Validation of an ELISA for the diagnosis of human trichinellosis

Running title: Serodiagnosis of human trichinellosis

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Abstract

Trichinellosis is a zoonotic disease caused by the consumption of raw or semi-raw 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 (ESA) to detect anti-*Trichinella* IgG 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 (AUC), which was equal to 0.999, indicating high accuracy. The coefficient of variation calculated on 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 of 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) (34). *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 (35).

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, 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. IgG antibodies can be detected from 15 to 60 days post infection (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 ELISA, given its sensitivity (13, 14, 19). This test was first developed using low specific crude worm extract (CWE) antigens prepared from L1 larvae (4, 11, 12, 45) and then the more specific excretory/secretory antigens (ESA) prepared from L1 larvae maintained in culture (1, 2, 5, 9, 10, 15, 20, 24, 26, 33, 42, 44, 47).
*Trichinella spiralis* muscle larva antigens have been classified into eight groups (TSL-1 – TSL-8) based on their recognition by different monoclonal and polyclonal antibodies (30). 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). In ESA, the most abundant antigen is TSL-1, which is stage-specific, originates from the stichosome (a glandular structure consisting of 50-55 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 immunodominant carbohydrate epitope (tyvelose, 3,6-dideoxy-D-arabinohexose), which is considered to be unique for parasites of the genus *Trichinella* (30).

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, 36). In highly specialized laboratories, the Western blot (Wb) assay is generally used as the confirmatory test for ELISA-positive sera (12, 19, 36, 40). 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 Gamble (18). Briefly, *T. spiralis* muscle larvae were washed three times in PBS 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 (DMEM) supplemented with penicillin (500 units/ml) and streptomycin (500µg/ml). Five thousand worms per ml were then resuspended in DMEM, supplemented with 1M HEPES, 200mM L-glutamine, 100mM Na-pyruvate, and 5000 units of penicillin/streptomycin (GIBCO, Grand Island, NY, USA), and incubated with 10% CO$_2$ in a 75 cm$^2$ culture flask (Corning Life Sciences, Pittsburgh, PA, USA) 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® (Amicon Inc., Billerica, MA, USA) pressure concentrating chamber. 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); 367 samples originated from the first drawing of blood (22-31 days post infection) or the second drawing (40-90 days post infection) 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 (37); 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 non-parasitic infections or with non-infective 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, trichiuriasis, ascaridiasis, ancylostomiais) 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 100µl/well of *Trichinella* ESA (5µg/ml) in carbonate buffered saline pH 9.6 ± 0.2. After incubation at 37°C for 1 h, the plates were washed 3 times with an automatic plate washer (Dynex Technologies, Denkendorf, Germany) with washing solution (0.5% Tween 20 in PBS pH 7.3 ± 0.2), blocked by adding 200 µl/well of blocking solution (0.5% BSA, 0.05% Tween 20), and incubated at 37°C for 1 h. After another washing, 100µl/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, 100µl/well of the diluted anti-human IgG peroxidase labeled antibodies (Kierkegaard and Perry Laboratories, Gaithersburg, MD, USA) were added, and the plates were incubated at 37°C for 1 h. Finally, after a last wash, 100µl/well of the substrate solution containing 3, 3′,5,5′-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 50µl/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, USA). 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 included in each run of the assay. This control must yield a result that is in the linear...
range of measurement (46). 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 an ELISA index ($I_E$) expressed as percentage of positivity was calculated according to the following equation:

$$I_E = \frac{\text{mean OD value of duplicate sample} - \text{OD blank}}{\text{mean OD value of the highest positive control} - \text{OD blank}} \times 100$$

**Western blot.** ESA were electrophoretically separated in a 10% resolving polyacrylamide gel and transferred onto nitrocellulose membranes. The non-specific binding of antibodies was blocked by incubating the membrane with 2% FCS 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 (Biorad, Hercules, CA, USA), diluted in blocking buffer, at RT for 1 h. The peroxidase substrate (3,3’-diaminobenzidine, Sigma, Saint Louis, MO, USA) 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 interpretation of the ELISA results when there are well-defined positive and negative populations available for analysis. The ROC curve is a graphical plot of the sensitivity vs. “1 – specificity” for a binary classifier system, using various cutoff values. This allows the cutoff value that gives the best
balance of sensitivity and specificity to be selected for the test being considered (49). The repeatability of the test was evaluated by comparing the differences between the OD values of the serum duplicates and their mean (6).

