Validation of an Enzyme-Linked Immunosorbent Assay for Diagnosis of Human Trichinellosis

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Trichinellosis is a zoonotic disease caused by the consumption of raw or semiraw meat from different animals harboring Trichinella larvae in their muscles. Since there are no pathognomonic signs, diagnosis can be difficult; for this reason, serology is important. The objective of this study was to validate an enzyme-linked immunosorbent assay (ELISA) using excretory/secretory antigens to detect anti-Trichinella immunoglobulin G antibodies in human sera. A total of 3,505 human serum samples were tested. A receiver-operator characteristic (ROC) curve analysis was performed. The accuracy of the test was determined by calculating the area under the curve, which was equal to 0.999, indicating high accuracy. The coefficient of variation calculated for data from four serum samples in eight working sessions was no higher than 5% for the positive sera or 14% for the negative sera. Moreover, the analysis of the differences in optical density between duplicates indicated a high repeatability for the ELISA. At the ROC optimized cutoff, the sensitivity and specificity of the test were, respectively, 99.2% and 90.6% (specificity of 95.6% when excluding the samples from multiparasitized persons from Tanzania). The validated ELISA showed good performance in terms of sensitivity, repeatability, and reproducibility, whereas the specificity was limited. These results suggest that this test is suitable for detecting anti-Trichinella antibodies in human sera for diagnostic purposes, whereas its use in epidemiological surveys could be questionable.

Trichinellosis (formerly known as trichinosis or trichiniasis) is the human form of the disease induced by nematode worms of the genus Trichinella (formerly known as Trichina) and is acquired through the consumption of raw or undercooked meat or meat products (e.g., sausages and salami) that harbor Trichinella sp. larvae (13). Although pork is the most common source of infection, meat from a variety of other animals has been implicated, including omnivores (e.g., wild boars), herbivores (e.g., horses in France and Italy and sheep in China), and carnivores (e.g., bears, cougars, foxes, badgers, jackals, dogs, and walruses) (33). Trichinella sp. larvae have also been detected in omnivorous and carnivorous birds, crocodiles, and monitor lizards, though human infection has never been found to be associated with the consumption of the meat of these animals, except for a monitor lizard and a turtle in Thailand (34).

Since there are no pathognomonic signs or symptoms of trichinellosis, clinical diagnosis is difficult, and diagnosis should be based on three main criteria: patient history of exposure, clinical evaluation, and laboratory tests, including serology and/or the detection of larvae in a muscle biopsy (13). However, the collection of a muscle biopsy is invasive and painful, and the result is not always positive even when infection is present.

Serology has a great diagnostic value and is of extreme practical use. Immunoglobulin G (IgG) antibodies can be detected from 15 to 60 days postinfection (13) and may persist for more than 30 years after infection (17). Although a plethora of tests for the detection of IgG antibodies have been developed, the most commonly used test is an enzyme-linked immunosorbent assay (ELISA), given its sensitivity (13, 14, 19). This test was first developed using low-specificity crude worm extract antigens prepared from L1 larvae (4, 11, 12, 44) and then using more-specific excretory/secretory antigens (ESA) prepared from L1 larvae maintained in culture (1, 2, 5, 9, 10, 15, 20, 24, 25, 32, 41, 43, 47).

Trichinella spiralis muscle larva antigens have been classified into eight groups (TSL-1 to TSL-8) based on their recognition by different monoclonal and polyclonal antibodies (29). Given that the antigenic pattern of all of the currently recognized Trichinella species and genotypes is quite similar, the antigens prepared with one species or genotype can be used to detect specific antibodies in persons infected with a different species (19). For ESA, the most abundant antigen is TSL-1, which is stage-specific, originates from the stichosome (a glandular structure consisting of 50 to 55 discoid cells or “stichocytes” which occupies the anterior half of the L1 larva), and is present in the larval cuticular surface. TSL-1 antigens share an immuno-dominant carbohydrate epitope (tyvelose [3,6-dideoxy-o-arabinohexose]), which is considered to be unique for parasites of the genus Trichinella (29).

Although ELISA is the most commonly used serological test for diagnosing trichinellosis, it has not been standardized, and most of the commercial ELISA kits for human serology are unreliable (19, 35). In highly specialized laboratories, the Western blot (Wb) assay is generally used as the confirmatory test for ELISA-positive sera (12, 19, 35, 39). Thus, laboratories...
accredited according to the ISO/IEC 17025:2005 and undertaking serological tests have to validate the ELISA to confirm that the method is suitable for its intended use. Therefore, the aim of this work was to validate an ELISA using ESA to detect specific anti-Trichinella IgG antibodies in human sera.

