**Tehran University of Medical Sciences Publication**
**http:// tums.ac.ir**

**Iranian J Parasitol**

**Open access Journal at**
**http:// ijpa.tums.ac.ir**

**Iranian Society of Parasitology**
**http:// isp.tums.ac.ir**

**Original Article**

# The Use of Recombinant 31 kDa Antigens of *Trichinella spiralis* for Serodiagnosis of Experimental Trichinellosis

**Li WANG, Xiang Yu TIAN, Ge Ge SUN, Ruo Dan LIU, Li Na LIU, Xi ZHANG, Peng JIANG, *Zhong Quan WANG, *Jing CUI**

*Dept. of Parasitology, Medical College, Zhengzhou University, P. R. China*

*Received* 16 Feb 2014
*Accepted* 11 May 2015

***Keywords:***
*Trichinella spiralis*, Trichinellosis, Serodiagnosis

***Correspondence**
**Email:**
cuij@zzu.edu.cn
wangzq@zzu.edu.cn

***Abstract***

***Background:*** We have previously reported that a 31 kDa protein was screened from the excretory-secretory (ES) proteins of *Tichinella spiralis* muscle larvae (ML) by immunoproteomics using early infection sera, and the gene encoding a 31 kDa protein from *T. spiralis* was cloned and expressed in an *E. coli* expression system. In this study, the recombinant 31 kDa antigens were used for detection of anti-*Trichinella* antibodies in serum of experimentally infected mice by ELISA.

***Methods:*** Anti-*Trichinella* IgG antibodies in sera of mice infected with *Trichinella* were assayed by ELISA with recombinant 31 kDa antigens, and its sensitivity and specificity were compared with ELISA with ES antigen.

***Results:*** The sensitivity and specificity of ELISA with recombinant antigens was 96.67% (29/30) and 96.87% (62/64), compared with 100% (30/30) and 98.44% (63/64) of ELISA with ES antigens was ($P>0.05$). In heavily, moderately and lightly infected mice (500, 300 and 100 larvae/mouse), anti-*Trichinella* antibodies were firstly detected by ELISA with recombinant antigens at 8, 12 and 14 dpi, respectively; then increased rapidly with a detection rate of 100% respectively at 28, 22 and 30 dpi. While the antibodies were firstly detected by ELISA with ES antigens at 10, 8 and 10 dpi, respectively, the antibody positive rate reached 100% at 14, 12 and 22 dpi, respectively.

***Conclusion:*** The recombinant 31 kDa antigens of *T. spirali* had a good sensitivity and specificity for detecting anti-*Trichinella* antibodies and might be the potential diagnostic antigen for trichinellosis.

## Introduction

Trichinellosis is an important zoonotic disease caused by the nematode parasite *Trichinella*. The parasite is infective to a wide range of hosts including bears, mice, rat, swine, horses and humans (1, 2). Human trichinellosis has been documented in 55 countries of the world, and is considered as an emerging/re-emerging disease (3, 4). From 2004 to 2009, 15 outbreaks of human trichinellosis, consisting of 1387 cases and 4 deaths, were reported in China (5). Therefore, trichinellosis is a major food-borne zoonosis with health, social, and economic impacts in China.

However, the diagnosis of trichinellosis is rather difficult, because its clinical manifestations are nonspecific. ELISA using excretory–secretory (ES) antigens of *T. spiralis* muscle larvae is the most commonly used serological method for diagnosis of trichinellosis (6, 7). But, the main disadvantage of detection of anti-*Trichinella* antibodies is the occurrence of a high rate of false negative results during the early stage of infection, and the cross-reaction between *T. spiralis* ES antigens and sera of patients with other parasitic diseases (e.g., paragonimiasis, schistosomiasis, clornorchiasis, cysticercosis, anisakiasis and so on) (8, 9). The IgG specific for *Trichinella* are not positive in pig and mice infected experimentally until 3–4 weeks after infection (10-12). When the synthetic tyvelose antigens were used in serodiagnosis of trichinellosis, the cross-reaction may occur with cases of anisakiasis, schistosomiasis, etc. (13, 14). Hence, there is an urgent need to develop the new specific antigens for serodiagnosis of trichinellosis.

