Proteomic Analysis of Eluted Antigens from Crude Somatic Antigens of *Trichinella Spiralis* Muscle Larva by Igg-ELISA, Two-Dimensional Gel Electrophoresis, and Mass Spectrometry

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Research

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Abstract

Background: Trichinellosis is caused by *Trichinella spiralis* muscle larvae (ML), which in swine is the main source of transmission. Trichinellosis is detected by enzyme-linked immunosorbent assay (ELISA) using excretory–secretory antigens (ESAg). Preparation of ESAg is difficult, and it produces a low content. Furthermore, the sensitivity and specificity of the test depends on the quality of the antigen that is produced in each batch. Crude somatic antigens (CSAg) of *T. spiralis* ML at molecular weights (MWs) of 101, 79, and 43 kDa were previously positive for swine trichinellosis sera, with no cross-reaction observed for normal sera, except that 79 and 43 kDa each reacted with one of the coccidiosis and with mixed infections containing trichuriasis and coccidiosis. Therefore, the current study aimed to obtain eluted antigens of *T. spiralis* ML CSAg, which were analyzed by IgG-ELISA for detecting swine trichinellosis and identification by two-dimensional polyacrylamide gel electrophoresis (2-DE) and liquid chromatography-tandem mass spectrometry (LC MS-MS).

Methods: In this study, *T. spiralis* larvae CSAg at 101, 79, and 43 kDa (TsCSAg-101, TsCSAg-79, and TsCSAg-43, respectively) were eluted from sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The eluted antigens were analyzed by IgG-ELISA for sensitivities and specificities. In addition, three specific antigens were identified by 2-DE and LC MS-MS.

Results: Sensitivity of IgG-ELISA using three eluted antigens was persistent at 100%. Specificities were 90.63%, 95.54%, and 97.77% following TsCSAg-101, TsCSAg-79, and TsCSAg-43, respectively. The LC MS-MS results showed that 18/20 spots of the antigens were identified for 11 different proteins. One protein has several isoforms; for example, a serine proteinase and the phosphoenolpyruvate carboxykinase protein.

Conclusions: TsCSAg-43 showed the highest specificity compared to the other two eluted antigens, which indicated that these specific proteins (a 45k antigen–trichina [fragment], a DNA topoisomerase 2-alpha, an antigen targeted by protective antibodies, and a conserved hypothetical protein [gi339234223]) should be developed and produced in large volumes in further study.

Background

Trichinellosis is a major animal disease that is transferred to humans (i.e., a zoonotic disease), since the parasite can survive in various host species without the host exhibiting clinical symptoms. This disease is transmitted to humans via the consumption of raw meat that are contaminated with small roundworms in the genus *Trichinella*, especially *T. spiralis*. In Thailand, trichinellosis has been detected annually [1] and is an ongoing public health problem. Swine is the main cause of this disease [2]. Clinical signs were not observed in naturally infected swine [3] or in experimentally infected pigs with *T. spiralis* larvae [4]. Thus, determining a way to diagnose *T. spiralis* infection in swine could prevent the animal-to-human transmission. The direct method is also commonly used. However, diagnosis is based on the severity of parasitic infection. Serology is suitable for screening and follow-up diagnostics on farms [5].

Serological methods involve high sensitivity, take less time for diagnosis, and examine a high number of samples. Therefore, selection of an appropriate antigen can improve the quality of trichinellosis diagnosis [6]. Previous studies found that excretory–secretory antigens (ESAg) prepared from the secretions or excretions of larvae showed higher sensitivity and specificity than crude somatic antigens (CSAg) [7]. However, ESAg preparation is more complicated, involves a lower volume, requires greater expertise, and has higher costs. Therefore, development of CSAg (which involves easier preparation and yields a greater volume) in serodiagnosis can help confirm trichinellosis in swine. Immunoreactive proteins that were used to diagnose *T. spiralis* infection were characterized by a combination of two-dimensional electrophoresis (2-DE), immunoblot, and liquid chromatography-tandem mass spectrometry (LC MS-MS) analysis. These proteomic methods have been applied to determine the surface proteins of *T. spiralis* ML for the detection of trichinellosis in mice [8] and species-specific ES proteins of *T. spiralis* adult worms for the diagnosis of human trichinellosis [9]. A previous study of Tattiyapong and colleagues (Ph.D. thesis of Tattiyapong, Department of Helminthology) is focused on 1-DE and immunoblotting of the immune response of pigs against crude extracts of *T. spiralis* ML and other parasitic infections. The results showed that some proteins (101, 79, and 43 kDa) were recognized by all experimentally infected pigs [10]. These results indicated that these proteins are likely potential antigens for diagnostic methods and the development of a vaccine.