MATERIALS AND METHODS

Antigens. ESA were prepared from T. spiralis muscle larvae collected after HCl-pepsin digestion of infected mouse muscles, according to the method of Gamble (18). Briefly, T. spiralis muscle larvae were washed three times in phosphate-buffered saline (pH 7.2) with penicillin (500 units/ml) and streptomycin (500 μg/ml). The larvae were then washed four times by allowing them to settle in Dulbecco’s modified Eagle’s medium supplemented with penicillin (500 units/ml) and streptomycin (500 μg/ml). Five thousand worms per ml were then resuspended in Dulbecco’s modified Eagle’s medium supplemented with 1 M HEPES, 200 mM L-glutamine, 100 mM Na-pyruvate, and 5,000 units of penicillin/streptomycin (Gibco, Grand Island, NY), and incubated with 10% CO₂ in a 75-cm² culture flask (Corning Life Sciences, Pittsburgh, PA) at 37°C for 18 h. Once the worms settled to the bottom of the flask, the medium was transferred to 50-ml conical tubes. The medium was filtered through a 0.2-μm YM-5 filter, and the supernatant was concentrated 100 times in an Amicon pressure concentrating chamber (Amicon, Inc., Billerica, MA). To determine the protein concentration and to establish the high quality of the batch (i.e., no bacterial or somatic contamination), the optical density (OD) was evaluated at a 280/260-nm ratio; antigens with a ratio higher than 1.2 were used.

Human sera. A total of 3,505 human serum samples were analyzed. Of these, 1,159 were from healthy Italians, who, according to Italian law, were considered to be suitable for blood donation (negative reference population). A total of 367 samples originated from the first drawing of blood (22 to 31 days postinfection) or the second drawing (40 to 90 days postinfection) and were taken from persons with a confirmed diagnosis of trichinellosis according to the algorithm proposed by Dupouy-Camet and Bruschi (positive reference population) (13); these persons had acquired infection during T. spiralis or Trichinella britovi outbreaks in Italy (36). Another 83 samples were also collected during the same T. spiralis or T. britovi outbreaks in Italy, but the diagnosis was not tested by the algorithm because of the lack of clinical information. Of the 3,505 samples, 1,896 were from persons with other parasitic or nonparasitic infections or with noninfective pathologies confirmed elsewhere by blood smears, stool examinations, and/or other specific tests (Table 1). Of these, 1,518 samples were from highly multiparasitized persons (i.e., with at least two of the following infections: malaria, schistosomiasis, trichuriasis, ascaridiasis, and ancylostomiasis) from the Island of Pemba (Tanzania); these persons are practicing Muslims, and no pig or other food animals susceptible to Trichinella exist where they live. All of the participants provided oral informed consent to have blood samples drawn.

ELISA procedure. A standard protocol was used. Briefly, 96-well microtiter plates (Nunc, Roskilde, Denmark) were filled with 100 μl/well of Trichinella ESA (5 μg/ml) in carbonate-buffered saline (pH 9.6 ± 0.2). After incubation at 37°C for 1 h, the plates were washed three times with a standard plate washer (Dynex Technologies, Denkendorf, Germany) with washing solution (0.5% Tween 20 in phosphate-buffered saline [pH 7.3 ± 0.2]), blocked by adding 200 μl/well of blocking solution (0.5% bovine serum albumin, 0.05% Tween 20), and incubated at 37°C for 1 h. After another washing, 100 μl/well of each 1/200-diluted serum sample was added in duplicate, and the plates were incubated at 37°C for 30 min. After washing again, 100 μl/well of the diluted anti-human IgG peroxidase-labeled antibodies (Kierkegaard and Perry Laboratories, Gaithersburg, MD) were added, and the plates were incubated at 37°C for 1 h. Finally, after a last wash, 100 μl/well of the substrate solution containing 3,3′,5′-tetramethylbenzidine and 0.02% hydrogen peroxide in a citric acid buffer was added, and the plates were incubated at room temperature (RT). The reaction was stopped by adding 50 μl/well of 1 N HCl solution. The OD value was obtained by reading the reaction at 450 nm using an ELISA plate microtiter reader (Dynex Technologies, Chantilly, VA). Each plate contained four positive and four negative reference serum samples, each of which was tested in duplicate. Since raw OD values are absolute measurements that are influenced by ambient temperature, test parameters, and photometric instruments, the results were expressed as a function of the reactivity of the positive control serum sample with the highest value of the four sera in each run of the assay. This control must yield a result that is in the linear range of measurement (45). The mean OD values of the control sera, as well as the mean OD values of the duplicate test sera, were then calculated, and for each serum sample, an ELISA index (Iₑ) expressed as the percentage of positivity was calculated according to the following equation: Iₑ = [(mean OD value of duplicate sample – OD blank)/(mean OD value of the highest positive control – OD blank)] × 100.

