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Detection of a *Trichinella*-specific IgE in human trichinellosis – the creation of a new test

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Abstract: Trichinellosis is a parasitic disease of humans caused by nematodes from the genus *Trichinella*, predominantly *Trichinella spiralis*. If *Trichinella* infection is suspected based on an epidemiological link and clinical criteria within defined period of time, then finding of *Trichinella*-specific antibodies in the examined sera provides definitive proof of the establishment of infection. Detection of a *Trichinella*-specific IgE that could precede, coincide or follow IgG seroconversion not only confirms the existence of infection, but could narrow the time frame in which the infection occurred to a year or even less. Since there are no commercially available tests for monitoring the presence of a *Trichinella*-specific IgE during the course of the disease, the present work was aimed at establishing this kind of ELISA test. The specificity and sensitivity of hitherto described *Trichinella*-specific IgE ELISAs are not sufficiently satisfactory; the two major problems are poor discrimination between positive and negative results and cross reactivity with sera of patients with different parasitic diseases. In this study, a *Trichinella*-specific IgE capture ELISA was developed that overcomes the problems of specificity and sensitivity and enables the determination of *Trichinella*-specific IgE.

Keywords: *Trichinella spiralis*; *Trichinella*-specific IgE; capture ELISA.

INTRODUCTION

Trichinellosis is a parasitic disease of humans caused by nematodes from the genus *Trichinella*, predominantly *Trichinella spiralis*.[1] Humans become infected through consumption of raw or undercooked infected meat. Host defense is activated upon the parasitic invasion and could be monitored through changes in blood parameters, such as eosinophilia (short lasting phenomenon, *i.e.*, duration measurable in days) and the presence of anti-*Trichinella* antibodies (long lasting, *i.e.*, detectible over months or years, as the presence of specific IgG antibodies).

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However, the finding of *Trichinella*-specific IgG in human sera might not be proof of *Trichinella* infection *per se*,[2] as it indicates that infection with this parasite had occurred sometime in the past (antibodies can be detectable from 12 to 60 days post infection and may persist for more than 30 years after infection),[3] regardless of whether it was asymptomatic or with visible clinical signs of illness, and that live parasites are still present in the host organism. In the case of mild or asymptomatic trichinellosis, it is hard to distinguish between recently acquired and chronic *Trichinella* infection. The presence of *Trichinella*-specific IgE could help in narrowing the period in which the infection had occurred. Namely, specific IgE appears early, within the first 2 weeks from infection, and could be found in the sera of *Trichinella*-infected persons one year after infection.[4,5]

Although best known for its role in allergy, IgE has also been studied in parasitic diseases (helminth infections) that are accompanied with symptoms resembling allergy, as is the case with trichinellosis. It is known that *T. spiralis* infection induces the synthesis of a specific IgE, and its connection with eosinophilia and direct involvement in allergic manifestation was suggested.[6] Animal models of *Trichinella* infection revealed a protective role to the host of a specific IgE.[7] However, such a role, with the present knowledge, could not be ascribed to a specific IgE in humans, although there are epidemiological studies for other helminths that confirm protection from re-infection.[8,9] Infection with *Trichinella*, as with other helminths, also results in temporary elevation of the total IgE during the course of the disease, which may not exceed the normal range. The role of unspecific IgE is not as clear,[6,10–12] but there are findings that connect high concentrations of unspecific IgE with beneficial effects to the parasite.[13]

Numerous attempts have been made to detect the presence of a *Trichinella*-specific IgE, but the detection rates reported in the literature varied from 7–100 %, due to the differences in the type of the assay used, the reliability of the test used for this purpose, and the acuteness of the infection.[6] Problems in the detection of *Trichinella*-specific IgE antibodies by a classical indirect enzyme-linked immunosorbent assay (ELISA) arise from its unfavorable competition with IgG antibodies for binding to antigen coated wells. This is a consequence of the naturally low concentration of IgE in sera, compared to the many times higher values for IgG. This does not mean that *Trichinella*-specific IgE antibodies in the sera could not be detected, but the probability for their detection with classical ELISA is very low, due to reduced sensitivity of the technique, which causes false negative (FN) results.

The goal of this study was to develop a sensitive ELISA test for determination of *Trichinella*-specific IgE in human serum samples, since there is no such test on the market. Two types of ELISAs for *Trichinella*-specific IgE detection were formulated and their analytical validation and comparison with

classical indirect ELISA that uses untreated serum samples were performed. The most commonly employed method for overcoming the problem of the excess IgG concentration and maximizing IgE detection is pretreatment of the examined sera with various commercially available absorbents with the aim of removing IgG prior to specific-IgE determination.[14,15] Thus, one type of test was classical indirect ELISA using sera treated with an in-house designed IgG absorbent. The other, capture ELISA for *Trichinella*-specific IgE detection (capture *Trichinella* IgE ELISA), solved the problem of competition between the two specific antibody isotypes – IgE and IgG, by employing a specific approach, which paired two monoclonal antibodies, one that allowed the separation of IgE antibodies from all other classes of antibodies in human sera (anti-human IgE monoclonal antibody) and the other that recognized an epitope on *Trichinella* spp. muscle larvae excretory–secretory antigens (detection 7C2C5 monoclonal antibody).

