precursor as originally believed.

Like ES antigen, TsA-12 was localized to the stichocyte cells of the muscle larvae and its production within infected mice correlated with the kinetics of muscle larvae encystment (Fig 2); however, the level of reactivity of TsA-12 antigen with infection sera was not equal to that of ES antigen. It is likely that the epitopes on the naturally-derived antigen which are most strongly recognized by infection sera and which form the basis for present ELISA test are the product of post-translational events such as protein glycosylation. Since these processing events are not performed by bacteria during exogenous gene expression, future research must concentrate on re-expressing this and other *T.spiralis* genes in eukaryotic systems capable of post-translationally modifying the gene product in an attempt to generate an antigen which more closely resembles the native protein.

CONCLUSIONS

The prevalence of *T. spiralis* varies dramatically throughout the world presenting a serious public health problem in some localities. Because of this parasite's ability to persist, especially its capability of using sylvatic hosts as a reservoir, the eradication of *T. spiralis* has all but been abandoned as a means of reducing the risk to human health. Consequently, within the past 10 years, research efforts have been

directed toward the development of a specific and sensitive diagnostic test for slaughterhouse and epidemiologic use. Specifically, the application of monoclonal antibody and recombinant DNA methodologies has led to significant advances in this area of research. The studies described here clearly demonstrate the utility of purified *T. spiralis* ES antigen to diagnose the disease in naturally-infected animals and should be adopted and standardized for commercial use. Future improvements to the test will likely result from developing a sensitive recombinant antigen to replace or enhance naturally-derived ES antigen.

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