Western blotting. ESA were electrophoretically separated in a 10% resolving polyacrylamide gel and transferred onto nitrocellulose membranes. The nonspecific binding of antibodies was blocked by incubating the membrane with 2% fetal calf serum in TNT (Tris-HCl [pH 8.0] with 0.05% Tween 20) at RT for 1 h. After blocking, the membrane was incubated at RT overnight with 1/50-diluted serum. As controls, in each working session we used serum samples from four Trichinella-infected donors which yielded different levels of anti-Trichinella-specific IgG by ELISA and from one Trichinella-free donor. After washing, the nitrocellulose was exposed to 1:1,000 peroxidase-labeled goat anti-human IgG (Bio-Rad, Hercules, CA), diluted in blocking buffer, at RT for 1 h. The peroxidase substrate (3,3′-diaminobenzidine; Sigma, Saint Louis, MO) was then added to reveal the IgG/antigen interaction.

Statistical analysis. The receiver-operator characteristic (ROC) curve analysis was carried out using STATA software version 8.0. This procedure optimizes the

### TABLE 1. Sera from 1,896 donors with disorders other than Trichinella infection, tested by ELISA

| Infecting organism(s) or disorder | No. of infected patients (no. of positive sera) |
|----------------------------------|-----------------------------------------------|
| **Protozoa**                     |                                               |
| Leishmania sp.                   | 75 (4)                                        |
| Trypanosoma cruzi               | 25 (1)                                        |
| Plasmodium falciparum           | 1 (0)                                         |
| Toxoplasma gondii               | 33 (8)                                        |
| Total                            | 134 (13)                                      |
| **Cestodes**                    |                                               |
| Cysticercus cellulosus          | 19 (0)                                        |
| Echinococcus granulosus         | 15 (3)                                        |
| Echinococcus multilocularis     | 9 (0)                                         |
| Total                            | 43 (3)                                        |
| **Nematodes**                   |                                               |
| Loa loa                         | 17 (1)                                        |
| Mansonella perstans             | 25 (2)                                        |
| Wuchereria bancrofti            | 11 (0)                                        |
| Capillaria philippinensis       | 6 (1)                                         |
| Strongyloides stercoralis       | 10 (2)                                        |
| Toxocara sp.                    | 68 (6)                                        |
| Total                            | 137 (12)                                      |
| **Copepods**                    |                                               |
| HIV-Cryptosporidium sp.         | 20 (8)                                        |
| HIV-Enterocytozoon bieneusi     | 2 (1)                                         |
| **Multiparasitized**            | 1,518 (229)                                   |
| Total                            | 1,540 (238)                                   |
| **Other disorders**             |                                               |
| Rheumatoid arthritis            | 7 (0)                                         |
| Lupus erythematosus             | 4 (1)                                         |
| Myositis                        | 2 (0)                                         |
| Sjogren’s syndrome              | 1 (0)                                         |
| Systemic sclerosis              | 2 (0)                                         |
| Vasculitis                      | 1 (0)                                         |
| Antiphospholipid syndrome       | 1 (0)                                         |
| Connectivities                  | 1 (0)                                         |
| Ruphus                          | 1 (0)                                         |
| Hypereosinophilic syndrome      | 1 (0)                                         |
| Anti-DNA antibodies             | 21 (5)                                        |
| Total                            | 42 (7)                                        |

