World Applied Sciences Journal 8 (4): 457-461, 2010
ISSN 1818-4952

Comparison of Two Antigens for Diagnosis of Trichinellosis in Pigs

[1]Soad E. Hassan, [1,2]FaragalLa M. El-Moghazy and [1]Nagwa I. Toaleb

[1]Department of Parasitology and Animal Diseases, National Research Center, Dokki, Giza, Egypt
[2]Department of Biology, College of Education for Girls, Scientific Departments, Al-Kharj University, K.S.A

Abstract: Due to its role in human Trichinellosis, there are increasing global requirements for reliable diagnostic method for *Trichinella spiralis* infection in pigs. Consequently, there is a need for selection of potent diagnostic antigen that could be successfully utilized in the diagnosis. Currently, two antigens (somatic and excretory-secretory antigens of *T. spiralis* larvae) were evaluated for the diagnosis of pig trichinellosis using ELISA. The ES antigen exhibited highest diagnostic potential (88.3%) as compared to somatic antigen (68.3%). The diagnostic potential of ES antigen is attributed to five immunogenic bands identified by infected pig sera in immunoblot. These bands are of molecular weight 127, 58, 35, 20.5 and 12 KDa. While in somatic antigen six immunogenic bands of molecular weight 230, 89, 58, 27, 15 and 6 KDa were identified. In conclusion, monitoring for *T. spiralis* antibodies in swine farms should be based on screening pig sera by means of ELISA in which larval ES antigen was utilized.

Key words: *Trichinella spiralis* · Diagnosis · ELISA · Excretory-secretory antigen · Somatic antigen · Immunoblot

INTRODUCTION

Trichinellosis is one of the most important parasitic food borne zoonotic disease, caused by the consumption of insufficiently cooked or raw meat contaminated with genus *Trichinella* [1-3].

Pig is considered the main host for *Trichinella spiralis* and responsible for transmission to human [4]. Although humans are accidental hosts [5, 6], human trichinellosis is estimated to affect at least eleven million people in different countries [7, 8].

In domestic animals and wildlife, the meat digestion and microscopic inspection is considered to be the most useful method for detecting these parasites, but it is somewhat cumbersome to perform [9]. A variety of immunological assays has been described for the diagnosis of trichinellosis in domestic and wild animals [10]. Methods used for diagnosis depend upon immunofluorescence assay (IFA), immuno-electrotransfer blot (IEBT), western blot, enzyme immunohistochemical assay and enzyme-linked immunosorbent assay (ELISA). Except for the ELISA, these tests have not been standardized and reagents are not available for routine use. Nevertheless, the International Commission of Trichinellosis (ICT) has provided a uniform set of recommendations for the development and use of serological tests for the detection of circulating antibodies [10]. The ELISA is the only immunological assay endorsed by the ICT. It is only approved as an epidemiological surveillance tool to detect anti-*Trichinella* antibodies in pigs.

The sensitivity and specificity of ELISA are largely dependent on the quality of the antigen used in the test. Antigens that are specifically secreted from the stichocyte cells of living L1 larvae are recognized by antibodies in *Trichinella* infected animals. These antigens are found in all *Trichinella* species and thus can be used to detect infections with any species or genotype [10, 11]. The excretory-secretory (ES) products released from muscle larvae contain these antigens and are commonly used in ELISA. This antigen increases the sensitivity and specificity of the method and reduces false positive results [12, 13]. Thus IgG-ELISA using ES antigen of the *Trichinella* larval stage was useful not only for diagnosis, but also in evaluation of cure.

So, the current research was designed to compare the diagnostic potentials of *T. spiralis* somatic and ES larval antigens in diagnosis of trichinellosis in pigs by ELISA. Also, detection of immunogenic proteins which are responsible for these potentials in both antigens by immunoblot assay was another target.

Corresponding Author: Soad E. Hassan, Department of Parasitology and Animal Diseases, National Research Center, Dokki, Giza, Egypt, Tel: +202- 0123590225

MATERIALS AND METHODS

Parasite: Larvae of *T. spiralis* were obtained from muscles of the diaphragms of infected pigs proved to be heavily infected with *T. spiralis*, by trichinoscope in Cairo abattoir. The infected muscles was minced and digested by conventional method of artificial digestion with Pepsin-HCl according to Arriage *et al.* [14]. The larvae were collected to be used either for preparation of somatic or ES antigens.

Serum Samples: A total number of 60 random serum samples were collected from slaughtered pigs, aliquoted and stored at -20°C until use.