To determine which parameter provided the most accurate result (i.e., the mean OD of the duplicates or the I_E), the area under the ROC curve (AUC) was calculated for each parameter. The diagnostic specificity and sensitivity of the test were interpreted according to the World Animal Health (46) recommendations and the ROC analysis. The interassay variability was assessed by testing two negative and two positive control sera in eight different work sessions; the mean of the OD values was determined and used to calculate the coefficient of variation (CV).

**Accreditation.** The laboratory is accredited according to ISO/IEC 17025:2005 by the Italian accreditation body SINAL (www.sinal.it).

**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 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 $I_E$ 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 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 Wb. 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 profiles for four representative sera from *Trichinella*-infected persons with different IgG levels found by ELISA (OD values: 0.5, 0.4, 1.5 and 2.0) are shown in Figure 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 45-55 kDa. Four of the six serum samples from persons with toxocariasis and two samples from persons with filariosis (*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, filariosis (*Loa loa*), capillariosis
(*Capillaria philippinensis*), strongyloidiasis, coinfection with HIV-*Cryptosporidium* and
HIV-*Encetocytozoon bieneusi*, malaria, trypanosomiasis (*Trypanosoma cruzi*),
hypereosinophilic syndrome, and systemic lupus eritematosus, did not react to the Wb. Of the
229 ELISA-positive serum samples from Tanzania, 25 were tested by Wb. Of these, eight
recognized strong bands in the range of 45-55 kDa, whereas 19 reacted with ESA bands with
different patterns and intensity (Table 6).

**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, UK)
(38). 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 cut-off be defined. Thus the sample size has to be large enough to minimize the stochastic uncertainty in the cut-off selection (25). 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 cut-off values were set at 11.8% for $I_E$ 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 (49). In our test, the AUC was 0.99 (i.e., very close to 1) both when using the OD values and $I_E$ (Fig. 1), which suggests that both parameters are suitable for interpreting an ELISA and attaining a 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 ELISA can be considered as high (6). 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 cut-off 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%; 3 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 sero-converted 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 ELISA to detect anti-Trichinella IgG in human sera was reported to be 100% (1, 2, 24, 27, 28). 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, 27).

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 non-parasitic infections or with non-infective 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 (46). 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 cut-off 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% CI: 94.82-96.82%; Table 5) when we excluded the samples from persons with other parasitic or non-parasitic infections or with non-infective 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 and particularly when crude parasite extracts are used, and to a lesser extent 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, 26, 39, 41).

Various attempts have been made to increase the specificity of ELISA. TSL-1 antigens, the main components of ESA, have been purified by affinity chromatography using monoclonal antibodies in an indirect ELISA and in a capture ELISA, resulting in improved
specificity when compared to the CWE (15, 43). The immunodominant carbohydrate tyvelose
has been synthesized and successfully used to detect anti-Trichinella IgG in humans (7) and
in swine (16), yet though this antigen has the advantage of high specificity and stability, the
results regarding sensitivity are contrasting (19, 31, 32, 36). In any case, ELISA for human
diagnostic purposes using purified antigens have to date not been validated with a sufficiently
large sample size.

Since the Wb analysis allows specific Trichinella antigens to be distinguished from
cross-reactive antigens, it has been considered by some to be the gold standard for serology
(19, 40, 47). In our study, the representative ELISA-positive sera (with a high or low anti-
Trichinella IgG level) reacted with the 45–55 kDa proteins, with different band intensity,
independently of the OD value (Fig. 3). Since the ESA preparation has not yet been
standardized, it is difficult to compare our results with those from other laboratories.