EXPERIMENTAL

Antigen preparation

T. spiralis muscle larvae (L1 larvae) were recovered from Wistar rats (infected with 7000 *T. spiralis* L1 per rat, ISSN 161, sacrificed three months post infection) by digesting the carcasses in pre-warmed gastric juice (1 % pepsin in 1 % HCl, pH 1.6–1.8).[16] For the production of muscle larvae excretory–secretory antigens (ES L1), the larvae were kept under controlled conditions (37 °C, 5 % CO_2) in complete Dulbecco's modified Eagle's medium (Sigma–Aldrich, Germany) supplemented with 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate (all from Sigma–Aldrich, Germany) and 50 U mL^{-1} Pen/Strep (Galenika, Serbia) for 18 h. Culture supernatants containing ES L1 products of the muscle larvae were dialyzed, concentrated and stored at –20 °C until use.[17]

Preparation of HRP conjugates

Monoclonal antibody (mAb) 7C2C5 has been maintained by the Serbian National Reference Laboratory for Trichinellosis (SrNRLT) since 1986, due to a joint project and with agreement of Dr. Gamble HR, USA. This mAb is specific for the epitope unique for the muscle larvae of the whole genus *Trichinella*, which is present on 45, 49 and 53 kDa protein components of *T. spiralis* ES L1 antigens.[18,19] 7C2C5 mAb does not exhibit any cross-reactivity with antigens from other parasites.[18] MAbs, 7C2C5 and mouse anti-human IgE (Medix Biochemica, Finland), were labeled with horseradish peroxidase (HRP) according to the procedure described elsewhere.[20] In brief, activation of the reactive groups on antibodies and of the enzyme was performed by incubation with carbonate buffer and buffer with $NaIO_4$, respectively. After the incubation and blocking of the remaining reactive sites on the antibodies, separation of labeled from un-labeled fractions was performed on a Sephacryl S-200 column. The quality of 7C2C5-HRP conjugate was assessed by an in-house *Trichinella*-ELISA test, while the quality of the anti-human IgE–HRP product was assessed by an in-house ELISA for the quantitative determination of the total serum IgE.

Pre-treatment of serum samples with IgG absorbent

Purified sheep polyclonal antibodies to human IgG (γ-chain specific, INEP, Serbia) were used as the IgG absorbent. The key step in serum stripping was the determination of the optimal volume of polyclonal antibodies for efficient removal of IgG antibodies from human

sera. The optimal ratio between serum and IgG absorbent was determined by absorbing fixed volumes of sera with different volumes of IgG absorbent. Finally, 50 µL of sera were mixed and incubated with 100 µL of IgG-absorbent for 1 h at room temperature. The immune complexes were removed by centrifugation at 6500 g for 10 min. The remaining supernatants were transferred to new tubes and tested for the presence of IgG by radioimmunodiffusion plates (RID IgG INEP, Serbia) and assayed for *Trichinella*-specific IgE.

Serum samples

This study included 28 serum samples collected from patients with confirmed diagnosis of trichinellosis. All serum samples were from the bank of SrNRLT, INEP, and were declared positive for the presence of anti-*Trichinella* antibodies (true positive, TP). The positivity was confirmed by two methods, ELISA (in-house *Trichinella*-ELISA test, INEP, Serbia) and indirect immunofluorescence assay (FITC *T. spiralis* Antibody Detection Kit; INEP, Serbia), both designed and validated for detection of parasite specific antibodies in serum samples. An ELISA based on excretory–secretory antigens (ES L1) could yield false positive results (FP) due to potential cross-reactivity with sera of patients with other parasitic or allergic diseases. This study included a total of 70 *T. spiralis* non-infected persons, covering 40 serum samples from healthy blood donors used as negative controls, sera from 20 patients with toxoplasmosis and echinococcosis and 10 patients with allergic diseases, which were involved in the estimation of the analytical specificity, diagnostic sensitivity and diagnostic specificity. Analytical specificity refers to the ability of an assay to measure a particular substance, rather than others, in a sample (in the present case *T. spiralis* specific IgE); diagnostic sensitivity is the percentage of persons who have confirmed infection (trichinellosis) and are identified by the assay as positive for a given substance (specific IgE) – true positive; diagnostic specificity is the percentage of persons who do not have the indicated infection and who are identified as negative for a given substance (specific IgE) in the assay – true negative (TN).[21]

All sera samples were split into aliquots and stored at –20 °C until testing.