A different approach for preparing diagnostic antigens was expression of *T. spiralis* antigens in heterologous systems such as a 53 kDa protein (15, 16), 49 kDa protein (17), 35.5 kDa protein (18) and 21 kDa protein (19). The recombinant *Trichinella* antigens had a low sensitivity or cross reaction with the sera of patients with paragonimiasis, cysticercosis and echinococcosis (16, 18, 19).

In our previous studies, two-dimensional electrophoresis (2-DE) and Western blot combined with MALDI-TOF/TOF-MS were used to screen the diagnostic antigens from the excretory–secretory (ES) proteins of *T. spiralis* muscle larvae by sera of infected mice at 18 days post infection (dpi), and a 31 kDa protein of *T. spiralis* (GenBank Accession No. AAA20539) was identified and found to have the obvious immunogenicity (20-22). The 31 kDa protein belongs to the trypsin-like serine protease superfamily and may be involved in a large number of processes and play pivotal roles of the developmental process of *Trichinella* larvae. The 31 kDa protein of *T. spiralis* was cloned and expressed in an *E. coli* expression system (23).

The aim of this study was to detect anti-*Trichinella* IgG antibodies in sera of mice infected with *Trichinella* by ELISA with recombinant 31 kDa antigens, and its sensitivity and specificity were compared with ELISA with ES antigen.

## Materials and Methods

### *Parasites and experimental animals*

*T. spiralis* isolate (T1, ISS534) used in this study was obtained from a domestic pig in Nanyang City of Henan Province, China. The reference *Trichinella* isolates used in this study were *T. nativa* (T2, ISS10), *T. britovi* (T3, ISS100), *T. pseudospiralis* (T4, ISS13) and *T. nelsoni* (T7, ISS29), obtained from International *Trichinella* Reference Centre (ITRC; Rome, Italy). All of the *Trichinella* isolates were maintained by serial passages in Kunming mice in our laboratory. Specific pathogen free (SPF) female BALB/c mice aged 6 weeks were purchased from the Experimental Animal Center of Henan province (Zhengzhou, China).

All procedures of animal experiment of this study were approved by the Life Science Ethics Committee of Zhengzhou University.

### Serum samples

Mouse infection sera were obtained from BALB/c mice infected with 300 larvae of *T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis* and *T. nelsoni* at 42 dpi. Serum samples of mice infected with three spargana of *Spirometra mansoni* were collected at 30 dpi in our department. Serum samples of mice infected with *Toxoplasma gondii* were gifted by Prof. GR Yin of Shanxi Medical University.

Thirty female BALB/c mice were randomly divided into 3 groups (10 mice/group): heavily infected group (500 larvae/mouse), moderate infected group (300 larvae/mouse), and lightly infected group (100 larvae/mouse). The mice were orally inoculated with of *T. spiralis* ML, and about 50 µl of tail blood was collected on alternate days during 2-42 dpi (24). All of the serum samples had been stored at -80 °C until used.

### Collection of muscle larvae and preparation of ES antigens

*T. spiralis* muscle larvae were recovered from the infected mice at 42 days post-infection (dpi) by artificial digestion as described previously (25, 26). The ES antigens of the muscle larvae were prepared as previously described (27, 28). Briefly, after being washed thoroughly in sterile saline and serum-free RPMI-1640 medium supplemented with 100 U penicillin/ml and 100 µg streptomycin/ml, the larvae were incubated in the same medium at 5 000 worms/ml for 18 h at 37 °C in 5% $CO_2$. After incubation, the media containing the ES proteins were poured into 50-ml conical tubes and the larvae were allowed to settle for 20 min. The supernatant containing the ES products was filtered through a 0.2 µm membrane. The ES products were dialyzed and then lyophilized by a vacuum concentration and freeze-drying (Heto Mxi-Dry-Lyo, Denmark). The protein concentration (2.5 µg/ml) was determined by the Bradford assay (29).

### The recombinant 31 kDa antigens of T. spiralis

The recombinant 31 kDa antigens of *T. spiralis* were expressed in an *E. coli* expression system and characterized in our laboratory. The purified fusion protein consisted of the 31 kDa proteins and the N-terminal maltose-binding protein. The immune serum against recombinant 31 kDa antigens recognized the native 31 kDa protein by Western blotting of muscle larval crude or ES antigens. An immunolocalization analysis identified 31 kDa proteins in the cuticle and stichocytes of the parasite, demonstrating the 31 kDa antigens came from the ES products of *T. spiralis* muscle larvae (23).