At present, the antigens of interest, 101, 79, and 43 kDa, of *T. spiralis* ML were electroeluted from sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and were analyzed by IgG-ELISA. Then, the proteomic approach by 2-DE and LC MS-MS was utilized for characterization of those antigen candidates. These data are expected to provide valuable information for further production of a diagnostic antigen for swine trichinellosis.

Methods

Parasites and animals

The *T. spiralis* muscle larvae (ML) used in the present study were obtained by acid-pepsin digestion [11] of infected ICR mice at the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University. In the experiment, 200 ML per mouse were maintained for the new ICR mice, which were purchased from the National Laboratory Animal Center, Mahidol University, and then cared for at the Animal Care Center, Faculty of Tropical Medicine. *T. spiralis* ML were obtained from those infected mice after 2 months of infection by acid-pepsin digestion and were collected using Baermann's apparatus [11].

Antigen preparations

CSAg was prepared from frozen *T. spiralis* ML. Briefly, larvae were mixed with lysis buffer [12] and then subjected to an ultrasonic apparatus (Ultrasonic Processor XL 2020, USA). In collection of the supernatant, the homogenate of the larvae was centrifuged at 13,000 *g* for 60 min at 4°C. The protein concentration was determined by Coomassie® Plus Protein Assay Reagent Kit (Pierce, USA) and stored at −80°C until use.

The eluted antigens (molecular weights [MWs] 101, 79, and 43 kDa, named TsCSAg-101, TsCSAg-79, and TsCSAg-43, respectively), following Tattiyapong's study [10], were prepared by separating CSAg via SDS-PAGE and localized with E-Zinc™ Reversible Stain Kit (Pierce, USA) following the manufacturer's protocol. Electroelution was performed as described previously, but with some modification [13]. Briefly, each small piece of gel that contained an antigen was cut and transferred into each well of the electroelution apparatus (ATTO, Japan). Each well was filled with 3 mL enzyme-linked immunosorbent assay (ELISA) coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6). The eluted proteins were removed from the bottom of the wells at a constant power of 30 mA, for 3 h. After that, the eluted antigens (TsCSAg-101, TsCSAg-79, and TsCSAg-43) were collected, and the sensitivity and specificity were determined by IgG-ELISA. The eluted antigens of the supernatant were prepared and analyzed by 2-DE and immunoblotting. Proteins spots were submitted to LC MS-MS for peptide identification.

Serum samples

Swine serum samples were obtained from a previous study [11] but with additional sera. These swine sera were divided into three categories: positive trichinellosis sera from experimentally infected swine (n = 5), other parasitic infections with additional diseases (n = 192), and normal control (n = 30). Swine trichinellosis sera were selected at an increasing antibody level on day 20, which was tested using CSAg of *T. spiralis* larvae by IgG-ELISA. Detailed information of the serum samples is presented in Supplementary Table S1 (see Additional file 1).

SDS-PAGE and immunoblot

These antigens were subjected to 14% SDS-PAGE. After electrophoresis, one gel was stained with ProteoSilver™ Silver staining (Sigma-Aldrich, USA), whereas the other gel was electroblotted onto a nitrocellulose membrane (0.45 μm, PALL, Sigma-Aldrich, USA) at 400 mA for 5 h via Semi-Dry Transfer Cell (ATTO, Japan). The immunoblot was performed as described previously, with some modifications [14]. Subsequently, the membrane was blocked with 3% skim milk for 1 h on a rocking platform, which was cut into long strips and incubated with pooled swine serum at a dilution of 1:100 at room temperature, overnight. After washing with phosphate buffered saline 0.5% Tween 20 (PBST), strips were incubated with peroxidase-labeled affinity purified antibody to swine IgG (1:1,000 dilution) for 4 h. The immunoreaction was detected with a substrate solution (2,6-dichlorophenol indophenol). Finally, the membrane was washed with distilled water to stop the reaction. The separated antigens were analyzed by SDS-PAGE and immunoblot (Figs. S1a and b, see Additional file 2).

