HUMAN TRICHINELLOSION IN SOUTHERN SPAIN: SEROLOGIC AND EPIDEMIOLOGIC STUDY

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Abstract. An outbreak of trichinellosis caused by wild boar meat occurred in the Iruela (Jaén) in southern Spain in February 1996. Thirty-five people were diagnosed on the basis of epidemiologic data, but only 24 patients agreed to participate in this study. Twenty-three (96%) had symptoms suggestive of trichinellosis. Immunofluorescent and Western blot test results for trichinellosis were positive in 18 persons, and 15 had circulating Trichinella spiralis antigens. These findings suggest that results of tests for circulating antigens in conjunction with clinical presentation are useful for the diagnosis of trichinellosis.

Trichinellosis is acquired by ingestion of meat containing viable L1 larvae of the nematode Trichinella spp. Apart from infection with Trichinella spiralis, other species such as T. pseudospiralis, T. nativa, and T. britovi have been reported in New Zealand and France.1±4 Two outbreaks of trichinellosis occurred in the Cuenca and Teruel Provinces of Spain in 1993 and 1994. The sources of infection were raw sausages prepared with pork and wild boar meat. In both outbreaks, T. britovi was identified by the random amplified polymorphic DNA method.5

Although trichinellosis is a notifiable disease in Spain, it remains a major public health threat since the parasite is enzootic in domestic pigs and wild animals. From January 1980 to May 1990, 1,261 cases were reported, 601 (47.7%) in Andalusia region in southern Spain; 124 new cases were reported in 1996.7 The real incidence is likely to be greater since most cases are asymptomatic. We present epidemiologic and serologic data from an outbreak that occurred in the province of Jaen in southern Spain.

MATERIALS AND METHODS

Description of the outbreak. The outbreak occurred in February 1996 in the Iruela (Jaén) near the National Park of Cazorla, Segura, and Las Villas. Wild boars, foxes, wild cats, and deer are predominant in the park, and illegal hunting is common, although rules for hunting have been established. The source of infection was a mixture of meat (60% from inspected pork and 40% from non-inspected wild boar meat) that was used in the production of sausages and salami. According to a classification scheme,8 the outbreak was considered moderate. There were no severe complications and no patients died. The incubation period after consumption of infected meat ranged from 3 to 20 days. Patients were treated with mebendazole.

Thirty-five people who visited a local hospital were interviewed. They reported eating the suspected products and had signs and/or symptoms suggestive of trichinellosis. Twenty-four individuals participated in this study.

Source of infection. Parasite infestation of the sausage was documented at the Department of Public Health of the Andalusian Health Service in Jaen. The specimens were individually examined by acid-pepsin digestion,9 followed by microscopic examination of the sediment.

Study population. The 24 patients, who were members of 7 families: 10 females and 14 males with age range of 8–72 years, had serologic studies performed. Written consent was given by all persons prior to their enrollment in the study. Ethical review and approval of this study were obtained from the Institutions involved. Three sera samples per patient were examined, the first one at 30 days postinfection and the other 2 at monthly intervals.

Antigens. Trichinella spiralis M SUS/ES/59/LASO (deposited in the Istituto Superiore di Sanità, Rome, Italy, with the code ISS112) first-stage (L1) larvae, maintained in Swiss mice and recovered by acid-pepsin digestion,9 were used as the antigen source for an indirect immunofluorescence (IIF) test. Adult T. spiralis (72-hr old) worms were obtained from Swiss mice infected with 5,000 L1 larvae as previously described.10 Crude extracts were prepared by homogenization of L1 larvae and 72-hr-old adult worms in phosphate-buffered saline (PBS) containing 1mM phenylmethylsulfonyl fluoride and 5mM EDTA. The homogenate was sonicated and centrifuged at 12,000 × g for 30 min at 4°C. Excretory/secretory (ES) antigens were obtained by incubation of L1 larvae and 72-hr-old adult worms in Dulbecco’s minimal essential medium containing penicillin and streptomycin as previously described.11 The protein concentration was measured by the Bradford procedure.12 Adult worm extract and ES antigens were used for Western blotting.

Antisera. Antisera were produced by oral infection of rabbits with 10,000 larvae, followed by booster infections with 5,000 and 2,500 larvae at monthly intervals. Immunoglobulin G was obtained by precipitation of antisera with caprylic acid.13 Labeling with horseradish peroxidase was carried out using the periodate procedure.14

Seroologic tests. An IIF test with whole larvae15 was used to detect antibodies. Western blotting was performed using crude adult worm extract and ES subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (10% gel).16 Goat anti-human immunoglobulin conjugated to peroxidase (Bio-Rad, Hercules, CA) was used to detect antibody binding. An ELISA sandwich technique to detect circulating antigens17 was carried using serum obtained 30 days after infection. Briefly, high-binding plates were coated with 0.68 μg/well of anti-T. spiralis IgG (100 μl/well) for 48 hr at 4°C. Three samples of undiluted serum were added and incubated for 4 hr at 37°C. Rabbit anti-Trichinella IgG conjugated to peroxidase at a 1:10 dilution was added and incubated for 1 hr at 37°C. Plates were de-
veloped with azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (Sigma, St. Louis, MO) (1 mg/ml) and 0.003% hydrogen peroxide in 0.1 M citrate-phosphate buffer for 15 min in the dark and read at an absorbance of 405 nm. A limiting optical density (OD) value was calculated by adding to the mean of the OD values of 30 sera from healthy donors the results of multiplying the SD by 2.57; OD values above this limit were considered positive. Circulating antigen-positive and -negative sera were used as controls.