Antigens Preparation:

Somatic Extract of Infective Larvae: Extract was prepared according to Year *et al.* [15]. In brief, *T. spiralis* larvae were homogenized by a glass Potter-homogenizer using a teflon pestle and further disintegrated by sonication. in Tris-EDTA buffer, containing 40 mM Tris, 1 mM EDTA, 0.25 M sucrose and protease inhibitors (170 µg/ml phenylmethylsulfonyl fluoride "PMSF"). Cetyl-trimethyl ammonium bromide (CTAB) 0.25% (w/v) was added to the mixture [16]. The homogenate was centrifuged at 14.000 rpm for 30 minutes at 4°C. Supernatant was assayed for protein content by the method of Lowry *et al.* [17] aliquoted and stored at -20°C until use.

Excretory-secretory (ES) Antigen: The harvested larvae (10000/ml) were suspended in 0.15 M PBS PH 7.2. The buffer was supplemented with antibiotics (100U/ml penicillin; 100µg/ml streptomycin) according to Gomez-Priego *et al*. [18]. Glucose (2%) was added as nutritive agent. The supernatant which corresponded to the ES antigen was collected after 10 h., clarified by centrifugation at 10000 rpm for 15 min, assayed for protein content by the method of Lowry *et al.* [17], aliquoted and frozen at - 20°C until use.

Enzyme Linked Immunosorbant Assay (ELISA): Diagnostic potency of each prepared antigen was evaluated by ELISA which performed according to Santiago *et al*. [19] with light modification. ELISA plates were coated with ES and somatic larval antigens separately using carbonate buffer pH 9.6 (coating buffer). 0.1ml /per well of antigen was added and then the plates were incubated overnight at 4°C. Plates were then washed with PBS -0.05 % Tween-20 to get rid of excess unbound antigen and the remaining free binding sites were then blocked with bovine serum albumin (BSA) 200 µL / well and kept for 1 h at room temperature. Plates were then washed 3 times with PBS - 0.05 % Tween. Random serum samples and sera from non infected pigs (as control) were added to the plates in a final volume of 0.1ml /well. Plates were incubated for 90 minutes at 37°C. Plates then washed with PBS -0.05 % Tween - 20 to get rid of excess unbound sera. A volume of 0.1ml / well of protein A conjugated horseradish peroxidase was added to each well and incubated at 37°C for 1 h. After incubation, the plates were washed and a volume of 0.1ml of ortho - phenylene - diamine substrate working solution was added/well. The reaction with yellowish coloration was stopped. The optical density values (OD) were read at 450 nm with a micro -ELISA reader system. The cut off values were calculated according to Hillyer *et al.* [20].

Characterization of Antigens Using:

(SDS-PAGE): Protein components of the excretory-secretory antigen and somatic larval antigen were electrophoretically separated on SDS-PAGE according to the method of Laemmli [21]. After separation, the gel was stained with Commassie brilliant blue dye. Molecular weights of bands were calculated using marker supplied by Fermentas International INC. Canada.

Immunoblot: Immunoblot assay was utilized to identify the immunoreactive components recognized in somatic and excretory-secretory antigens by naturally infected pig sera. After electrophoresis, protein components of two antigens were immunoblotted onto nitrocellulose membrane according to Towbin *et al*. [22]. Nitrocellulose membrane was incubated with ELISA positive pig sera and protein A conjugate horseradish peroxidase. ECL western blotting detection reagent (Fermentas International INC. Canada) was used to visualize the immunoreactive bands.

RESULTS

Elisa: Comparative diagnostic potentials of *Trichinella* larval somatic and ES antigens in the detection of *T. spiralis* antibodies in pigs revealed that the ES antigen showed the highest diagnostic potentials and recorded (88.3 %) as compared to somatic antigen (68.3%) as depicted in Figures 1,2 and Table 1.

Table 1: Infection % of trichinellosis in pigs by somatic and ES antigens using ELISA

Antigen	No. of samples	Positive samples	Negative samples	Infection percentage
Somatic antigen	60	41	19	68.3
ES antigen	60	53	7	88.3