Indirect ELISA

The protocol of the indirect ELISA followed a previously described method [14], but with minor modifications. The optimal antigen, dilutions of serum, and conjugate obtained from checkerboard titration were 1 μg/mL, 1:400, and 1:4,000, respectively. Each 50 μL of diluted antigen with coating buffer in the duplicate well was filled, incubated at 37°C for 1 h, and left overnight at room temperature. The unbinding sites with antigens in wells were blocked by 75 μL 0.5% skim milk in PBS at 37°C for 1 h. Antigens reacted with antibodies in 50 μL of diluted serum at 37°C for 1.30 h. Each 50 μL of diluted

conjugate (peroxidase-labeled affinity purified antibody to swine IgG) was added and incubated as described above. Then, the color reaction was established by adding 50 μL of 2,2-azino-di-(3-ethyl-benzothiazoline sulfonate) containing H_2O_2 (ABTS) substrate and left for 30 min. The reaction was stopped by adding 75 μL 1% SDS, and the absorbance was determined by an ELISA microplate reader (Tecan, Austria) at 405 nm.

The cut-off value of IgG-ELISA was evaluated for the three eluted antigen preparations on the basis of a receiver operating characteristic analysis performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, La Jolla, California, USA, www.graphpad.com).

2-DE and immunoblot

CSAg (100 μg) was adsorbed onto 7 cm immobilized pH gradient 3–10 NL (IPG) strips (GE Healthcare, USA) and then focused on an Ettan IPGphor 3 (GE Healthcare, USA). After the equilibration steps, IPG strips were placed onto 12% SDS-PAGE gels and run at 400 mAm, after which the gels were silver-stained. Alternatively, the separated spots were transferred to nitrocellulose membranes following standard protocols. Swine sera were used in 1:100 dilution. Immunoreactive spots were detected using goat anti-swine IgG antibody conjugated with horseradish peroxidase at 1:1,000 dilution and an immunoreaction detection system with a substrate solution.

Mass spectrometric analysis

Protein bands recognized by swine trichinellosis sera were excised from the parallel gel and subjected to in-gel tryptic digestion following the protocols of Reamtong et al. [15]. Briefly, the gels were destained with 50 mM NH_4HCO_3 and 200 μL acetonitrile (ACN). Then, the colorless gels were incubated with 100 μL of 4 mM dithiothreitol (DTT) (GE Healthcare, UK) in 50 mM NH_4HCO_3 at 60°C for 15 min and alkylated with 250 mM iodoacetamide (IAM) (GE Healthcare, UK) in Milli-Q water at RT for 30 min in the dark. Next, reactions were quenched by 4 mM DTT in Milli-Q water and left for 5 min. Consequently, the gels were dehydrated with 100 μL ACN and shacked for 15 min. Afterward, the supernatant was removed, and the gels were completely gel dried in a fume hood. Subsequently, the gel pieces were digested with 10 ng/200 μL trypsin in 50 mM NH_4HCO_3 (Sigma-Aldrich, USA). The reactions were incubated overnight at 37°C. The amount of peptides in each small gel piece was achieved by extraction with 50% (V/V) ACN and dried using a vacuum evaporator (Labconco, USA). Then, the proteins were separated by high-performance liquid chromatography and analyzed by tandem MS. The LC MS-MS data were searched against the *T. spiralis* protein database from NCBI using Mascot version 2.4.1 (Matrix Science, UK).

Bioinformatics analysis

The signal peptide, transmembrane domains, and subcellular localization of the identified proteins were predicted by using online tools SignalP [16], TMHMM [17], and CELLO v.2.5 [18], respectively.

