

## Investigation of Cross-Reactions against *Trichinella spiralis* Antigens by Enzyme-Linked Immunosorbent Assay and Enzyme-Linked Immunoelctrotransfer Blot Assay in Patients with Various Diseases

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**Data regarding cross-reactions against *Trichinella spiralis* in humans are scarce and controversial. For this reason, we tested serum samples from patients with typhoid fever, brucellosis, toxoplasmosis, amoebiasis, cysticercosis, trichocephaliasis, ascariasis, and onchocerciasis against an antigenic extract of *T. spiralis* infective larvae in an enzyme-linked immunosorbent assay (ELISA) and an enzyme-linked immunoelctrotransfer blot (EITB) assay. All except one serum sample from the group of patients with onchocerciasis were negative in the ELISA; in the EITB assay, only faint bands were observed with the samples from patients with onchocerciasis and ascariasis and negative results were obtained with the samples from patients with other diseases. In conclusion, cross-reactions were found only in the groups of patients with other nematode infections and were of very low magnitude, most of them virtually negative.**

Trichinellosis is a worldwide zoonosis acquired by the ingestion of undercooked meat containing the infective larvae (L<sub>1</sub>) of the nematode *Trichinella spiralis* (8). The incidence of this disease remains high in some underdeveloped countries because of inadequate sanitary inspection of pork carcasses (12, 13). In Mexico, where pork is the main source of animal proteins, transmission of trichinellosis is favored because pigs are often free roaming, eating trash and dead animals. Clinical symptoms of trichinellosis are diverse and include abdominal pain, diarrhea, fever, myalgia, malaise, and periorbital edema, so trichinellosis could be confused with other infections (7, 9, 16, 17). Several methods for immunodiagnosis have been developed to detect antibodies in human and pig sera, with different sensitivities and specificities (2-6, 15, 18, 19). Possible cross-reactions have been investigated in a few studies: cross-reactive antibodies were present in pigs infected with *Ascaris suum* as determined by hemagglutination, bentonite agglutination, enzyme-lined immunosorbent assay (ELISA), or enzyme-linked immunoelctrotransfer blot (EITB) assay (3, 5). In other studies, *Trichinella* cross-reactive antibodies were found by agar precipitation in a serum sample from one typhoid fever patient (23) and by ELISA in up to 20% of patients with non-*Trichinella* parasitic diseases (4, 18). Nevertheless, to our knowledge, no systematic search for cross-reactive antibodies in defined groups of patients has been performed. The aim of this work was to analyze the presence of *Trichinella* cross-reactive antibodies by ELISA and EITB assay in the sera of patients with infectious diseases other than trichinellosis, which is prevalent in Mexico, since it would be of help to establish the usefulness of these techniques for differential diagnoses.

*T. spiralis* was maintained in male Wistar rats. The L<sub>1</sub> larvae were isolated by the HCl-pepsin method. The antigenic extract was prepared by the method described by Parkhouse et al.

(20). Larvae were suspended in 10 mM Tris (pH 8.0) containing proteinase inhibitors (TPCK [tolylsulfonyl phenylalanyl chloromethyl ketone], TLCK [*N*α-*p*-tosyl-L-lysine chloromethyl ketone], and phenylmethylsulfonyl fluoride; Sigma Co., St. Louis, Mo.) and homogenized on ice for 15 min. A 1/4 volume of 10% sodium deoxycholate (Sigma) was added, and the mixture was homogenized for another 15 min. After 20 min of incubation on ice, the mixture was centrifuged at 15,000 × *g* for 30 min at 4°C. After protein quantification, the antigen was aliquoted and stored frozen until use. Serum samples from patients with different diseases were kindly donated by several physicians and included samples from apparently healthy persons who went to local laboratories for conventional tests in order to obtain health certificates for marriage, sports, or job applications (*n* = 35; see acknowledgments) and specimens from patients with trichinellosis (*n* = 25), brucellosis (*n* = 31), toxoplasmosis (*n* = 16), typhoid fever (*n* = 12), amoebiasis (*n* = 8), trichocephaliasis (*n* = 1), cysticercosis (*n* = 9), ascariasis (*n* = 20), and onchocerciasis (*n* = 17). The ELISA was modified from a technique described elsewhere (2). Briefly, highly binding polystyrene plates (Costar, Cambridge, Mass.) were coated with 5 μg of antigen per ml at 4°C overnight. Wells were washed four times with 0.05% Tween 20 (Sigma) in 0.01 M phosphate-buffered 0.15 M saline (PBS), pH 7.2, and blocked with 1% human serum albumin (Gerencia General de Biológicos y Reactivos, SSA, México D. F., México) in PBS-Tween for 2 h at 37°C. Serum samples diluted 1:1,000 in PBS-Tween were incubated for 2 h at 37°C. A goat anti-human immunoglobulin-peroxidase conjugate (KPL, Gaithersburg, Md.) diluted 1:3,000 was added, and the mixture was incubated for 2 h at 37°C in PBS-Tween. Finally, a substrate-chromogen solution containing *O*-phenylenediamine (Sigma) and H<sub>2</sub>O<sub>2</sub> (Merck, Darmstadt, Germany) was used to develop the reaction, which was then stopped by the addition of 100 μl of 2 N H<sub>2</sub>SO<sub>4</sub>. A<sub>490</sub>s were obtained with an ELISA reader (Boehringer, Mannheim, Germany). The cutoff point was defined as the average of the absorbance values plus 3 standard deviations obtained with 35