### ELISA for detection of anti-Trichinella antibodies

ELISA was performed as previously described (23,30). In brief, 96-well ELISA plates (Corning, USA) were coated with purified recombinant antigens (2.5 µg/ml) or ES antigens (2.5 µg/ml) in 100 µl of bicarbonate buffer (pH 9.6) overnight at 4°C. After being blocked in PBS-0.1% Tween 20 (PBST) containing 5% skimmed milk for 2 h at 37°C, the following reagents were sequentially added and incubated for 1 h at 37°C: (1) mouse sera diluted at 1:100 in PBST, and (2) HRP-conjugated anti-mouse IgG (Sigma, USA) diluted at 1: 5 000. The reactions were detected by addition of the substrate o-phenylenediamine dihydrochloride (OPD; Sigma, USA) plus $H_2O_2$ and stopped with 50 µl/well of 2M $H_2SO_4$. Optical density (OD) values at 490 nm were measured with a microplate reader (TECAN, Austria). All samples were run in duplicate. The ratio <2.1 of samples to be tested/negative sample (OD values of the samples to be tested divided by OD of the negative, S/N < 2.1) were regarded as negative, and S/N ≥2.1 as positive (31). The cut-off values of ELISA with recombinant antigens

and ELISA with ES antigens for detection of mouse sera were 0.26 and 0.21, respectively.

### *Statistical analysis*

All statistical analyses of data were done with SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL). Chi-square test and repeated measures of analysis of variance (ANOVA) were used to determine the difference between antibody levels at various periods post-infection, the difference between the heavily, moderately, and lightly infected group, and the difference at different time points in the same group with respect to the detection rate and serum level antibody. $P < 0.05$ was considered statistically significant.

## Results

### *Detection of specific antibodies in mice infected with Trichinella and other parasites*

The sensitivity of both ELISA with recombinant antigens and ELISA with ES antigens for detecting anti-*Trichinella* antibodies was 96.67% (29/30) and 100% (30/30) ($\chi^2$=1.017, $P>0.05$) when the sera of mice experimentally infected with *T. spiralis* (Table 1). No cross-reactions of both ELISA were observed with sera of mice infected *T. gondii* and normal mice. When the serum samples of mice infected with *S. mansoni* were determined, the cross-reaction rate was 7.14% (2/28) by ELISA with recombinant antigens ELISA and 3.57% (1/28) by ELISA with ES antigens ($\chi^2$=0.352, $P>0.05$). The specificity of both ELISA with recombinant antigens and ELISA with ES antigens for detecting anti-*Trichinella* antibodies was 96.87% (62/64) and 98.44% (63/64) ($\chi^2$=0.341, $P>0.05$).

The specific serum anti-*Trichinella* IgG antibodies in mice infected with other species of *Trichinella* were also assayed by ELISA, and the results are shown in Table 2. The detection rate of anti-*Trichinella* antibodies by ELISA with recombinant antigens and ELISA with ES antigens was no significant difference among the mice infected with *T. spiralis, T. nativa*, *T. britovi* and *T. nelsoni* ($\chi^2_{T1}$=1.017, $\chi^2_{T2}$=3.158, $\chi^2_{T3}$=1.034, $\chi^2_{T7}$=1.32, $P>0.05$) . However, the antibody detection rate (40.91%) of mice infected with *T. pseudospiralis* by ELISA with recombinant antigens was obviously lower than 90.91% by ELISA with ES antigens ($\chi^2$=12.239, $P<0.05$).

### *Serum anti-Trichinella antibody dynamics in mice experimentally infected with different-level infections*

The levels of anti-*Trichinella* antibodies in sera from 3 groups of infected mice at different time points after infection were determined by ELISA with recombinant antigens and ELISA with ES antigens (Fig. 1). In heavily, moderately and lightly infected mice, anti-*Trichinella* antibodies were firstly detected by ELISA with recombinant antigens at 8, 12 and 14 dpi, respectively; the antibody positive rate reached 100% at 28, 22 and 30 dpi, respectively.