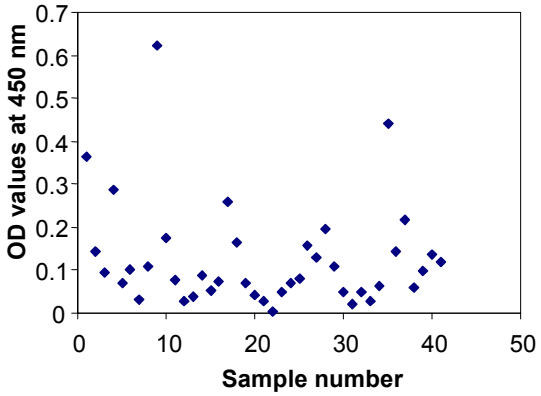

Fig. 1: Diagnostic potential of somatic antigen for trichinellosis in pigs

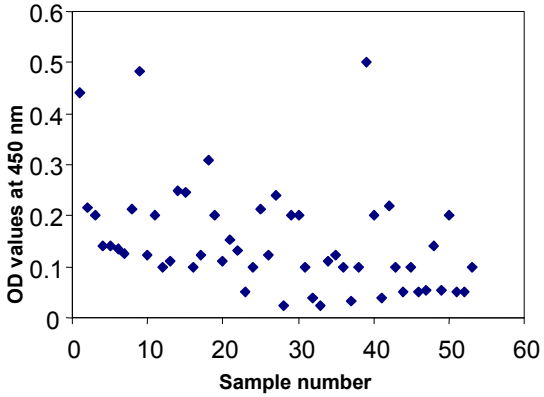

Fig. 2: Diagnostic potential of ES antigen for trichinellosis in pigs

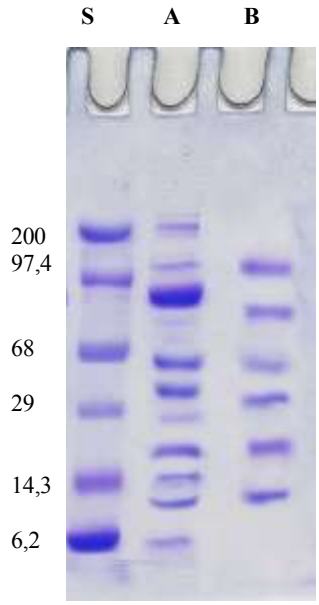

Fig. 3: Comparative electrophoretic profile of *T. Spiralis*. lane A: Somatic antigen. lane B: ES antigen. lane S: Molecular weight standards in KDa

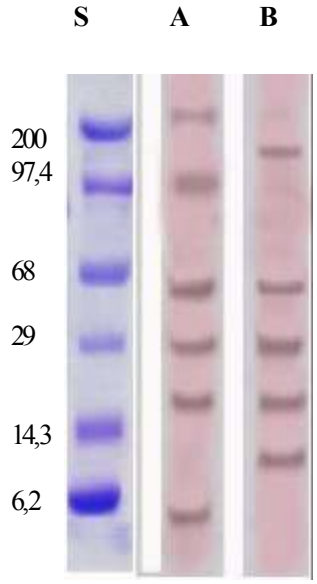

Fig. 4: Immunoreactive bands identified by pig infected sera. Lane A : somatic antigen, lane B: ES antigen. Lane S:Molecular weight standards in KDa.

Characterization of Antigens:

SDS-PAGE: Electrophoretic profile of both *Trichinella* antigens is shown in Figure 3. The ES antigen was characterized into six polypeptide bands of molecular weight 127., 85, 58, 35., 20.5 and 12. KDa as compared with complex profile of somatic extract which revealed ten polypeptide bands of molecular weight 230, 119, 89, 58, 39.6, 27, 20, 15, 11 and 6. KDa.

Immunoblot Assay: The immuno-reactive bands of both *T. spiralis* antigens (ES and somatic larval antigens) were identified with *T spiralis* positive sera by immunoblot assay. The recognized immunogenic bands of the ES antigen were 127, 58, 35, 20.5 and 12 KDa. While the somatic larval antigen revealed six immunogenic bands with molecular weight of 230, 89, 58, 27, 15 and 6 KDa (Figure 4).

DISCUSSION

In the surveillance of trichinellosis, the serological methods for the detection of *Trichinella* - specific antibodies using ELISA offer a sensitive and relatively specific tool [23]. In the current research, two *T. spiralis* antigens (somatic and ES) were evaluated for the diagnosis of trichinellosis in pigs using ELISA. The ES antigen proved higher diagnostic potential than somatic antigen. This result was previously introduced [24,25].Moreover, the present results were confirmed by the observation of Moller *et al.* [23], Forbes *et al*. [26] and Gamble *et al*. [27]. These authors recorded higher sensitivity of ES antigen over a synthetic glycan antigen, β-tyvelose. Additionally, ES sensitivity and specificity reached to 98% in the diagnosis of trichinellosis in pigs [13] and in pigs and horses [23, 28 ,29] The ES antigen not only increase the specificity of ELISA, but also it reduced false positive results [30]. All previous studies together with the current work prove the importance of ES antigen in accurate diagnosis of trichinellosis in pigs.