ELISA procedure

Classical indirect ELISA. To detect *Trichinella*-specific IgE antibodies, the standard test configuration with a 3-step procedure was used. Briefly, 96-well polystyrene microtiter plates (Immulon Microtiter 96-well plates and strips, Thermo Scientific) were coated with 100 µL per well of ES L1 antigens (5 µg mL^{-1} in 0.1 M carbonate buffer, pH 9.6) and incubated overnight at 4 °C. The plates were washed with washing solution (0.05 % Tween 20 in phosphate buffer (PBS), pH 7.2), and the remaining binding sites were blocked with 1 % BSA in PBS pH 7.2 for 1 h at room temperature. After the washing step, 100 µL per well of each 1/3 diluted serum sample was added in duplicate and incubated for 18 h at 4 °C. After extensive washing, 100 µL per well of anti-human IgE mAb–HRP was added to each well and incubated for 3 h at room temperature. After a final wash, 100 µL per well of chromogenic solution (3,3′,5,5′-tetramethylbenzidine hydrogen peroxide–TMB substrate) was added and the plates were incubated at room temperature for 10 min. After stopping the reaction with 2 M H_2SO_4, the optical densities (*OD*) were measured at 450 nm using a Diamedix (Miami, FL, USA) microassay BP-12 ELISA reader.

To reduce the IgG concentration in the sample and mitigate the IgG/IgE antibody competition, the serum samples were pretreated with IgG absorbent, as previously explained. After removal of the immune complexes, the remaining supernatants were transferred to new tubes and used for the detection of specific IgE in a classical indirect ELISA.

Capture Trichinella *IgE ELISA*. The test configuration was achieved through a 4-step procedure. The 96-well plates were coated with 100 µL of mouse anti-human IgE mAb (2 µg mL^{-1} in 0.1 M carbonate buffer, pH 9.6), and incubated overnight at 4 °C. The plates were washed with washing solution (0.05 % Tween 20 in PBS, pH 7.2) and the remaining binding sites were blocked with 1 % BSA in PBS, pH 7.2, 200 µL per well, for 1 h at room temperature. After washing, the serum samples, diluted 1:3 in PBS, were added in duplicate and incubated for 2 h at 37 °C. The plates were again washed and ES L1 antigens (2 µg mL^{-1}, 100 µL per well) were added for 2 h at room temperature, with mixing. After removal of unbound ES L1 antigens with washing, 100 µL of HRP labeled 7C2C5 mAb (1:500 dilution) was added to each well for 1 h at room temperature. The optimal concentrations of the reagents (anti human-IgE mAb, serum dilution and ES L1 antigens) had previously been determined. After a final wash, 100 µL per well of chromogenic solution (3,3′,5,5′-tetramethylbenzidine hydrogen peroxide–TMB substrate) was added and the plates were incubated at room temperature for 10 min. After stopping the reaction with 2 M H_2SO_4, the *OD* was measured at 450 nm using a Diamedix (Miami, FL, USA) microassay BP-12 ELISA reader.

Statistics

Receiver operating characteristic (ROC) analyses[22] were performed using MedCalc software, version 13.1 (MedCalc Software, Ostend, Belgium). Other results were analyzed using GraphPad Prism software, version 6 (GraphPad Software Inc., La Jolla, CA, USA).

Accreditation

The INEP Laboratory (that includes SrNRLT) has accredited services for human laboratory medicine according to UNI CEI EN ISO/IEC 17025:2005.

RESULTS

Classical indirect ELISA for the detection of Trichinella*-specific IgE using untreated and treated serum samples*

The results for the determination of the presence of anti-*Trichinella* IgE in untreated sera by classical indirect ELISA are given in Fig. 1A. The obtained absorbance (*A*) values were low (between 0.04 and 0.1). The ranges of *A* values for the positive and negative samples were very similar and, consequently, discrimination between them and interpretation of such results could not be performed.

Using serum samples treated with IgG absorbent, this kind of ELISA was able to detect *Trichinella*-specific IgE in 93 % (26 out of 28) in *Trichinella* antibody positive sera. The obtained absorbance values for the positive sera were higher than those obtained in the Classical Indirect ELISA using unprocessed sera, but still the range of the measured absorbance remained low (with a highest *OD* value of 0.340). The results are shown in Fig. 1B. An ROC curve was built with data from the positive reference population (28 samples from patients with a confirmed diagnosis of trichinellosis) and the negative reference population (40 samples from blood donors) (Fig. 1C). According to the ROC analysis, the best cut-off *OD* value was 0.078; based on this cut-off value, the sensitivity and specificity of the test were 92.9 and 100 %, respectively. The area under the ROC

curve (*AUC*), which indicates accuracy, determined for the best cut-off *OD* value was equal to 0.98.