**Table 1:** Detection of serum anti-*Trichinella* IgG antibodies in mice infected with other parasites by ELISA

| Sera of mice infected with | No. of serum samples | ELISA with recombinant antigens | | ELISA with ES antigens | |
|---|---|---|---|---|---|
| | | OD value | No. of positive serum samples (%) | OD value | No. of positive serum samples (%) |
| *Trichinella spiralis* | 30 | 0.50±0.11 | 29 (96.67) | 0.80±0.05 | 30 (100) |
| *Spirometra mansoni* | 28 | 0.17±0.05 | 2（7.14） | 0.16±0.04 | 1（3.57） |
| *Toxoplasma gondii* | 6 | 0.15±0.02 | 0 | 0.14±0.01 | 0 |
| Normal mice | 30 | 0.12±0.01 | 0 | 0.10±0.02 | 0 |

**Table 2:** Detection of serum anti-*Trichinella* IgG antibodies in mice infected with other species of *Trichinella* by ELISA

| Sera of mice infected with | No. of serum samples | ELISA with recombinant antigens | | ELISA with ES antigens | |
|---|---|---|---|---|---|
| | | OD value | No. of positive serum samples (%) | OD value | No. of positive serum samples (%) |
| *T. spiralis* | 30 | 0.50±0.11 | 29 (96.67) | 0.80±0.05 | 30 (100) |
| *T. nativa* | 30 | 0.43±0.15 | 27 (90.00) | 0.72±0.13 | 30 (100) |
| *T. britovi* | 15 | 0.40±0.17 | 14 (93.33) | 0.79±0.08 | 15 (100) |
| *T. pseudospiralis* | 22 | 0.22±0.16 | 9 (40.91) | 0.52±0.21 | 20 (90.91) |
| *T. nelsoni* | 16 | 0.40±0.16 | 15 (93.75) | 0.76±0.08 | 16 (100) |

While the antibodies were firstly detected by ELISA with ES antigens at 10, 8 and 10 dpi, respectively; the antibody positive rate reached 100% at 14, 12 and 22 dpi, respectively (Fig. 2). The anti-*Trichinella* antibody levels in 3 groups of infected mice increased rapidly at 8 dpi and reached their peak at 32 dpi by both ELISA. When ELISA with recombinant antigens was used, the antibody levels in 3 groups of infected mice was not significant different ($F$=2.049, $P$>0.05), but the antibody levels at different time points after infection was statistically significant ($F$=219.924, $P$<0.05); there was interaction between detection time and inoculation dose ($F$=3.311, $P$<0.05).

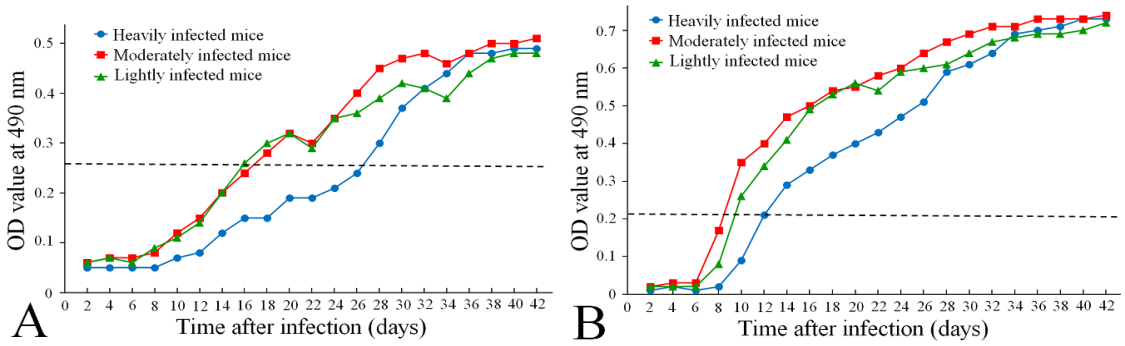


**Fig. 1:** Kinetics of anti-*Trichinella* antibodies in sera of heavily (infected with 500 *T. spiralis* larvae), moderately (infected with 300 *T. spiralis* larvae), and lightly (infected with 100 *T. spiralis* larvae) infected mice. The anti-*Trichinella* IgG antibodies were detected by ELISA with recombinant antigens (A) and ELISA with ES antigens (B), and cut-off value is represented by the dotted line