Results

Indirect ELISA

The results of the three eluted antigens (TsCSAg-101, TsCSAg-79, and TsCSAg-43) using IgG-ELISA are summarized in Table 1. The cut-off values of IgG-ELISA with those three antigens for swine trichinellosis were 0.221, 0.262, and 0.276, respectively. The highest sensitivity using the three antigens was specified as 100%. Among the three eluted antigens, TsCSAg-43 performed the highest specificity (97.77%); meanwhile, TsCSAg-79 and TsCSAg-101 were 95.54% and 90.63%, respectively. The TsCSAg-43 resulted in five false IgG-ELISA positives of three diseases. TsCSAg-101 gave the highest cross-reactivity, with 22 sera of five diseases: trichuriasis, coccidiosis, toxoplasmosis, trypanosomiasis, and mixed infection of coccidiosis and trichuriasis. This antigen was highly cross-reactive to 13 of 83 coccidiosis in comparison to coccidiosis among three antigens. In contrast, TsCSAg-43 and TsCSAg-79 did not react with trypanosomiasis or mixed infection of coccidiosis and trichuriasis. None of the antigens reacted with several different diseases, such as single infections with *Strongyloides*, *Ascaris suum*, *Oesophagostomum dentatum*, *Cryptosporidium*, *Balantidium*, or several mixed infections, except coccidiosis combined with trichuriasis (Table 2). In this study, antibodies of six samples of mixed infections against *Ascaris*, other parasites, and coccidia did not react with these eluted antigens. In addition, negative results were found from these antigens to antibodies of several mixed infections with those parasites: *Ascarop dentata*, *Bourgelatia diducta*, *Cysticercus cellulosae*, *C. tennuicollis*, *Echinostoma malayanum*, Filariidae, unidentified GI-nematodes, *Globocephalus* sp., *Gnathostoma doloresi*, *Hyostrongylus* spp., *O. dentatum*, *Physocephalus sexalatus*, and *Pseudanoplocephala* sp. The results of the false positives and true negatives that were demonstrated from the reactions of antibodies of other parasitic infections with the three eluted antigens following their cut-off values are shown in Tables 1 and 2. Among the three antigens, TsCSAg-43 is the most favorable antigen based on specificity. Regarding quality of antigen, TsCSAg-79 can serve as a screening antigen for swine trichinellosis because of its specificity of 95.54% and the same cross-reactivity to trichuriasis, coccidiosis, and toxoplasmosis as that using TsCSAg-43, except with larger serum numbers.

Table 1
Comparison of the eluted antigens using IgG-ELISA

Antigen	Cut off value	%Sensitivity	%Specificity	Area (95% CI)	Std. Error	P-value
TsCSAg-101	0.221	100	90.63	0.9313	0.01733	0.0009855
TsCSAg-79	0.262	100	95.54	0.9813	0.009782	0.0002368
TsCSAg-43	0.276	100	97.77	0.9866	0.007425	0.0002016

Table 2
False positive cases of other diseases by IgG-ELISA against the eluted antigens

Diseases (total number)	Number of false positive cases		
	TsCSAg-101	TsCSAg-79	TsCSAg-43
1. Trichuriasis (n = 11)	2	1	1
2. Coccidiosis (n = 83)	13	6	2
3. Toxoplasmosis (n = 23)	5	3	2
4. Trypanosomiasis (*T. evansi*) (n = 8)	1	-	-
5. Mixed infection of coccidiosis and trichuriasis (n = 12)	1	-	-
Total	**22**	**10**	**5**

2-DE and immunoblot

The separation patterns of CSAg of *T. spiralis* as detected by 2-DE were approximately 300 protein spots stained by silver stain (Fig. 1). They mostly comprised the pI between 4 and 8. The 20 protein spots, which showed the lowest cross-reaction with other parasitic infection diseases using 1-DE immunoreaction, were compared and selected from previous results [11]. Spot Nos. csa 1–5, csa 6–17, and csa 18–20 belong to TsCSAg-101, TsCSAg-79, and TsCSAg-43, respectively. After that, all spots were cut and identified by using MS.