Searching for the components responsible for diagnostic potentials in both antigens, immunoblot showed 6 bands associated with somatic antigen 230, 89, 58, 27, 15 and 6KDa using pig infected sera. While, 5 immunogenic bands were associated with ES antigen of molecular weight 127, 58, 35, 20.5 and 12KDa. Although, there is a common immunogenic band between both antigens (58KDa) the other bands are specific to each one. This variation is responsible for their different diagnostic potentials. Previously, different profile of seven immunogenic bands (229,108, 64, 45, 41 and 33-38KDa) were identified in *T. spiralis* ES by infected big sera in immunoblot [12] Moreover, five immunogenic bands were recognized in Es antigen of *T. spiralis* by infected pig sera of molecular weight 102, 66, 61, 47 and 43 KDa [31]. This variation in bands pattern profile is probably attributed to differences in antigen preparation, differences in the type of gel and molecular marker used or to the method of calibration.

In conclusion, the current study recommended the use of ES antigen of *T. spiralis* larvae in the diagnosis of trichinellosis in pigs rather than somatic one. Bands responsible for this diagnostic potential are of molecular weight 127, 58, 35, 20 and 12 KDa

REFERENCES

1. Dupouy-Camet, J., 2000. Trichinellosis:at world wide zoonosis. Vet. Parasitol., 93: 191-200.
2. Jung, D., J.P. Teifke, A. Karger, K. Michael, S. Venz, W. Wittman, K. Kindermann, K. Nockler and E. Mundt, 2007. Evaluation of baculovirus-derived recombinant 53-KDa protein of *Trichinella spiralis* for detection of *Trichinella-specific* antibodies in domestic pigs by ELISA. Parasitol. Res., 100: 429-437.
3. Mahdavi, M., 2009. Trichinellosis in Iran. Iranian J. Publ Health, 38: 131-133.
4. Leiby, D.A., C.H. Duffy, K.D. Murrell and G.A. Schad, 1990. *Trichinella spiralis* in agricultural ecosystem: transmission in the rat population. J. Parasitol, 76: 360-364.
5. Caner, A., M. Doskaya, A. Degrimenci, H. Can, S. Baykan, A. Uner, G. Basdemir, U. Zeybek and Y. Guruz, 2008. Comparison of the effects of Artemisia vulgaris Artemisia *absinthium* growing in western Anatolia against trichinellosis (spirals) in rats. Experimt. Parasitol., 119: 173-179.
6. Kennedy, A.D., R.L. Hall, S.P. Montgomery, S.G. Pyburn and J.L. Jones, 2009. Trichinellosis surveillance-United-States, 2002-2007. MMWR Surveill Summ., 58: 1-7.
7. Pozio, E., M.A. Gomez Morales and J. Dupouy Camet, 2003. Clinical aspects, diagnosis and treatment of Trichinellosis. Expert. Rev. Anti-infect. Ther., 1: 471-482.
8. Kapel, C.M.O., P. Webster and R. Gamble, 2005. Muscle distribution of salyvatic and domestic *Trichinella* larvae in production animals and wildlife. Vet. Parasitol., 132: 101-105.
9. Gamble, H.R., A.S. Bessonov, K. Cuperlovic, A.A. Gajadhar, F. Van Knapen, K. Noeckler, H. Schenone and X. Zhu, 2000. International Commission on Trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. Vet. Parasitol., 93: 393-408.
10. Gamble, H.R., E. Pozio, F. Bruschi, K. Nockler, C.M. Kapel and A.A. Gajadhar, 2004. International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and man. Parasite, 11: 3-13.
11. Kapel, C.M.O. and H.R. Gamble, 2000. Infectivity, persistence and antibody response to domestic and sylvatic *Trichinella spp*. in experimentally infected pigs. Int. J. Parasitol., 30: 215-221.