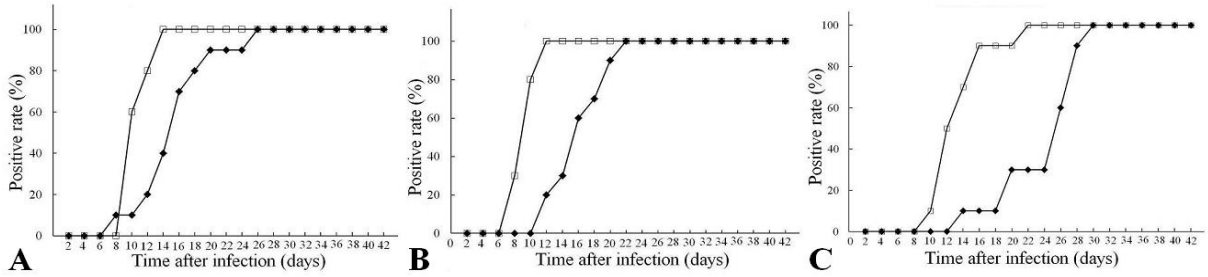


**Fig. 2:** Comparison of detection rate of anti-*Trichinella* IgG antibodies in sera from heavily (A), moderately (B), and lightly (C) infected with *T. spiralis* at different time intervals post-infection by ELISA with recombinant antigens (■) and ELISA with ES anigens (□)

## Discussion

The ES antigens of *T. spiralis* muscle larvae is the most commonly used serodiagnostic antigens for trichinellosis recommended by the International Commission on Trichinellosis (ICT) (11). However, the reparation of ES antigens requires collection of muscle larvae from experimentally infected laboratory animals, which is practically inconvenient in terms of cost, labor, and time. Recombinant proteins are a good alternative to the ES antigens as they can be produced easily in large amounts by using a bacterial expression system and can be used as an antigen in a sensitive, specific, and standardized ELISA for serodiagnosis of trichinellosis. Therefore, development of sensitive and specific recombinant *Trichinella* antigens will improve the serodiagnosis of this disease. In the present study, the sensitivity and specificity of ELISA with recombinant antigens for detecting anti-*Trichinella* antibodies in serum of experimentally infected mice was 96.67% (29/30) and 96.87% (62/64), respectively; the results were similar with that of ELISA with ES antigens, suggesting that the recombinant 31 kDa antigens of *T. spiralis* could be the alternative antigens of ES antigens for diagnosis of trichinellosis.

Moreover, there was no significant difference in the antibody detection rates in sera of mice infected with *T. nativa*, *T. britovi*, and *T. nelsoni* by ELISA with recombinant antigens and ELISA with ES antigens ($P>0.05$). But, the antibody detection rate (40.91%) of mice infected with *T. pseudospiralis* by ELISA with recombinant antigens was obviously lower than 90.91% by ELISA with ES antigens ($P<0.05$). The 31 kDa proteins might expressed at high levels in encapsulated species (such as *T. spiralis*, *T. nativa*, *T. britovi*, and *T. nelsoni*), but at a low level in non-encapsulated species (*T. pseudospiralis*) (32, 33). The results suggested that the most epitopes of 31 kDa antigens recognized by mouse infection sera were common to the encapsulated species of *Trichinella* (34).

Our ELISA results also showed the antigenic epitopes of 31 kDa proteins were recognized by infection sera at 8-14 dpi, suggesting that the 31 kDa proteins might be secreted by the parasite into the peripheral blood circulation of host at early infection stage, induced an early antibody response continuing to the muscle larval stage. In the present study, the specific anti-*Trichinella* IgG antibodies were firstly detected at 8, 12 and 14 dpi in heavily, moderately and lightly infected mice, and continued to grow up to the end of this experiment (42 dpi). Similarly, other some recombinant proteins (such as the recombinant 53 kDa protein, rTs21, rTsDAF-21) were firstly recognized by the sera of mice infected with *T. spiralis* at 8-14 dpi (19, 35, 36). However, the levels of anti-*Trichinella* IgG antibodies did not increased proportionally with the different infected doses. Similar observation regarding the kinetics of anti-*T. spiralis* newborn larva antibodies and the infection dose effects has also been observed in experimentally infected rats (37).