* Sera from multiparasitized persons from the Island of Pemba (Tanzania) (with at least two of the following infections: malaria, schistosomiasis, trichuriasis, ascaridiasis, and ancylostomiasis).
TABLE 2. Descriptive indices of ELISA results to detect anti-Trichinella IgG in sera from persons with a confirmed diagnosis of trichinellosis (infected) and from healthy persons (noninfected)

| Statistical indices | Infected subjects\( (n = 367) \) | Noninfected subjects\( (n = 1,159) \) |
|---------------------|---------------------------------|-------------------------------------|
| OD\(^a\)            | \(I_E\)                         | OD\(^a\)                           | \(I_E\)                           |
| Mean                | 1.2368                          | 0.063                              | 3.2                               |
| SD\(^b\)            | 0.646                           | 0.055                              | 2.8                               |
| p50                 | 1.1785                          | 0.0445                             | 2.4                               |
| p25                 | 0.717                           | 0.0275                             | 1.5                               |
| p75                 | 1.691                           | 0.08150                            | 4.1                               |
| Minimum             | 0.230                           | 0                                  | 0                                 |
| Maximum             | 2.946                           | 0.517                              | 28.4                              |

\(^a\) The OD was recorded at 450 nm.

\(^b\) SD, standard deviation.

RESULTS

ROC curves were built with data from the positive reference population and the negative reference population. The descriptive indices of ELISA results are shown in Table 2. The AUC was 0.999 for both the mean OD value of the duplicates and the \(I_E\), indicating that the two parameters provided equally accurate results (Fig. 1). Regarding repeatability, the differences in the OD between the duplicates did not increase with increases in their mean (Fig. 2).

With regard to the interassay variability, the CV, which was calculated with data from eight different working sessions, did not exceed 5% for the two positive serum samples or 14% for the two negative samples (Table 3). According to the ROC analysis, the best cutoffs were 11.8% for the \(I_E\) and 0.233 for the OD values; based on these cutoff values, the sensitivity and specificity were 98.7% and 98.4%, respectively.

The diagnostic specificity and sensitivity of the ELISA, when including the multiparasitized Tanzanian population and when excluding them, are summarized in Tables 4 and 5. When including the multiparasitized population, 3 false-negative and 295 false-positive serum samples were found, with a sensitivity and specificity of 99.2% and 90.6%, respectively (positive predictive value \(= 55.7\%\); negative predictive value \(= 99.9\%\)) (Table 4).

When excluding the 1,518 samples taken from the multiparasitized population, 229 (15%) of which yielded positive results (all of which were considered to be false positives), the specificity increased to 95.6% (positive predictive value \(= 84.9\%\); negative predictive value \(= 99.81\%\)) (Table 5).

Given that 273 (14.3%) samples cross-reacted with ESA in the ELISA (Table 1), the Wb analysis was used to further investigate the nature of the cross-reactivity. Of the 367 ELISA-positive samples from persons with confirmed trichinellosis, 280 were tested by a Wb assay. All sera consistently recognized sharp bands at 45, 50, and 55 kDa, independently of the IgG level detected by ELISA; some of the sera also recognized additional bands at different kDa (data not shown). The Wb

![FIG. 1. Scatter plot of the differences between the OD (recorded at 450 nm) of the serum duplicates and their means. Mean + 2SD, mean plus two standard deviations; Mean - 2SD, mean minus two standard deviations.](image)

![FIG. 2. The ROC curve built for 1,159 serum samples from healthy blood donors and 367 samples from persons with confirmed trichinellosis. The AUC, which indicates accuracy, was determined for \(I_E\) and for the mean of the OD duplicates and was equal to 0.999.](image)

TABLE 3. ELISA reproducibility\(^a\)

| Serum sample | \(I_E\) mean ± SD | CV (%) |
|--------------|------------------|-------|
| 1            | 98 ± 4.4         | 5     |
| 2            | 99 ± 3.9         | 4     |
| 3            | 5.3 ± 0.67       | 13    |
| 4            | 5.5 ± 0.76       | 14    |

\(^a\) Data from two Trichinella-positive (1 and 2) and two Trichinella-negative (3 and 4) serum samples obtained in eight different working sessions. SD, standard deviation.
profiles for four representative sera from *Trichinella*-infected persons with different IgG levels found by ELISA (OD values of 0.5, 0.4, 1.5, and 2.0) are shown in Fig. 3.

We focused the study of Wb cross-reactivity on sera from persons with health disorders different from trichinellosis, which were ELISA positive and had recognized *Trichinella* epitopes in the range of 45 to 55 kDa. Four of the six serum samples from persons with toxocariasis and two samples from persons with filariasis (*Mansonella perstans*) yielded strong bands at 45 and 50 kDa, whereas four samples from persons with visceral leishmaniasis showed faint bands at 45 and 55 kDa. Only one of the eight samples from persons with toxoplasmosis showed bands lower than 45 kDa. Two of the samples from persons with anti-DNA antibodies showed a 45-kDa band and a band higher than 55 kDa, whereas three samples showed bands lower than 45 kDa.