12. Figueroa, B.R.A., C.R.B. Garfias, G. Rogas, M.E. De Nova, O.I. Rodriguez and F.M. Gomez, 2000. Experimental swine trichinellosis:Use of dot ELISA and Werstern blot with excretion /secretion antigens (ES) from infective larvae to detect anti *Trichinella spiralis* antibodies.Revista Latinoame ricana de Microbiologia, 42: 57-62.
13. Gajadahar, A.A., E. Pozio, H.R. Gample, K. Nokler, C.M. Hyttel, LB. Forbes, I.R. Vallee, P. Rossi, A Marinculic and P. Boireau, 2009. *Trichinella* diagnostics and control:Mandatory and best practices for ensuring food safety. Vet. Parasitol., 159: 197-205.
14. Arriage, C., L. Yepez-Mulla, N. Viveros, L.A. Adame, D.S. Zarienga, J.R. Lichtenfels, E. Benitez, M.G. Ortega-Pierres, 1995. Detection of *Trichinella spiralis* muscle larvae in naturally infected horses. J. Parasitol., 81: 781-783.
15. Yera, H., S. Andiva, C. Perret, D. Limonne, P. Boireau and J. Dupouy-Camet, 2003. Development and evaluation of a Western blot kit for diagnosis of human trichinellosis. Clin. Diagn. Lab. Immunol., 10: 793-796.
16. Bolas-Frenandez, F. and D. Wakelin, 1990. Infectivity, antigenicity and host responses to isolates of the genus *Trichinella*. Parasitology, 100: 491-497.
17. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biological Chemistry, 193: 265-275.
18. Gomez-Priego, A., L. Crecencio-Rosales and J. De-La-Rosa, 2000. Serological evaluation of Thin-Layer immunoassay-enzyme- linked immunosorbent assay for antibody detection in human trichinellosis. Clinical and Diagnostic Laboratory Immunol., 7: 810-812.
19. Santiago, N., G.V. Hillyer, M. Garcia-Rosa and M.H. Morales, 1986. Identification of functional *Fasciola hepatica* antigens in experimental infections in rabbits. Int. J. Parasitol., 14: 197-206.
20. Hillyer, G.M., M. Soler De Galanes, J. Rodriguez-Perez, J. Bjorland, M.S. De Lagrava, S.R. Guzman and R.T. Bryan, 1992. Use of the falcon assay screening test enzyme-linked immunosorbent assay (FAST-ELISA) and the enzyme-linked immunoelectrotransfer blot (EITB) to determine the prevalence of human fascioliasis in the Bolivian Altiplano. Am. J. Trop. Med. Hyg., 46; 603-609.
21. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.
22. Towben, H., T. Stahelin and J. Gordon, 1979: electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets and some applications. Proceedings of the National Academy of Sci. U.S.A., 76: 4350-4354.
23. Moller, L.N., E. Petersen, H.R. Gamble and C.M.O. Capel, 2005. Comparison of two antigens for demonstration of *Trichinella* spp. Antibodies in blood and muscle fluid of foxes, pigs and wild boars. Vet. Parasitol., 132: 81-84.
24. Zarlenga, D.S. and H.R. Gamble, 1990. Molecular cloning and expression of an immunodominant 53 KDa excretory-secretroy antigen from Trichinella spiralis muscle larvae. Mol. Biochem. Parasitol., 42: 165-174.
25. Karn, S.K., F. Horchner, L. Srikitjakarn, M. Baumann and K. Nockler, 2008. Cross-sectional study of *Trichinella* spp. in pigs in CDR,Nepal using pepsin digestion and ELISA serology.Southeast Asian J. Trop. Med. Public. Health, 39: 795-799.
26. Forbes, L.B., G.D. Appleyard and A.A. Gajadhar, 2004. Comparison of synthetic tyvelose antigen with excretory-secretory antigen for the detection of trichinellosis in swine using enzyme-linked immunosorbent assay. J. Parasitol., 90(4): 835-40.
27. Gamble, H.R., N. Wisnewski and D.L. Wassom, 1997. Diagnosis of trichinellosis in swine by enzyme immunoassay using synthetic glycan antigen. Am. J. Vet. Res., 58: 1417-1421.
28. Yepez-Mulia, L., C. Arriaga, N. Viveros, A. Adame, E. Benitez and M.G. Ortega-Pierres, 1999. Dtection of *Trichinella* infection in slaughter horses by ELISA and western blot analysis. Vet. Parasitol., 81: 57-68.
29. OIE(World organization for Animal Health), 2008. Trichinellosis.In:Manual of Diagnostic Tests and Vaccines for Terrestrial Animals,6th ed. Office International des Epizooties, Paris Chapter 2.1.16.
30. Bieñ, J., 2007. The usefulness of ELISA test for early serological detection of *Trichinella* spp. infection in pigs Wiad Parazytol., 53(2):149-00.
31. Nockler, K., S. Reckinger, A. Broglia, A. Mayer-Scholl and P. Bahn, 2009. Evaluation of a western blot and ELISA for the detection of anti *Trichinella* IgG in pigs sera. Vet. Parasitol., 163: 341-347.