## Conclusion

The recombinant 31-kDa antigens of *T. spirali* had a good sensitivity and specificity for detecting anti-*Trichinella* antibodies in serum of experimentally infected mice, and might be a potential diagnostic antigen for trichinellosis.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81271860 and 81141084). The authors declare that there is no conflict of interest.

## References

1. Murrell KD, Pozio E. Worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerg Infect Dis. 2011; 17:2194-2202.

2. Cui J, Jiang P, Liu LN, Wang ZQ. Survey of *Trichinella* infections in domestic pigs from northern and eastern Henan, China. Vet Parasitol. 2013; 194:133-135.
3. Cui J, Wang ZQ, Kennedy MW. The re-emergence of trichinellosis in China? Trends Parasitol. 2006; 22:54-55.
4. Dupouy-Camet J. Presidential address of ICT12 Conference: "*Trichinella* and trichinellosis-a never ending story". Vet Parasitol. 2009; 159:194-196.
5. Cui J, Wang ZQ, Xu BL: The epidemiology of human trichinellosis in China during 2004-2009. Acta Trop. 2011; 118:1-5.
6. Gomez-Morales MA, Ludovisi A, Amati M, Cherchi S, Pezzotti P, Pozio E: Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis. Clin Vaccine Immunol. 2008; 15:1723-1729.
7. Gottstein B, Pozio E, Nockler K: Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin Microbiol Rev. 2009; 22:127-145.
8. Mahannop P, Setasuban P, Morakote N, Tapchaisri P, Chaicumpa W. Immunodiagnosis of human trichinellosis and identification of specific antigen for *Trichinella spiralis*. Int J Parasitol.1995; 25:87-94.
9. Yera H, Andiva S, Perret C, Limonne D, Boireau P, Dupouy-Camet J: Development and evaluation of a Western blot kit for diagnosis of human trichinellosis. Clin Diagn Lab Immunol. 2003; 10: 793-796.
10. Kapel CM and Gamble HR. Infectivity, persistence, and antibody response to domestic and sylvatic *Trichinella* spp. in experimentally infected pigs. Int J Parasitol. 2000; 30:215–221.
11. Gamble HR, Pozio E, Bruschi F, Nockler K, Kapel CM, Gajadhar AA. International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and man. Parasite. 2004; 11:3-13.
12. Liu LN, Jing FJ, Cui J, Fu GY, Wang ZQ. Detection of circulating antigen in serum of mice infected with *Trichinella spiralis* by an IgY-IgM mAb sandwich ELISA. Exp Parasitol. 2013; 133:150-155.
13. Bruschi F, Moretti A, Wassom D, and Piergili-Fioretti D. The use of a synthetic antigen for the serological diagnosis of human trichinellosis. Parasite. 2001; 8:S141-S143.
14. Dea-Ayuela MA, Romarís F, Ubeira FM, Rama-Iñiguez S, Martínez-Fernández AR, Bolás F. Possible presence of common tyvelose-containing glycans in *Trichinella* L1 larvae and embryonated eggs of several nematodes. Parasite. 2001; 8:S120-S122.
15. Zarlenga DS, Gamble HR. Molecular cloning and expression of an immunodominant 53-kDa excretory–secretory antigen from *Trichinella spiralis* muscle larvae. Mol Biochem Parasitol. 1995; 72:253.
16. Jung D, Teifke JP, Karger A, Michael K, Venz S, Wittmann W, Kindermann K, Nöckler K, Mundt E. Evaluation of baculovirus-derived recombinant 53-kDa protein of *Trichinella spiralis* for detection of Trichinella-specific antibodies in domestic pigs by ELISA. Parasitol Res. 2007; 100: 429-437.
17. Su XZ, Prestwood AK, McGraw RA. Cloning and expression of complementary DNA encoding an antigen of *Trichinella spiralis*. Mol Biochem Parasitol. 1991; 45:331–336.
18. Wang B, Wang ZQ, Jin J, Ren HJ, Liu LN, Cui J. Cloning, expression and characterization of a *Trichinella spiralis* serine protease gene encoding a 35.5 kDa protein. Exp Parasitol. 2013;134:148-154.
19. Wang R, Wang ZQ, Cui J. Immunodiagnostic value and immune protection of the recombinant Ts21 antigen of *Trichinella spiralis*. Chin J Parasitol Parasit Dis. 2009; 27: 17-21.
20. Arasu P, Ellis LA, Iglesias R, Ubeira FM, Appleton JA. Molecular analysis of antigens targeted by protective antibodies in rapid expulsion of *Trichinella spiralis*. Mol Biochem Parasitol. 1994; 65: 201-211.
21. Wang L, Wang ZQ, Hu DD, Cui J. Proteomic analysis of *Trichinella spiralis* muscle larval excretory-secretory proteins recognized by early infection sera. Biomed Res Int. 2013; 2013:139745.
22. Wang L, Cui J, Hu DD, Liu RD, Wang ZQ. Identification of early diagnostic antigens from major excretory-secretory proteins of *Trichinella spiralis* muscle larvae using immunoproteomics. Parasit Vectors. 2014; 7:40.
23. Cui J, Wang L, Sun GG, Liu LN, Zhang SB, Liu RD, Zhang Xi, Jiang P, Wang ZQ. Characterization of a *Trichinella spiralis* 31 kDa protein and its potential application for the serodi-