Serum samples from persons with hydatidosis, filariasis (*Loa loa*), capillariasis (*Capillaria philippinensis*), strongyloidiasis, coinfection with human immunodeficiency virus (HIV)-*Cryptosporidium* and HIV-*Enterocytozoon bieneusi*, malaria, *trypanosomiasis* (*Trypanosoma cruzi*), hyperesinophilic syndrome, and systemic lupus erythematosus did not react to the bands with different patterns and intensities (Table 6).

Table 5. Diagnostic sensitivity, specificity, and positive and negative predictive values (cutoff of 11.8%, 95% confidence interval [CI]) in different populations, excluding the multiparasitized Tanzanian population

| ELISA result | Trichinella infection | Present | No. of infected subjects | Absent | No. of noninfected subjects | Total |
|--------------|----------------------|---------|--------------------------|--------|-----------------------------|-------|
| Positive     | True positive        | 371     | False positive           | 295    | 666                         | 3,131 |
|              | False negative       | 3       | True negative            | 2,836  | 2,839                       | 6,131 |
| Total        |                      | 374     |                          | 3,131  | 3,505                       | 6,505 |

* Sensitivity = 99.20% (95% CI, 97.67 to 99.83%), calculated as TP/(TP + FN). Specificity = 90.58% (95% CI, 89.50 to 91.58%), calculated as TN/(TN + FP). Positive predictive value = 55.71% (95% CI, 51.84 to 59.52%), calculated as TP/(TP + FP). Negative predictive value = 99.89% (95% CI, 99.69 to 99.98%), calculated as TN/(TN + FN). TP, the number of true positives; FN, the number of false negatives; TN, the number of true negatives; FP, the number of false positives.

**FIG. 3.** Western blot profiles obtained with sera from *Trichinella*-infected persons with different IgG levels by ELISA. A, molecular mass markers in kDa; lane 1, serum sample that yielded an OD of 2.0; lane 2, sample that yielded an OD of 1.5; lane 3, sample that yielded an OD of 0.5; lane 4, sample that yielded an OD of 0.4.

**DISCUSSION**

For human parasitic diseases, no diagnostic test or reference materials have been standardized, except for a human serum of anti-*Toxoplasma* IgG (human TOXM, NIBSC, United Kingdom) (37). According to the laboratory accreditation process of ISO/IEC 17025:2005, it is first necessary to validate a serological test and then proceed to the standardization process. If the test shows an acceptable performance in terms of sensitivity, specificity, accuracy, and reproducibility, the test results could be combined with other laboratory findings and with clinical and epidemiological data to make a final diagnosis.

With specific regard to trichinellosis, although several methods have been used for serological diagnosis, ELISA has and continues to be the most commonly used method because of its high sensitivity (14, 19). However, to the best of our knowledge, no serological test has been validated using a large-enough panel of serum samples from healthy persons, persons with confirmed trichinellosis, and persons with health disorders other than trichinellosis.

In validating a serological test, it is fundamental that the cutoff be defined. Thus, the sample size has to be large enough to minimize the stochastic uncertainty in the cutoff selection (ROCKIT 0.9B beta version; C. E. Metz [www.radiology.uchicago.edu/krl/toppage11.htm]). To select a positive and a negative reference population, a gold standard must be available, yet in the case of trichinellosis, no such gold standard has...
been found. To this end, we tested 1,159 serum samples from presumably healthy persons and 367 from persons with trichinellosis confirmed on the basis of the algorithm proposed by Dupouy-Camet and Bruschi (13). Based on the ROC analysis, the cutoff values were set at 11.8% for OD values and 0.233 for OD; based on these values, the sensitivity and specificity were 98.7% and 98.4%, respectively.

It is assumed that a test with a perfect discrimination (i.e., with no overlap between the two populations [in this case healthy persons and persons with confirmed trichinellosis]) has an ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity) and an AUC equal to 1; thus, as the ROC plot moves closer to the upper left corner, the accuracy becomes greater (48). In our test, the AUC was 0.99 (i.e., very close to 1) when using both the OD values and $I_p$ (Fig. 1), which suggests that both parameters are suitable for interpreting an ELISA and attaining high performance in identifying the presence/absence of infection.

Furthermore, since more than 95% of the differences between the OD values of the serum duplicates did not exceed two standard deviations, the ability to replicate the ELISA can be considered high (6). The reproducibility of the ELISA was also high, as indicated by the interassay variability, with a CV that did not exceed 5% for the positive sera or 14% for the negative sera (Table 3).