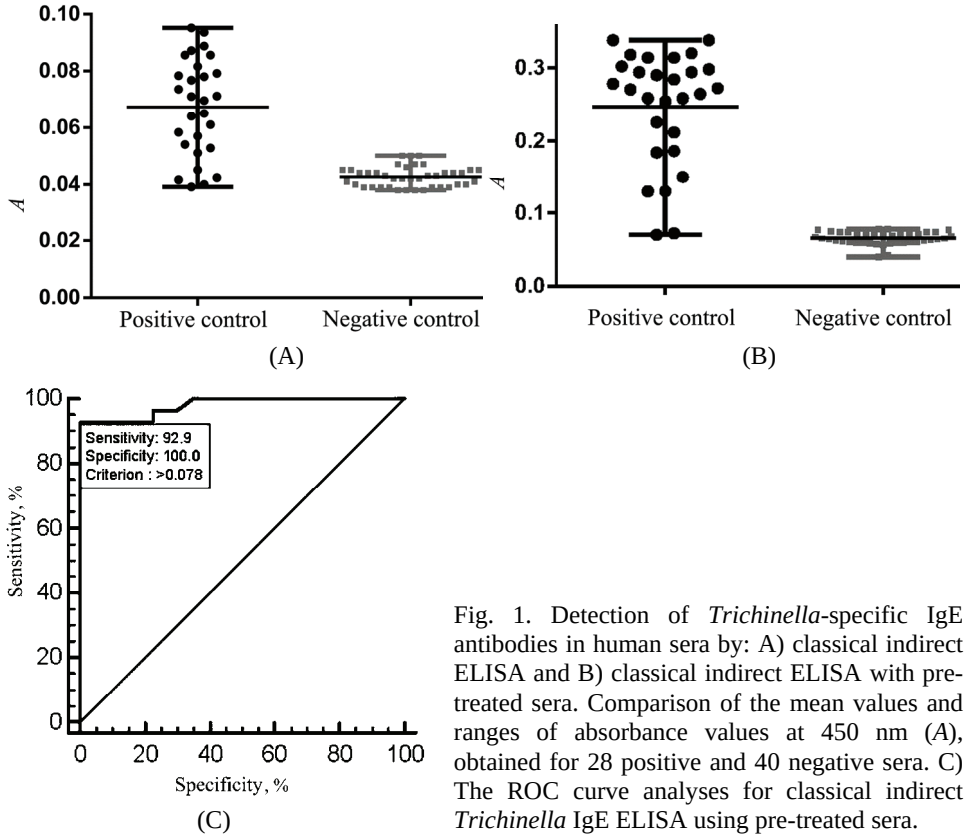

Fig. 1. Detection of *Trichinella*-specific IgE antibodies in human sera by: A) classical indirect ELISA and B) classical indirect ELISA with pre-treated sera. Comparison of the mean values and ranges of absorbance values at 450 nm (*A*), obtained for 28 positive and 40 negative sera. C) The ROC curve analyses for classical indirect *Trichinella* IgE ELISA using pre-treated sera.

Preliminary estimation of repeatability was determined using five positive and five negative sera from the mentioned panel of sera. The intra- and inter--assay variability statistics are given in Table I. Results are shown as mean values of standard deviations and coefficients of variation obtained in five times performed intra- and inter-assays (*CV* / % = *SD*/mean values of samples absorbance). Coefficients of variation with values less than 20 % indicate adequate

TABLE I. Intra- and inter-assay repeatability for classical indirect *Trichinella* IgE ELISA using pre-treated sera; times run: 5

Assay	Serum	*SD*	*CV* / %
Intra	Negative	0.0025	8.2
	Positive	0.00258	2.93
Inter	Negative	0.0061	18.23
	Positive	0.005	6.0

repeatability at this stage of the assay development (validation).[23] All of the obtained results were within the acceptable range of error.

Analytical specificity was assessed by use of panel of sera collected from people suffering from parasitic (toxoplasmosis or echinococcosis) or other (allergic) diseases where the presence of parasite-specific IgE or increased total IgE, respectively, may influence the detection of *Trichinella*-specific IgE. Three out of ten sera of patients with toxoplasmosis gave positive results in the test, and it is already well known that there is some degree of cross-reactivity between specific antibodies to *Toxoplasma gondii* and the ES L1 antigen of *T. spiralis*.[24,25] None of the sera of patients with echinococcosis were reactive in this type of ELISA. The analytical specificity of the indirect *Trichinella* IgE ELISA was 90 %.

Diagnostic sensitivity and specificity were estimated from the results of the testing of 28 serum samples of patients with confirmed diagnosis of trichinellosis (TP), 40 sera samples from healthy blood donors – as the negative control (TN), and 30 sera from patients with other known infection or allergy (having potential to cross-react with the ES L1 antigen of *T. spiralis*). The results obtained by testing the sera by indirect *Trichinella* IgE ELISA are summarized in Table II. The diagnostic specificity was 95 %, calculated as $TN/(TN+FP)$, and the sensitivity was 89 %, calculated as $TP/(TP+FN)$. The positive predictive value was 93 %, calculated as $TP/(TP+FP)$, and the negative predictive value was 95 %, calculated as $TN/(TN+FN)$, where *TP* is the number of true positives, *FN* the number of false negatives, *TN* the number of true negatives and *FP* the number of false positives.