agnosis of trichinellosis. Acta Trop. 2015; 142:57-63.
24. Wang ZQ, Fu GY, Jing FJ, Jin J, Ren HJ, Jiang P, Cui J. Detection of *Trichinella spiralis* circulating antigens in serum of experimentally infected mice by an IgY-mAb sandwich ELISA. Foodborne Pathog Dis. 2012; 9:727-933.
25. Gamble HR, Bessonov AS, Cuperlovic K, Gajadhar AA, van Knapen F, Noeckler K, Schenone H, Zhu X. International Commission on Trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. Vet Parasitol. 2000; 93:393-408.
26. Li F, Cui J, Wang ZQ, Jiang P. Sensitivity and optimization of artificial digestion in the inspection of meat for *Trichinella spiralis*. Foodborne Pathog Dis. 2010; 7:879-885.
27. Wang SW, Wang ZQ, Cui J. Protein change of intestinal epithelial cells induced in vitro by *Trichinella spiralis* infective larvae. Parasitol Res.2011; 108:593-599.
28. Hu DD, Cui J, Wang L, Liu LN, Wei T, Wang ZQ. Immunoproteomic analysis of the excretory-secretory proteins from *Spirometra mansoni* sparganum. Iran J Parasitol. 2013; 8:408-416.
29. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72: 248-254.
30. Cui J, Li N, Wang ZQ, Jiang P, Lin XM. Serodiagnosis of experimental sparganum infections of mice and human sparganosis by ELISA using ES antigens of *Spirometra mansoni* spargana. Parasitol Res. 2011; 108: 1551-1556.
31. Wang ZQ, Lin XM, Zhang HW, Xu BL, Zhang X, Jiang P, Cui J. Serological survey for sparganum infection in people of central China. Helminthiologia. 2014: 51:158-161.
32. Zhang YW, Lee DL, Smith JE. Biochemical characterisation of *Trichinella spiralis* and *T. pseudospiralis* stichocyte antigens. Appl Parasitol.1993; 34:291-294.
33. Kapel CM, Gamble HR. Infectivity, persistence, and antibody response to domestic and sylvatic *Trichinella* spp. in experimentally infected pigs. Int J Parasitol. 2000; 30: 215-221.
34. Appleton JA, Bell RG, HOMAN W, Van Knapen F. Consensus on *Trichinella spiralis* antigens and antibodies. Parasitol Today. 1991; 7: 190-192.
35. Nagano I, Wu Z, Takahashi Y. Species-specific antibody responses to the recombinant 53-kilodalton excretory and secretory proteins in mice infected with *Trichinella* spp. Clin Vaccine Immunol. 2008; 15:468-473.
36. Yang Y, Qin W, Qiu H, Liu Y. Characterization of TsDAF-21/HSP90 protein from the parasitic nematode *Trichinella spiralis*. Parasitol Res. 2014;113:2209-2217.
37. Salinas-Tobon Mdel R, Navarrete-Leon A, Mendez-Loredo BE, Esquivel-Aguirre D, Martínez-Abrajan DM, Hernandez-Sanchez J. *Trichinella spiralis*: strong antibody response to a 49 kDa newborn larva antigen in infected rats. Exp Parasitol. 2007; 115: 160-167.