The immunoreactions of swine sera are depicted in Fig. 2a (negative swine) and Fig. 2b (swine trichinellosis). Seventy-five spots were positively recognized by swine trichinellosis sera when conjugated anti-swine IgG was used. Major antigenic spots of the selected 20 protein spots were located in the acidic range (pH 3–7) migrating at 101–43 kDa.

Mass spectrometry

A total of 20 protein spots (TsCSAg-101 = spots 1–5, TsCSAg-79 = spots 6–17, and TsCSAg-43 = spots 18–20) were selected from the immunoreactive spots of 2-DE immunoblot. The spots were excised from 2-DE gels, and a proteomic analysis of the selected spots was carried out using LC MS-MS. The 18 spots (csa 2 and csa 4–20) were successfully identified. csa 1 and 3 could not be identified with any *Trichinella* sequence in the NCBI database. The results were carried out using MASCOT (Matrix Science) and are summed up in Tables 3–5 (see Additional file 3).

The 20 protein spots were selected and identified by LC MS-MS. Of these, 18 protein spots were identified, which represented 11 different proteins. Two different proteins from TsCSAg-101, one spot (csa 2), were identified as a conserved hypothetical protein (gi339235735) protein. Two spots (csa 4 and 5) were identified as a putative fasciclin domain protein. They have similar MWs but different pIs. Five different proteins from TsCSAg-79, six spots (csa 6 and csa 10–14), were identified as a serine protease protein, which has different MWs and pIs. Two spots (csa 7–8) were identified as a heat shock protein (A

protein). Three spots (csa 7–9) were identified as a muscle cell intermediate filament protein (OV71 protein). Three spots (csa 8–10) were identified as an intermediate filament protein (ifa-1 protein). Five spots (csa 6 and csa 14–17) were identified as a phosphoenolpyruvate carboxykinase protein. The spots have similar MWs, but different pIs. Of the four different proteins from TsCSAg-43, one spot (csa 18) was identified as having two different proteins and is thus associated as an antigen targeted by protective antibodies and a 45k antigen–trichina (fragment) protein. Three spots (csa 18–20) were identified as a DNA topoisomerase 2-alpha protein. Three spots (csa 18–20) were identified as two different proteins, which were DNA topoisomerase 2-alpha protein and a conserved hypothetical protein (gi339234223). They have similar MWs, but different pIs. Results of the signal peptide, transmembrane domain, and subcellular localization prediction are shown in Tables 3–5 (see Additional file 3).

Discussion

At present, the antigen used for immunodiagnosis was derived mainly from *T. spiralis* ML, during the infective stage. The advantage of this stage is that it easily increases the number of larvae in mice, which is a sensitive experimental host for the propagation of *T. spiralis* ML. Most studies of *T. spiralis* antigens have focused on antibodies of human trichinellosis; there have been few reports on antigens to swine antibodies. It was found that the antibody of the experimental swine infected with *Trichinella* larvae increased and was thus detected at 2 weeks post-infection [19]. Swine serum samples in the current study were selected on approximately day 20 of infection, at which time the antibody had already increased. Since larvae can reside in the muscle of intermediate hosts, especially in swine, for a long time, larvae are important for surveillance of disease.