TABLE II. Data for the determination of the diagnostic specificity and sensitivity for classical indirect *Trichinella* IgE ELISA using pre-treated sera

ELISA result	Serum	Number of infected subjects	Serum	Number of *Trichinella* non-infected subjects
Positive	True positive	26	False positive	3
Negative	False negative	2	True negative	67
Total		28		70

Capture Trichinella *IgE ELISA*

Capture ELISA solved the problem of the competition between the excess amounts of IgG and the low concentration IgE for the same binding site on ES L1. By using monoclonal antibodies against human IgE bound to the solid phase, this test, in the first step, ensures separation of IgE antibodies in the serum from all other classes of antibodies before determination of *Trichinella*-specific IgE antibodies. *Trichinella*-specific IgE could then be detected using ES L1 antigens as specific ligands. ES L1 antigens bound to specific IgE could be revealed by the interaction with HRP-labeled 7C2C5 mAb. Capture ELISA produced higher *OD* signals than the indirect ELISA, providing significantly better discrimination

between samples from patients with trichinellosis and healthy persons (Fig. 2A). ROC curve analysis was performed with the same panel of sera as in the previously described ELISA. According to the ROC analysis (Fig. 2B), the best cut-off for the *OD* values was 0.115; based on this cut-off value, the sensitivity and specificity were both 100 %. The *AUC*, which indicates accuracy, was determined for the *OD* values and was equal to 1.

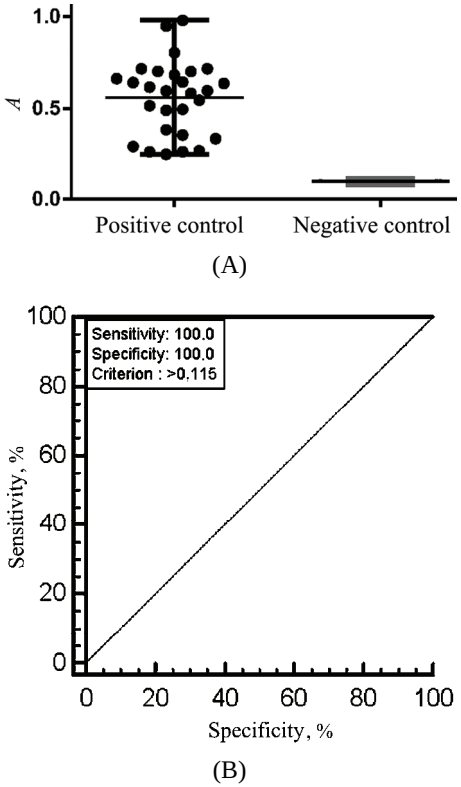

Fig. 2. Detection of *Trichinella*-specific IgE antibodies in human sera by capture ELISA. A) Comparison of the mean values and ranges of absorbance values at 450 nm (*A*), obtained for 28 positive and 40 negative sera. B) The ROC curve analyses for capture *Trichinella* IgE ELISA.

Preliminary estimation of repeatability was again determined according to the results of intra- and inter-assay testing of five positive and five negative sera. The intra- and inter-assay variability statistics are presented in Table III. Since differences between the absorbance values of the serum duplicates did not exceed *CV* values of 2 % for the positives and 6 % for the negatives in the intra assay, and 4% for the positives and 8 % for the negatives in inter assay testing, it is clear that constructed capture *Trichinella* IgE ELISA test possessed great reproducibility.

The analytical specificity was also assessed by the use of the same panel of sera as in previously described ELISA (patients with toxoplasmosis, echinococcosis and sera of patients with allergic diseases). This type of ELISA technique

shows no cross-reactivity with any of the tested serum, with an analytical specificity of 100 %.

TABLE III. Intra- and inter-assay repeatability for capture *Trichinella* IgE ELISA; times run: 5

Assay	Serum	*SD*	*CV* / %
Intra	Negative	0.0025	5.5
	Positive	0.0006	1.28
Inter	Negative	0.0044	7.71
	Positive	0.017	3.5

Diagnostic specificity and sensitivity were derived from testing samples with known infection status – 28 serum samples with confirmed diagnosis of trichinellosis, 40 samples from blood donors as negative controls, and 30 sera with potential cross-reactivity with the ES L1 antigen of *T. spiralis* (total of 70 *T. spiralis* non-infected persons). The results obtained by testing the above-mentioned sera with the capture *Trichinella* IgE ELISA are summarized in Table IV. The diagnostic specificity was 100 %, the diagnostic sensitivity was 100 %, the positive predictive value was 100 % and the negative predictive value was 100 %.