However, as in the study of Yepez-Mulia et al. (48), we considered the 45–55 kDa pattern as
a distinctive profile for Trichinella infection, because most of the anti-TSL-1 antibodies
specific for tyvelose recognize antigens in this range. Nuñez et al. (29) identified 13 bands
ranging from 14 to 66 kDa; of these, those of 14, 29, 36, and 55 kDa proved to be Trichinella-
specific and only the 55 kDa band was detected in all of the tested sera. In another study, the
43-44 kDa band and the 64 kDa band were detected in all patients in the course of acute
trichinellosis (47). In our study, the samples from persons with toxocariasis, persons with
filariosis due to M. perstans, and multiparasitized persons recognized antigenic bands of 45-
55 kDa. The Wb analysis confirmed the non-specific reactivity by ELISA to sera from
persons with hydatidosis, filariosis due to L. loa, capillariasis, strongyloidiasis, HIV-
Cryptosporidium and HIV-E. bieneusi co-infection, malaria, trypanosomiasis,
hypereosinophilic syndrome, and systemic lupus eritematosus.
However, there still exists the problem of the cross-reaction of sera from persons with disorders other than *Trichinella* sp. For this reason, ELISA is not suitable for epidemiological purposes, especially in areas where other parasites are present. In a previous study we used tyvelose (a highly selected antigen) and observed an improvement in specificity, yet the sensitivity decreased (19), not to mention the fact that tyvelose is quite costly. The validated ELISA shows an optimal performance in terms of sensitivity, repeatability, and reproducibility and an acceptable specificity. Consequently, the method is suitable for detecting anti-*Trichinella* antibodies in human sera and could mainly be used for diagnostic purposes, constituting an important part of the diagnostic algorithm.
ACKNOWLEDGMENTS

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REFERENCES


Figure legends

FIG. 1. Scatter plot of the differences between optical density (OD, recorded at 450 nm) of the serum duplicates and their means.

FIG. 2. The receiver-operator characteristic (ROC) curve built for 1,159 serum samples from healthy blood donors and 367 samples from persons with confirmed trichinellosis. The area under the ROC curve (AUC), which indicates accuracy, was determined for the ELISA index (I_E, expressed as percentage of positivity, see text) and for the mean of the OD duplicates, and was equal to 0.999.

FIG. 3. Western blot profiles obtained with sera from *Trichinella*-infected persons with different IgG levels by ELISA: A, molecular weight 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; and lane 4, sample that yielded an OD of 0.4.
TABLE 1. Sera from 1,896 donors with disorders other than *Trichinella* infection, tested by ELISA. In brackets, ELISA-positive sera.

<table>
<thead>
<tr>
<th>Protozoa</th>
<th>Cestodes</th>
<th>Nematodes</th>
<th>Co-infections</th>
<th>Other disorders</th>
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<tr>
<td>Leishmania sp.</td>
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<td>Loa loa</td>
<td>HIV-Cryptosporidium</td>
<td>Rheumatoid arthritis Antiphospholipid</td>
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<td>cellulose 19 (0)</td>
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<td><em>bienesi</em> 2 (1)</td>
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<td>Wuchereria</td>
<td>Multiparasitised*</td>
<td>Myositis</td>
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<td><em>falciparum</em> 1 (0)</td>
<td><em>multilocularis</em> 9 (0)</td>
<td><em>bancrofti</em> 11 (0)</td>
<td>1,518 (229)</td>
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| *Trypanosoma*     | *Plasmo...
TABLE 2. Descriptive indices of ELISA results to detect anti-\textit{Trichinella} IgG in sera from persons with a confirmed diagnosis of trichinellosis (infected), and from healthy persons (non-infected)

<table>
<thead>
<tr>
<th>Statistical indices</th>
<th>Infected (367)</th>
<th>Non-infected (1,159)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD$^a$</td>
<td>IE$^b$</td>
</tr>
<tr>
<td>Mean</td>
<td>1.2368</td>
<td>55.7</td>
</tr>
<tr>
<td>SD</td>
<td>0.646</td>
<td>26</td>
</tr>
<tr>
<td>p50</td>
<td>1.1785</td>
<td>57</td>
</tr>
<tr>
<td>p25</td>
<td>0.717</td>
<td>32.5</td>
</tr>
<tr>
<td>p75</td>
<td>1.691</td>
<td>78</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.230</td>
<td>11.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>2.946</td>
<td>110</td>
</tr>
</tbody>
</table>