The present study analyzed three eluted antigens following their IgG-ELISA conditions, which were obtained in the same manner. Specificity was different in the range of 90–98%. Only five false positives of the three parasitic infections were found by using TsCSAg-43, as follows: 1 of 11 trichuriasis cases and 2 each of coccidiosis (n = 83) and toxoplasmosis (n = 23). In contrast, TsCSAg-101 had more cross-reactive molecules than those of TsCSAg-79 and TsCSAg-43, because the antibody to coccidiosis could react with 13 of 83 samples. In a comparison with a previous study [11], CSAg of muscle larvae gave 91.8% specificity and showed a cross-reaction with trichuriasis (2/11), coccidiosis (5/86), and mixed infections (8/39); strongyloidiasis and coccidiosis (1/4); *Oesophagostomum* spp.-like GI-nematode and ascariasis (1/1); *Oesophagostomum* spp.-like GI-nematode and coccidiosis (1/7); *Oesophagostomum* spp.-like GI-nematode, strongyloidiasis, and coccidiosis (2/3); *Oesophagostomum* spp.-like GI-nematode, strongyloidiasis, cryptosporidiosis, and coccidiosis (1/1); and unidentified GI-nematode and coccidiosis (2/3). It indicated that antibodies against *Oesophagostomum* spp.-like GI-nematode in mixed infections promote cross-reactivity to TsCSAg, because single infections with *Ascaris*, *Cryptosporidium*, and *Strongyloides* did not react with TsCSAg of their study. However, with coccidiosis in their study, only 5 of 86 samples (6%) performed little cross-reactivity with TsCSAg, but a few mixed infections with coccidiosis were negative to TsCSAg. It is possible that the antibody against *Oesophagostomum* spp. reacts with this antigen. Regarding the three eluted antigens, antibodies against *Oesophagostomum* parasites of single and mixed infections did not perform cross-reactivity. Our results indicated that cross-

reactivity can be reduced during the detection of swine trichinellosis by the preparation of the eluted antigen, which was in comparison with the study of Tattiyapong et al. [11], which used several diseases and serum samples. TsCSAg-43 is the best antigen among the three antigens, based on specificity, which was determined by several diseases and a high number of serum samples.

The 11 different proteins of 20 spots were identified using LC MS-MS from the NCBI database. Some proteins showed several isoforms, which were identified differing in pI; for instance, six spots (csa 6 and csa 10–14) were identified as serine proteinase, and five spots (csa 6 and csa 14–17) were assigned to phosphoenolpyruvate carboxykinase. This result indicates that one protein has multiple isoforms. There were several possibilities for describing different isoforms of the protein, such as post-translation, variation, and protein processing [20]. They also play a role in biological processes, for example, parasite survival, immune evasion mechanisms, and immunopathogenesis. Moreover, these proteins may be the same members of the family that share functional domains [21]. The signal peptide was identified by SignalP. The three different proteins have a signal peptide that serves as a serine proteinase, which is an antigen targeted by protective antibodies, a DNA topoisomerase 2-alpha, and a conserved hypothetical protein (gi339234223). Furthermore, out of the 11 total proteins, only DNA topoisomerase 2-alpha was found in the transmembrane helices of the proteins. Two protein spots (csa 1 and csa 3) could not be used to determine the type of protein, which may be because of their low protein concentration or because they could not produce high-level spectrometric data. Therefore, the proteins had not yet been described and were thus not included in the database [8].

Conclusions

In the present study, TsCSAg-43 showed the highest specificity compared to the three eluted antigens, which indicated that these specific proteins (a 45k antigen–trichina [fragment], a DNA topoisomerase 2-alpha, an antigen targeted by protective antibodies, and a conserved hypothetical protein [gi339234223]) should be developed and produced in large volumes in the diagnostic study. We demonstrated that IgG-ELISA based on eluted TsCSAg-43, performed with the highest sensitivity and specificity as a diagnostic tool, and TsCSAg-79 is an appropriate screening test for swine trichinellosis monitoring in farms.

Abbreviations

ELISA: enzyme-linked immunosorbent assay; ESAg: excretory–secretory antigens; MW: molecular weight; CSAg: crude somatic antigen; ML: muscle larvae; 2-DE: two-dimensional polyacrylamide gel electrophoresis; LC MS-MS: liquid chromatography-tandem mass spectrometry; SDS-PAGE: sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Declarations

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Ethics approval

This study was approved by the Animal Ethics Committee from the Faculty of the Tropical Medicine, Mahidol University, with approval number FTM-ACUC No. 016/2017.

Consent for publication

Not applicable.

Availability of data and materials

Data supporting the conclusions of this article are included within the article and its additional file.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

US (PhD student) performed all experiments, provided data analysis and results, and contributed to manuscript preparation. DW participated in manuscript preparation. OR supervised US in performing 2-D gel electrophoresis and mass spectrometry and bioinformatic analysis. YL contributed to the animal experiments. PA was involved in immunological and bioinformatic analysis. LZ participated in technological guidance and analysis. PD supervised the immunological techniques and analysis, including interpretation of the results and manuscript. All authors read and approved the final manuscript.