TABLE IV. Data for the determination of the diagnostic specificity and sensitivity for capture *Trichinella* IgE ELISA

ELISA result	Serum	Number of infected subjects	Serum	Number of *Trichinella* non-infected subjects
Positive	True positive	28	False positive	–
Negative	False negative	–	True negative	70
Total		28		70

Since the level of non-specific IgE also increases during helminth infection and because this test utilizes anti-human IgE mAb for capturing IgE from human sera, the effect of non-specific IgE on the result of the assay for *Trichinella*-specific IgE was estimated. No obvious decrease in the absorbance values for *Trichinella*-specific IgE was observed after addition of non-specific IgE-containing serum in range 0–180 kIU L^{-1} (Table V).

To summarize all the data from three previously described ELISAs, their characteristics were compared. The results for the absorbance ranges and the mean values, presented in Fig. 3, clearly show that the range of the obtained absorbance values in the capture *Trichinella* IgE ELISA (0.260–0.960) was much broader than in the classical indirect ELISA using treated sera (0.074–0.340) and untreated sera (0.04–0.10), which enables better interpretation of the results and increases the sensitivity of the test. Data obtained for the classical indirect ELISA using treated sera and the capture ELISA are compared in Table VI and it is obvious that the capture ELISA test for *Trichinella*-specific IgE exhibited very good performances.

TABLE V. The effect of non-specific IgE on the *Trichinella*-specific IgE determination by capture *Trichinella* IgE ELISA

Concentration of added nonspecific IgE in serum sample, kIU L^{-1}	$A_{450\ nm}$
0	0.377
3	0.357
7	0.378
10	0.368
30	0.377
60	0.369
120	0.366
180	0.369

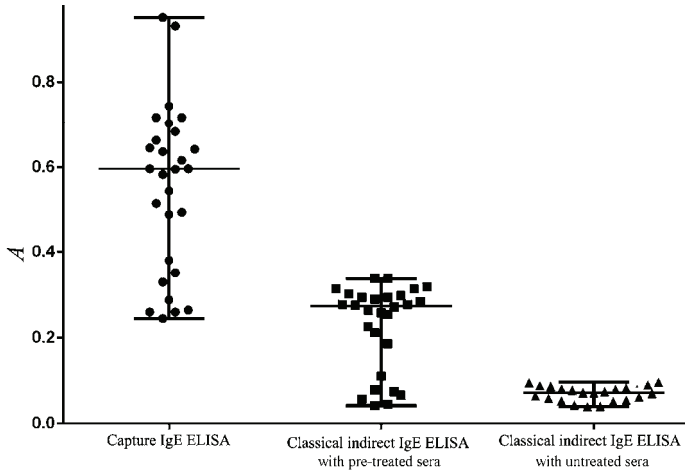

Fig. 3. Comparison of the mean values and ranges for the absorbance results obtained in ELISAs for the detection of *Trichinella*-specific IgE: capture *Trichinella* IgE ELISA, classical indirect *Trichinella* IgE ELISA with pre-treated sera and classical indirect ELISA with untreated sera.

TABLE VI. Comparison of the characteristics of classical indirect *Trichinella* IgE ELISA with pre-treated sera and capture *Trichinella* IgE ELISA for the detection of *Trichinella*-specific IgE antibodies in human sera

Characteristic	Classical indirect *Trichinella* IgE ELISA with pre-treated sera	Capture *Trichinella* IgE ELISA
$A_{450\ nm}$ range	0.074–0.340	0.260–0.960
Intra-assay repeatability for positive samples, %	2.93	1.28
Inter-assay repeatability for positive samples, %	6	3.5
Analytical specificity, %	90	100
Diagnostic specificity, %	95	100
Diagnostic sensitivity, %	89	100

DISCUSSION

The herein presented investigation was focused on creating ELISA tests that could give reliable results concerning the presence of *T. spiralis* specific IgE in the examined sera. One approach was to utilize the formerly established classical indirect ELISA but with serum samples treated with an IgG absorbent to increase the sensitivity of the test, and the other was to develop a new test, *i.e.*, a capture ELISA with performances adequate for the discrimination between specific IgE positive and negative samples. The classical indirect ELISA using untreated serum samples proved to be inadequate for the detection of *Trichinella*-specific IgE antibodies, giving false negative results because of the large quantities of specific IgG that masked the presence of a low-level of specific IgE.