Eosinophil levels were measured and a muscular enzymatic profile (creatine phosphokinase [CPK] and lactate dehydrogenase [LDH] levels) (normal values, 195 U/L and 500 U/L, respectively) was done in sera obtained 30 days postinfection.

RESULTS

Samples of sausage and salami analyzed by acid-pepsin digestion revealed *Trichinella* spp. larvae. Since pork was previously inspected and showed negative results, it was assumed that the non-inspected wild boar meat used in the mixture was the source of infection.

Of the 24 patients studied, 23 (96%) had symptoms of trichinellosis: 23 (96%) had myalgias, 20 (83.3%) had fever, 20 (83.3%) had periorbital edema, 13 (54.1%) had diarrhea, and 12 (50%) had peripheral edema. Eosinophil levels were >10% of the total leukocytes in 20 (83.3%) patients and >40% in 4 (16.6%) of them. Levels of CPK were elevated (range = 298–6,540 U/L) in 14 (58.3%) subjects. Thirteen (56.5%) of 23 patients had elevated levels of LDH, ranging from 526 to 1,375 U/L.

Results of serum antibody tests are summarized in Figure 1. Initial samples from 18 (75%) patients were positive by the IIF test (titers range = 1:80–1:5,120, the most common titer was 1:640) (Figure 2). Eighteen patients positive by the IIF test were also positive by Western blotting. The ES from adult worms was hardly reactive by Western blotting; only a 43.1-kD band was seen. Two different responses to the rest of the antigens were observed. The first was for patients with low IIF titers (1:80–1:160). This response was directed mainly to *T. spiralis* larvae group 1 (TSL-1) antigens of ES products (Figure 3, patient 8) and to 33–215-kD polypeptides in crude extracts from adult worms (Figure 4, patient 8). A similar pattern was observed with crude extract from *L. larvae* (Figure 5, patient 8). The other response was observed...
in patients with higher IIF titers (1:320–1:5,120). These showed antibody responses to polypeptides with apparent sizes < 31 kD both in ES products (Figure 3, patient 11) and crude adult worm extracts (Figures 4 and 5, patient 11). The sandwich ELISA result for antigen detection was positive in 15 (62.5%) patients. The OD value was > 0.5 in all serum samples (Figure 6). Serologic results from symptomatic patients are shown in Table 1.

DISCUSSION

Both _T. spiralis_ and _T. britovi_ infections have been reported in Spain. According to the criteria given by some investigators, the outbreak that took place in 1990 may have been caused by _T. britovi_. It is remarkable that the epidemic described here occurred in Andalusia, which is where most cases of trichinellosis in Spain are reported. The increasingly widespread habit of eating wild boar meat and poor understanding of how trichinellosis is transmitted may account for occurrence of the epidemic. The epidemiology and ecology of trichinellosis in the European Union have been related to the ingestion of meat from imported animals and the eating habits of certain sectors of the population. Infections due to the consumption of pork are rare, except in Spain where wild boar and pork are the main sources of infections. The increase in the wild boar population in Europe over the past 20 may also account for many cases of trichinellosis in France, Germany, and Italy.

Our clinical findings did not differ from those reported by others. Immune response was evaluated by the IIF test for the detection of antibodies against cuticular _L_1 larvae, and Western blotting was used to detect antibodies to somatic and ES antigens. A good correspondence between the results of the IIF test and Western blotting was found. All sera negative by the IIF test were also negative by Western blotting. Similarly, there was a good correspondence between IIF titers and the number of polypeptides shown by Western blotting. Crude extract from the adult stage has not been frequently used in diagnosis or seroepidemiologic studies, and, as far as we know, this is the second time these types of antigens have been used. Prominent adult antigens > 94 kD and < 43 kD were detected by other investigators; our findings differ slightly from their results. In fact, prominent polypeptides < 31 kD were observed. We have also detected these low molecular weight antigens in other epidemic outbreaks (Rodriguez M and others, unpublished data). The ES products from _L_1 larvae have been widely used for diagnosis in both human and pigs with excellent results. Some of these antigens (TSL-1), contain the tyvelose epitope that is dominant in the immune response and is highly specific.

The ES antigens from the adult stage had not been previously studied. The data reported here show that the response to these antigens was very low and only a 43.1-kD antigen was detected. After a primary infection, it is likely that few or no adult worms develop in the host gut and that most exposure is to antigens from larvae. Also, the antigens most likely to be detected would be those that share epitopes with antigens from larvae. Cuticular antigens from 72-hr-old worms and antibody recognition of them by sera from in-

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<th>Table 1</th>
<th>Serologic results in symptomatic patients*</th>
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<td>No. of patients</td>
<td>IIF+ Western blot</td>
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* All tests were performed on sera taken at 30 days postinfection. IIF = indirect immunofluorescence.
fected rats have been characterized. Reacting antibodies appeared at about the same time as those reacting with infective larvae but were decreased at 20 days postinfection. It could be speculated that the same thing occurred with ES antigens, which contain cuticular antigens, and this could be reason antibodies are not detected. Antigens from the adult stage have an important advantage because by the third day postinfection adult worms can easily be obtained from the host intestine. Thus, its preparation is less time-consuming than that of larvae, which require at least a month of infection. On the other hand, it is necessary to determine the sensitivity and specificity of the 72-hr-old worm extract for a reliable diagnosis.

Antigen was detected in 62.5% of the patients. This percentage is similar to that previously reported by our group\textsuperscript{27,28} and other investigators\textsuperscript{29} and in agreement with our previous findings, the higher OD values were obtained in patients who, in spite of having eaten infected meat, did not develop detectable antibodies during the time of the study. The present data show that the detection of circulating antigens as a diagnostic tool for trichinellosis is worth considering, especially in association with other methods.

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REFERENCES