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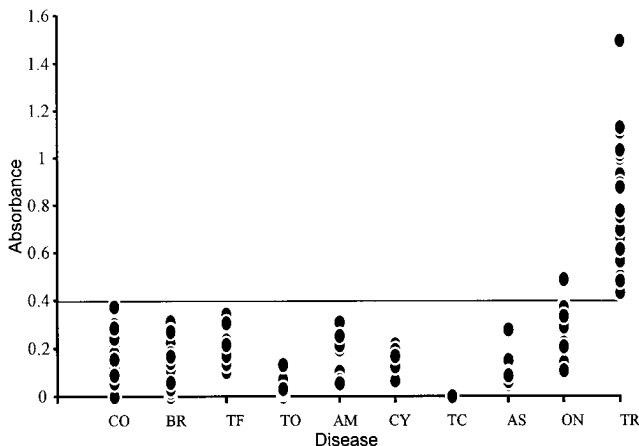


FIG. 1. *T. spiralis* antibodies in sera from patients with different diseases analyzed by ELISA. Samples were from normal controls (CO) or from patients with brucellosis (BR), typhoid fever (TF), toxoplasmosis (TO), amoebiasis (AM), cysticercosis (CY), trichocephaliosis (TC), ascariasis (AS), onchocerciasis (ON), or trichinellosis (TR). The line represents the cutoff point. The numbers of cases of each disease are listed in the text.

with peroxidase-conjugated goat antibodies against human immunoglobulins (KPL) diluted 1:1,000 in PBS-Tween for 2 h at room temperature; afterwards, the substrate-chromogen solution containing 50 mg of 4-chloro-1-naphthol (Sigma), 10 ml of methanol, 50 ml of PBS, and 50  $\mu$ l of 3% H<sub>2</sub>O<sub>2</sub> (Merck) was added. Reactions were stopped with tap water.

All serum samples from patients with brucellosis, toxoplasmosis, typhoid fever, amoebiasis, trichocephaliosis, ascariasis, and cysticercosis were negative by ELISA, and only 1 of 17 serum samples from the patients with onchocerciasis was positive. In contrast, all sera from patients with trichinellosis were positive by this method (Fig. 1). In the EITB assay, sera from patients with trichinellosis recognized the homogeneous band pattern previously described (1) (Fig. 2). The sera from most patients with onchocerciasis and ascariasis reacted by this method, although very faint bands were observed; there was no correlation between the absorbance of each serum sample as determined by ELISA and the number or intensity of the bands in the strips. However, an ELISA-positive sample from a patient with onchocerciasis reacted in the EITB assay (Fig. 2, second strip from left); a 77-kDa band was observed. One sample from the patients with ascariasis was also positive in the EITB assay (Fig. 2, leftmost AS Strip), although it was negative in the ELISA. No cross-reactive bands were found with serum samples from patients with the other diseases (not shown).

serum samples from healthy people. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out with Bio-Rad (Hercules, Calif.) equipment. A low-molecular-weight standard (Bio-Rad) was used as a marker. The antigenic preparation was solubilized under reducing conditions by the method of Laemmli (14). Proteins were transferred to nitrocellulose membranes (Bio-Rad) according to the procedure developed by Towbin et al. (21); for immunological reactions, the nitrocellulose membranes were blocked with 5% skim milk (Sveltes-Nestlé, México D. F., México) in PBS-Tween overnight at 4°C and strips 0.5 cm wide were cut. Each strip was incubated for 2 h at room temperature with one serum sample diluted 1:50 in PBS-Tween. After three washes with PBS-Tween and two washes with PBS (5 min each), bound antibodies were fixed to the antigen with 0.25% glutaraldehyde (Sigma) in cold PBS for 15 min by the method reported by Ikegaki and Kenett (11). The blots were washed once with PBS and blocked again with 0.1% bovine serum albumin (Sigma) in cold PBS (pH 8.5) for 20 min. The strips were then incubated

Weiner and Price observed cross-reactions against a saline extract of *T. spiralis* larvae in a micro-precipitin ring test when they tested one sample from a clinical case of typhoid fever (23). The different results obtained in the present work are due partially to the antigenic preparation, since the one used in our study is enriched in surface components, and partially to the greater sensitivity of both enzymatic techniques, as well as the specificity of the EITB assay. We conclude that false-positive reactions for the sera of patients with typhoid fever do not occur in the ELISA or the EITB assay. Besides the report cited above (23), there are no studies that systematically analyze the presence of circulating cross-reactive antibodies against *T. spiralis* in patients with other diseases; exceptions are data for patients and pigs with nematode infections, although they are scarce and controversial. Some authors have suggested that the reliability of serological methods for porcine trichinellosis depends on the quality and specificity of the *T. spiralis* antigen used and that the occurrence of false-positive reactions is due