After the cutoff was established, the sample size was increased by testing 83 serum samples from persons involved in an outbreak of trichinellosis yet without a confirmed diagnosis, 1,518 samples from multiparasitized persons from Tanzania, and 378 samples from persons with different health disorders. The ELISA showed a good sensitivity (99.2%; three false negatives out of a total of 374 samples [0.8%]) (Table 4). These three samples originated from persons involved in a trichinellosis outbreak, who seroconverted some days after the first blood sample was drawn, as shown by a second drawing of blood. Since the WB analysis performed on these samples was also negative (data not shown), it is reasonable to assume that at the time the first blood sample was drawn, the IgG level was below the detectable threshold.

In some previous studies, the sensitivity of the ELISA to detect anti-Trichinella IgG in human sera was reported to be 100% (1, 2, 24, 26, 27). However, fewer samples were analyzed than in the present study, and they were collected 1.5 and 4 months after infection. In studies in which the samples were collected shortly after infection, the sensitivity ranged from 75% to 94% (4, 5, 20, 26).

The specificity of ELISA is greatly influenced by the panel of sera tested. Of the 3,505 samples, 295 (8.4%; Table 4) showed a false-positive reaction. However, when considering separately the different populations from which the samples were drawn, the specificity differed. In particular, only 22 (1.9%) of the samples from healthy persons showed a false-positive reaction, which is a lower percentage than that observed in other studies (1, 20). When considering persons with other parasitic and nonparasitic infections or with noninfective pathologies, there was a high percentage of false-positive reactions (14.3% [273/1,896]). Of these 273 samples, 229 were from multiparasitized persons from the Island of Pemba (Tanzania). These samples were included in this study to assess specificity under “extreme conditions” (i.e., in populations in which a number of infections are present).

Moreover, the positive and negative predictive values of the test, which depend on the prevalence of the infection in the population under study, may vary as the population changes (45). In fact, in our study, when there were many false positives and few false negatives, a positive ELISA result was in itself a poor indicator of the presence of infection, whereas a negative result was a good indicator of the lack of infection (Table 4 and 5).

Using a cutoff value of 11.8% allows weakly positive sera to be detected, though it also increases the risk of attaining false-positive results and thus a low specificity because of cross-reactivity with other organisms. In fact, the specificity of our ELISA increased from 90.58% to 95.6% (95% confidence interval, 94.82 to 96.82%; Table 5) when we excluded the samples from persons with other parasitic or nonparasitic infections or with noninfective pathologies. We also found a high percentage of false-positive reactions for samples taken from Italians with anti-Toxoplasma IgG, which has a high seroprevalence in Italy (21). Similarly, of importance is the high percentage (14.3%) of false-positive reactions for samples from persons living on the Island of Pemba, an area with poor health conditions and a high diffusion of a number of different infections.

Cross-reactivity is a major problem in the serological diagnosis of parasitic infections, especially those caused by nematodes, particularly when crude parasite extracts are used, and to a lesser extent with ESA. In fact, the presence of shared antigens of Trichinella sp. has been widely documented for other parasites and pathogens (3, 8, 12, 22, 23, 25, 38, 40).

Various attempts have been made to increase the specificity of ELISA. TSL-1 antigens, the main components of ESA, have

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### TABLE 6. Sera from persons with parasitic infections other than trichinellosis or with other health disorders recognizing Trichinella antigenic fractions of different molecular masses by Western blotting

| Disease                         | No. of sera | No. of sera recognizing bands of indicated molecular mass (kDa)* |
|---------------------------------|-------------|-------------------------------------------------------------------|
|                                 | <45         | 45                                                                |
|                                 | 50          | 55                                                                |
|                                 | >55         |
| Leishmaniasis                   | 4           | 4 (faint)                                                         |
| Toxoplasmosis                   | 8           | 4 (strong) 4 (strong)                                             |
| Toxocariasis                    | 6           | 2 (sharp) 2 (sharp)                                               |
| Filariasis (M. perstans)        | 2           | 2 (sharp) 2 (sharp)                                               |
| Anti-DNA antibodies             | 21          | 2 (sharp)                                                        |
| Multiparasitized<sup>b</sup>    | 25          | 8 (sharp) 5 (sharp)                                               |

*The band intensities are indicated in parentheses.

<sup>b</sup>Sera from persons from the Island of Pemba (Tanzania).
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