$^a$ OD, optical density recorded at 450 nm

$^b$ IE, ELISA index expressed as percentage of positivity
TABLE 3. ELISA reproducibility. Data from two *Trichinella*-positive (N. 1 and 2) and two *Trichinella*-negative (N. 3 and 4) serum samples, obtained in eight different working sessions.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_E (^a^) mean ± SD</td>
<td>98 ± 4.4</td>
</tr>
<tr>
<td>CV (%)</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a^I_E^,\) ELISA index expressed as percentage of positivity
TABLE 4. Diagnostic sensitivity\(^a\), specificity\(^b\), and positive\(^c\) and negative\(^d\) predictive values (cut-off of 11.8%, 95% CI) in different populations, including the multiparasitized Tanzanian population

<table>
<thead>
<tr>
<th>ELISA result</th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>True positive</td>
<td>371</td>
<td>False positive</td>
</tr>
<tr>
<td>Negative</td>
<td>False negative</td>
<td>3</td>
<td>True negative</td>
</tr>
<tr>
<td>Total</td>
<td>374</td>
<td>3,131</td>
<td>3,505</td>
</tr>
</tbody>
</table>

\(^a\)Sensitivity = 99.20% (95% CI: 97.67-99.83%), calculated as TP/(TP+FN), where TP and FN are the numbers of true positives and false negatives, respectively

\(^b\)Specificity = 90.58% (95% CI: 89.50-91.58%), calculated as TN/(TN+FP), where TN and FP are the numbers of true negatives and false positives, respectively

\(^c\)Positive predictive value = 55.71% (95% CI: 51.84-59.52%), calculated as TP/(TP+FP)

\(^d\)Negative predictive value = 99.89% (95% CI: 99.69-99.98%), calculated as TN/(TN+FN)
TABLE 5. Diagnostic sensitivity\(^a\), specificity\(^b\) and positive\(^c\) and negative\(^d\) predictive values (cut off 11.8%, 95% CI) in different populations, excluding the multiparasitized Tanzanian population

<table>
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<td></td>
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<td>3</td>
<td>True negative</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>374</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Sensitivity = 99.20% (95% CI: 97.67-99.83%), calculated as TP/(TP+FN), where TP and FN are the numbers of true positives and false negatives, respectively

\(^b\)Specificity = 95.6% (95% CI: 94.82-96.82%), calculated as TN/(TN+FP), where TN and FP are the numbers of true negatives and false positives, respectively

\(^c\)Positive predictive value = 84.90% (95% CI: 81.19-88.12%), calculated as TP/(TP+FP)

\(^d\)Negative predictive value = 99.81% (95% CI: 99.43-99.96%), calculated as TN/(TN+FN)
TABLE 6. Sera from persons with parasitic infections other than trichinellosis or with other health disorders recognizing *Trichinella* antigenic fractions of different molecular weights by Western blot

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of sera</th>
<th>No. of sera recognizing bands of different molecular weights of sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;45</td>
</tr>
<tr>
<td>Leishmaniosis</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>8</td>
<td>4 (faint)(^a)</td>
</tr>
<tr>
<td>Toxocariosis</td>
<td>6</td>
<td>4 (strong)(^a)</td>
</tr>
<tr>
<td>Filariosis (\textit{M. perstans})</td>
<td>2</td>
<td>2 (sharp)</td>
</tr>
<tr>
<td>Anti-DNA antibodies</td>
<td>21</td>
<td>3 (faint)</td>
</tr>
<tr>
<td>Multiparasitised(^b)</td>
<td>25</td>
<td>11 (sharp)</td>
</tr>
</tbody>
</table>

\(^a\) Band intensity

\(^b\) Sera from persons from the Island of Pemba (Tanzania)
Figure 2.

- Sensitivity
- Specificity

[Graph showing a ROC curve with sensitivity on the y-axis and 1-specificity on the x-axis.]