Other authors using the same type of ELISA were able to detect *Trichinella*--specific IgE in a small number of patients (7 %) or in percentages ranging from 13 to 46 %.[4,6] Bruschi *et al.*[26] amplified the indirect ELISA and reported detection rates of 80 % after two months and 20 % after one year for specific IgE determination. Although the amplified method was more sensitive than the classical indirect test, this kind of ELISA does not solve the problem of competition, which was the reason for the contradictory results presented in the literature.[6,27,28] The present attempts to eliminate competition included pre-treatment of serum samples with IgG absorbent, with the aim of removing excess IgG. Many studies, however, showed that commercially available absorbents were not sufficient to improve IgE detection,[29] and the technique with protein A and G agarose beads did not give consistent results in the assay.[30,31] It was observed that these processing techniques resulted in a loss of certain amounts of IgE (data not shown), which may cause variations between tests. In addition, commercial IgG absorbents were not suitable due to the dilution effect. To avoid these problems, our own IgG absorbent was created and used in the Classical Indirect ELISA. Range of absorbance values achieved by this test was higher than obtained by classical indirect ELISA. However, the measured absorbance values remained relatively low and the problem of discrimination between positive and negative results still existed. According to the ROC analyses, the test expressed good sensitivity (92 %) and specificity (100 %), which is better than results obtained with Amplified ELISA.[26] As in other ELISA procedures, the problem of cross-reactivity remained (analytical specificity 90 %). Cross reactivity is in fact the major obstacle in serological diagnosis of various parasites and pathogens because of the shared epitopes among them. The presence of shared antigens of *Trichinella* spp. has been widely documented for other parasites and pathogens.[22,25] Hitherto, different laboratories have dealt with this problem, but they all had a common approach and that was to use different antigens or their isolated components in attempts to increase specificity. With tyvelose (carbohydrate epitope specific for

Trichinella spp) coated plates, Gamble *et al.*[32] obtained good specificity, however, the sensitivity of the test was not satisfactory.

Here, a completely new approach to solve the problems concerning the determination of *Trichinella*-specific IgE is offered. First obstacle was to remove the high levels of *Trichinella*-specific IgG that cause false negative results for specific IgE, and second to eliminate cross-reactivity. This challenge resulted in the development of a capture *Trichinella* IgE ELISA that ensured the capturing of the IgE antibodies by immobilized anti-human IgE mAb, and detection of *Trichinella*-specific IgE among them by introducing ES L1 antigens and HRP labeled 7C2C5 mAb. 7C2C5 mAb recognizes the immunodominant epitopes on ES L1 antigens bound to specific IgE. Using HRP labeled 7C2C5 mAb, the signal was successfully amplified, thus solving the problem of low absorbance values and ranges that were found in the application of previously described ELISA tests. More importantly, the obtained specificity of the capture *Trichinella* IgE ELISA test was 100 % and false positive results were eliminated.

CONCLUSIONS

The herein presented results indicate the very good performances of the capture ELISA test for *Trichinella*-specific IgE detection that, once validated, could contribute to a better understanding of the diagnostic significance of specific IgE detection in patients with trichinellosis. Extended studies with this kind of test could confirm whether specific IgE is suitable as a marker for the early phase of infection with *Trichinella* or, at least, whether it could provide additional data for estimating the time when the infection occurred, and as such, whether it could be used as a tool for differentiating between newly acquired and chronic infection.

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ИЗВОД

ОТКРИВАЊЕ СПЕЦИФИЧНИХ IgE АНТИТЕЛА У ТРИХИНЕЛОЗИ КОД ЉУДИ – СТВАРАЊЕ НОВОГ ТЕСТА

МАРИЈА ДЕВИЋ, АЛИСА ГРУДЕН-МОВСЕСИЈАН и ЉИЉАНА СОФРОНИЋ-МИЛОСАВЉЕВИЋ

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Трихинелоза је паразитско обољење људи које изазива нематода из рода *Trichinella*, најчешће *Trichinella spiralis*. Налаз *Trichinella*-специфичних антитела у серуму је дефинитивни доказ постојања инфекције овом нематодом у случају када је инфекција суспектна на основу клиничке слике и постојећих лабораторијских и епидемиолошких података. Присуство IgE антитела специфичних за *Trichinella* у серуму пацијената може бити знак недавне инфекције или пак последица инфекције која се десила у неком тренутку у прошлости. Позитиван налаз *Trichinella*-специфичних IgE антитела би, с друге стране, могао да укаже на време које је протекло од инфекције, с обзиром на то да се

присуство IgE антитела може пратити у ограниченом временском интервалу, највише до годину дана након инфекције. Постоји, међутим, проблем у детектовању специфичних IgE антитела, који се јавља услед незадовољавајуће сензитивности и специфичности постојећих тестова од којих ниједан није комерцијално доступан. Наиме, до сада описани тестови су слабо разграничавали позитивне од негативних серума и нису решавали појаву лажно позитивних резултата услед унакрсне реактивности са другим паразитским болестима. Ова студија је била посвећена стварању новог теста сендвич ELISA за детекцију специфичних IgE антитела који превазилази проблеме у вези са специфичношћу и сензитивношћу и омогућава поуздано откривање *Trichinella*-специфичних IgE антитела.