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Figures

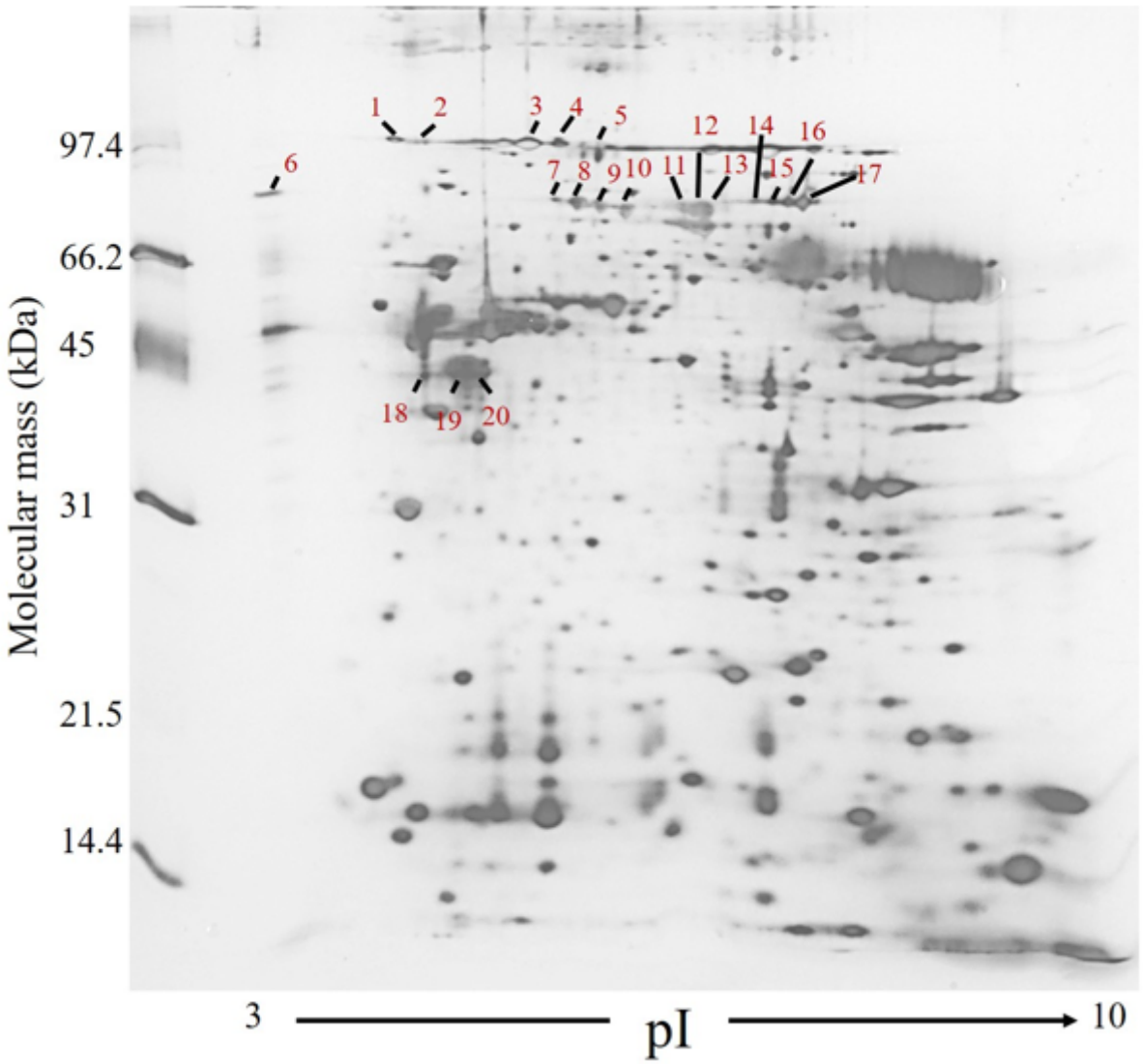

Figure 1

2-DE pattern of CSAg proteins of T. spiralis. 2-DE was performed in nonlinear IPG with a pI range of 3–10 stained with silver-stained. Labeled spots were immunodetected using swine serum and identified by MS. (TsCSAg-101 = spots 1–5, TsCSAg-79 = spots 6–17, and TsCSAg-43 = spots 18–20)