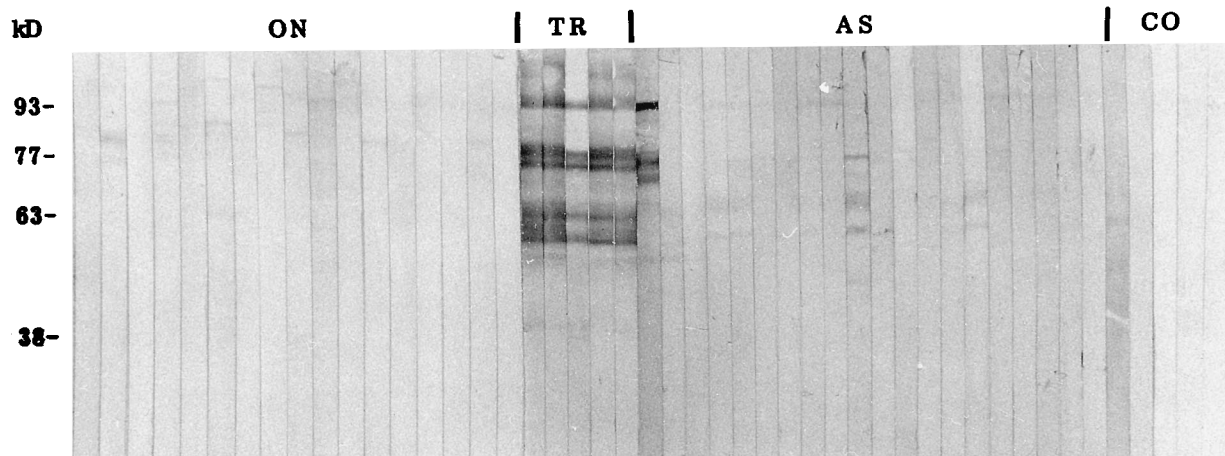


FIG. 2. *T. spiralis* antibodies in sera from patients with different diseases determined by EITB assay. Nitrocellulose strips for patients with onchocerciasis (ON), trichinellosis (TR), or ascariasis (AS) and for normal controls (CO) are shown. Molecular mass markers are indicated in kilodaltons on the left. The numbers of cases of each disease are given in the text.

to the use of crude parasite extracts (5, 10). This is supported by the results of Arriaga et al., who observed high absorbance values when they assayed serum samples from pigs experimentally infected with *A. suum* or *Trichuris suis* against a total soluble extract of *T. spiralis* larvae, while low values were detected when they used purified antigens from either surface-stichosomal or excretory-secretory components (3). Conversely, van Knapen et al. failed to detect cross-reactions in ELISA against a crude antigen of *T. spiralis* in pigs infected with an *Ascaris* sp., an *Oesophagostomum* sp., and a *Balantidium* sp. (22). More recently, a recombinant excretory-secretory antigen of *T. spiralis* that does not bind serum antibodies from animals experimentally infected with *A. suum* or with *T. suis* was produced (24). In the present study, we found cross-reactive bands in the EITB assay only with the samples from patients with onchocerciasis and ascariasis; these reactions were expected, since *Onchocerca*, *Ascaris*, and *Trichinella* parasites are nematodes and common epitopes could be present. However, the responses observed were very faint in comparison with the strong response observed for the patients with trichinellosis; moreover, all samples from these cases except for one were negative in the ELISA, and the sample from the patient with trichocephaliosis was negative in both the ELISA and the EITB assay. However, Au et al. analyzed an outbreak by ELISA and found three cross-reactive samples, from patients with clonorchiasis, hookworm, and ascariasis (4), and Morakote et al. found that 21 and 9% of patients with intestinal nematode and cestode infections were found to be positive for Immunoglobulin G and M antibodies, respectively (18). These studies were performed with a saline crude antigen of the parasite that could have more cross-reactive epitopes than the antigen used in the present work (see reference 3). Absorbance values of the cross-reactive sera were around the cutoff point, except for those from two cases of capillariasis, a disease not tested in this study because it is not found in Mexico. The patient with onchocerciasis, whose serum reacted positively in both the ELISA and the EITB assay, could also be infected with other helminths (including a *Trichinella* sp.), because the sample was also positive when tested against *Taenia solium* larvae in the ELISA (17a). Although the patient was diagnosed as having *Onchocerca volvulus* infection, other parasitic infections could not be excluded. The lack of cross-reactions found for most patients with onchocerciasis and ascariasis in the ELISA could be explained by cross-reactive antibodies which are present in low concentrations or which are of low affinity; either possibility is supported by the faint bands observed in most strips in the EITB assay. Thus, cross-positivity fell below the cutoff point in the ELISA, allowing discrimination between trichinellosis and other nematode infections.

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samples were given to us by Margarita Guerrero (Centro de Salud; M. González-DDF. México D. F.). We acknowledge Ana Flisser and Peter Schantz for critical review of the manuscript and María Teresa Negrete for manuscript preparation assistance.

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