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REFERENCES

1. E. Pozio, E. Hoberg, G. la Rosa, D. S. Zarlenga, *Infect. Genet. Evol.* **9** (2009) 606
2. J. Dupouy-Camet, F. Bruschi, in *FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis*, 1st ed. FAO/WHO/OIE, Paris, 2007, p. 37
3. W. Fröscher, F. Gullott, M. Saathoff, W. Tackmann, *Eur. Neurol.* **28** (1988) 221
4. K. D. Murrell, F. Bruschi, *Prog. Clin. Parasitol.* **4** (1994) 117
5. F. Bruschi. L. Chiumiento, *Endocr., Metab. Immune Disord.: Drug Targets* **12** (2012) 4
6. N. Watanabe, F. Bruschi, M. Korenaga, *Trends Parasitol.* **21** (2005) 175
7. M. F. Gurish, P. J. Bryce, H. Tao, A. B. Kisselgof, E. M. Thornton, H. R. Miller, D. S. Friend, H. C. Oettgen, *J. Immunol.* **172** (2004) 1139
8. D. W. Dunne, A. E. Butterworth, A. J. C. Fulford, H. C. Kariuki, J. G. Langley, J. H. Ouma, A. Capron, R. J. Pierce, R. F. Sturrock, *Eur. J. Immunol.* **22** (1992) 1483
9. P. Rihet, C. E. Demeure, A. Bourgois, A. Prata, A. J. Dessein, *Eur. J. Immunol.* **21** (1991) 2679
10. D. Negrão-Corrêa, *Rev. Inst. Med. Trop. Sao Paulo* **43** (2001) 291
11. N. Watanabe, in *Progress of Medical Parasitology in Japan*, Vol. 7, M. Otsuru, S. Kamegai, S. Hayashi, Eds., Meguro Parasitological Museum, Tokyo, 1999, p. 321
12. *Principles of validation of diagnostic assays for infectious diseases*, in *Manual of diagnostic tests and vaccines for terrestrial animals*, World Organisation for Animal Health, Paris, 2008, p. 34
13. N. Watanabe, *Parasitol. Int*. **63** (2014) 332
14. A. M. Atta, A. D'Oliveira Júnior, J. Correa, M. L. B. Atta, R. P. Almeida, E. M. Carvalho, *Am. J. Trop. Med. Hyg.* **59** (1998) 426
15. W. G. Jaoko, M. Lund, E. Michael, P. E. Simonsen, *Acta Trop.* **80** (2001) 51
16. H. R. Gamble, A. S. Bessonov, K. Cuperlovic, A. A. Gajadhar, F. van Knapen, K. Noeckler, H. Schenone, X. Zhu, *Vet. Parasitol.* **93** (2000) 393
17. I. Radovic, A. Gruden-Movsesijan, N. Ilic, M. Mostarica-Stojkovic, Lj. Sofronic-Milosavljevic, *Mem. Inst. Oswaldo Cruz* **107** (2012) 503
18. R. Gamble (Holder), United States Patent, Number 4,670,384 (1987)
19. M. G. Ortega-Pierres, L. Yepez-Mulia, W. Homan, H. R. Gamble, P. L. Lim, D. I. Wassom, J. A. Appleton, *Parasite Immunol.* **18** (1996) 273
20. P. Tijssen, *Techniques in biochemistry and molecular biology*, Elsevier, Amsterdam, 1985, p. 171
21. R. H. Jacobson, *Rev. Sci. Tech.* **17** (1998) 469
22. M. H. Zweig, G. Campbell, *Clin. Chem.* **39** (1993) 561

23. A. Pryseley, K. Mintiens, K. Knapen, Y. Van der Stede, G. Molenberghs. *J. Appl. Stat*. **37** (2010) 1729
24. M. A. Gomez-Morales, A. Ludovisi, M. Amati, S. Cherchi, P. Pezzotti, E. Pozio, *Clin. Vaccine Immunol*. **15** (2008) 1723
25. J. L. De la Rosa, P. Alcantara, D. Correa, *Clin. Diagn. Lab. Immunol*. **2** (1995) 122
26. F. Bruschi, C. Tass, E. Pozio, *Am. J. Trop. Med. Hyg*. **43** (1990) 186
27. F. Van Knapen, J. H. Franchimont, A. R. Verdonk, J. Stumpf, K. Undeutsch, *Am. J. Trop. Med. Hyg*. **31** (1982) 973
28. I. Ljungstrom, L. Hammarstrom, W. Kocieck, C. I. E. Smith, *Clin. Exp. Immunol*. **74** (1988) 230
29. R. L. Mamoni, C. L. Rossi, Z. P. Camargo, M. H. S. L. Blotta, *Am. J. Trop. Med. Hyg*. **65** (2001) 237
30. Y. Kadooka, T. Idota, H. Gunji, M. Shimatani, H. Kawakami, S. Dosako, T. Samori, *Int. Arch. Allergy Immunol*. **122** (2000) 264
31. M. L. Souza-Atta, M. I. Araújo, A. D'Oliveira Júnior, A. Ribeiro-de-Jesus, R. P. Almeida, A. M. Atta, E. M. Carvalho, *Braz. J. Med. Biol. Res*. **32** (1999) 1101
32. H. R. Gamble, E. Pozio, F. Bruschi, K. Nocler, C. M. O. Kapel, A. A. Gajdhar, *Parasite* **11** (2004) 3.