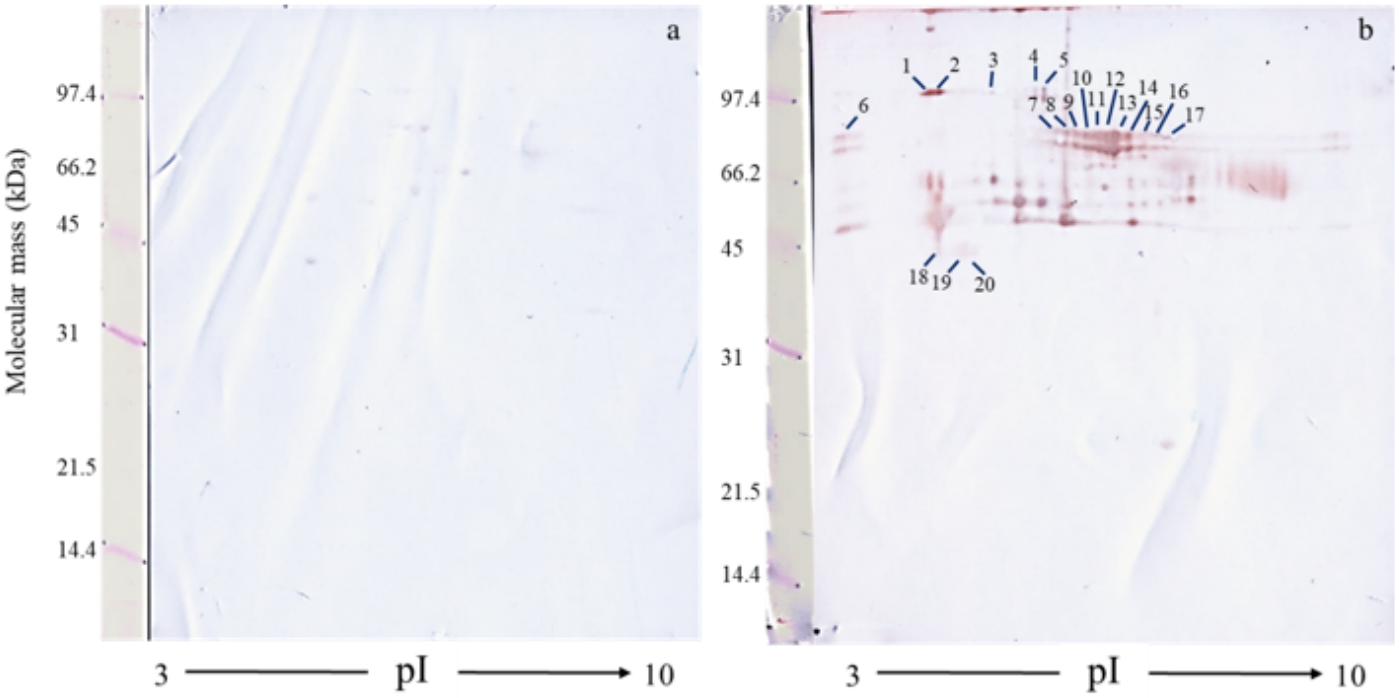

Figure 2

Immunoblot analysis of CSAg against antibody of pooled swine sera [(a) = negative swine serum and, (b) = positive trichinellosis serum] using anti-swine IgG conjugate. (TsCSAg-101 = spots 1–5, TsCSAg-79 = spots 6–17, and TsCSAg-43 = spots 18–20)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1TableS130421.docx
- Additionalfile2FigS130421.docx
- Additionalfile3Table3530421.docx
- Graphicalabstract30421.docx