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Serodiagnosis of *Trichinella spiralis* infections in pigs by enzyme-linked immunosorbent assays*

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Abstract

Antibodies to Trichinella spiralis *are detectable by enzyme-linked immunosorbent assays even in the earliest days after infection. A modification of the test, which is suitable for application in the slaughterhouse, is described with horseradish peroxidase as the marker enzyme.*

The detection of *Trichinella spiralis* infections in pigs at slaughter is mainly by direct tests (trichinoscopy or digestion method), in which the parasite itself is demonstrable (3, 5). Since trichinoscopy is not sufficiently sensitive and the digestion method is too time-consuming for routine use in a slaughterhouse laboratory, a different test, possibly by a serologic (i.e. indirect) method, was clearly needed. To be acceptable, the test should satisfy the following criteria: (*a*) simplicity of technique, (*b*) rapidity of operation, (*c*) ease of evaluation, and (*d*) reliability, with particular attention to sensitivity. No serologic method so far has been sensitive enough to detect antibodies shortly after the onset of a *T. spiralis* infection, so that a highly infected animal could escape detection during slaughterhouse inspection (4). The desired serologic method should therefore identify the parasite at least as early as 17 days after infection, since at that time *T. spiralis* larvae are infective, i.e., able to resist the digestive action in the human stomach.

The enzyme-linked immunosorbent assay (ELISA) seems to meet all the criteria mentioned above (1, 6, 7). In this immuno-enzyme method, specific antibodies are measured by enzyme-labelled anti-immunoglobulin in antigen-coated tubes. The enzyme remaining in the tubes after washing provides a measure of the amount of specific antibodies in the serum.

Materials and method

For the assay, a saline extract was prepared from *T. spiralis* larvae, isolated from the muscle of rats, which had been infected with 2 000–3 000 larvae about 4–5 weeks previously. The larvae were washed with saline and homogenized by ultrasonic vibration in a sodium carbonate buffer (0.1 mol/l, pH 9.6) with 0.02% sodium azide. The suspension of homogenized larvae was incubated on a magnetic stirrer at 4° C for 2 days. To obtain the soluble antigens the extract was centrifuged at 45 000 *g* for 1 h; the supernatant was decanted and the amount of protein was determined. In the assay, a quantity of 5 μg protein per ml was used.

Horseradish peroxidase (PO) conjugated to sheep anti-porcine IgG was prepared as reported by Kawaoi & Nakane (2). The enzyme preparation used was horseradish peroxidase Sigma type VI,[a] and the substrate was 5-amino-2-hydroxybenzoic acid (5AS) and hydrogen peroxide. This substrate was prepared by dissolving 80 mg of 5AS in 100 ml of hot distilled water. Directly prior to use the pH of this stock solution was brought to 6.0 with sodium hydroxide (1 mol/l), and to 9 ml of this 5AS was added 1 ml of 0.05% hydrogen peroxide.

The assay was performed as follows. Disposable polystyrene tubes (11 by 55 mm) [b] were coated with antigen by adding 1 ml of the antigen solution (in sodium carbonate buffer (0.1 mol/l, pH 9.6) with 0.02% sodium azide) and incubating the tubes in a water bath at 37°C for 3 h. The tubes were then emptied and refilled with 2 ml of a 4% bovine serum albumen (BSA) solution (in sodium carbonate buffer (0.1 mol/l, pH 9.6) with 0.02% sodium azide) and incubated with the BSA solution at 4°C overnight. Before the assay, the tubes were washed with tap water (3 times for 5 min each). The sera

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were diluted with phosphate-buffered saline (PBS), and 1 ml of diluted serum was added to each tube. The tubes were incubated at 37°C for 30 min and then washed with tap water (3 times for 5 min each). The tubes were incubated at 37°C for 30 min after the addition of 1 ml of a 1% BSA solution in distilled water to each one. The tubes were again washed with tap water (3 times for 5 min each). One ml of conjugate, diluted to the proper dilution in PBS with 1% BSA, was added. The tubes were incubated at 37°C for 30 min and then washed with tap water (3 times for 5 min each). One ml of substrate was added and the tubes were incubated at room temperature for 1–2 h. The reaction was stopped with 0.1 ml sodium hydroxide (1 mol/l). The reaction product (a brown colour) was measured in a spectrophotometer at 449 nm. The amount of antibodies present in the serum was expressed as the internal transmission density value. The assays were performed on semi-automated equipment for enzyme analysis.[a]

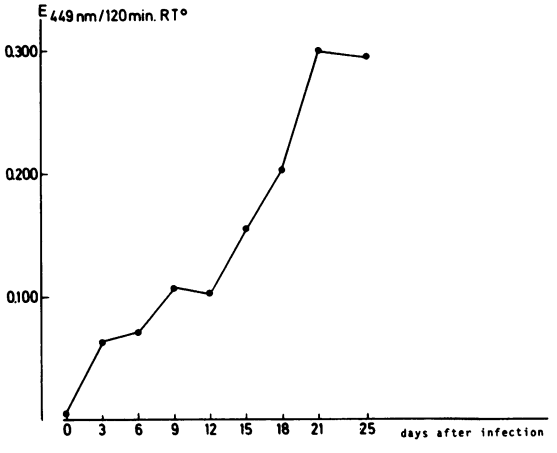

Fig. 1. Results of enzyme-linked immunosorbent assay in 1 pig infected with 5 000 *T. spiralis* larvae.

Results and conclusions

ELISA was tested in SPF (specific pathogen free) pigs experimentally infected with *T. spiralis* larvae. A typical example is presented in Fig. 1. Specific antibodies could be detected from 3 days after infection onwards, which suggests that the test is extremely sensitive.

The major advantage of ELISA is the speed with which the assay may be performed. Automation of all steps is feasible so that as many as 4 000 sera could be examined daily, even with the present macro-system originally developed for application in a clinical chemistry laboratory. Future research will be focused on the development of a micro-system in order to make ELISA even more convenient for routine use.

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[a] Olli System 3000; Olli, Kivenlahti, Finland.