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Protection against *Taenia pisiformis* larval infection induced by a recombinant oncosphere antigen vaccine

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ABSTRACT. *Taenia pisiformis* larvae cause significant health problems to rabbits. At present, it is not known whether the recombinant antigen from the *T. pisiformis* oncosphere is able to confer protective immunity against *T. pisiformis* larval infection. The full-length cDNA was cloned into a pET32a (+) vector, and the recombinant protein was then expressed in BL21 (DE3) cells. Vaccination with the purified *r*TpUbc2 coupled with QuilA was carried out in New Zealand rabbits to evaluate the immunoprotective effect against *T. pisiformis* infection. The full-length open reading frame of the TpUbc2 gene was 444 bp, and encoded a 16.63-kDa protein. Finally, *r*TpUbc2 was used to evaluate the ability to induce immunoprotective responses in rabbits. A 79.3-90.8% reduction ($P < 0.01$) in the recovery of larvae was observed in the experimental group compared to the control group. Specific anti-*r*TpUbc2 antibodies from immunized rabbits had significantly higher levels of IgG ($P < 0.01$) compared to the control group; however, no significant difference in IgA levels was found between groups

($P > 0.05$). Our data support the use of *r*TpUbc2 as a potential candidate to develop a vaccine against *T. pisiformis* larvae.

Key words: *Taenia pisiformis*; Ubiquitin-conjugating enzyme; Immunization; Vaccine

INTRODUCTION

The adult stage of *Taenia pisiformis* (Cestoidea; Cyclophyllidea; Taeniidae) parasitizes the small intestine of canines and felines (Saeed et al., 2006; Lahmar et al., 2008; Bagrade et al., 2009; Jia et al., 2010). *T. pisiformis* larvae infect the liver capsule, greater omentum, and mesentery of rabbits, which serve as an intermediate host. *T. pisiformis* is widely distributed across the world (Foronda et al., 2003; Martinez-Moreno et al., 2007; Zhou et al., 2008). Infections may occur when canines ingest the internal organs of rodents infected with *T. pisiformis* larvae, or when lagomorphs consume food contaminated with the proglottids of *T. pisiformis*. *T. pisiformis* causes significant health problems to its intermediate and definitive hosts (Rajasekariah et al., 1985).

Recombinant antigen has been proposed as candidates for a future vaccine against a variety of helminth parasites. Since the early 1990s, more than 80 different recombinant antigens from 22 different helminth species have been studied (Geldhof et al., 2007). Effective recombinant vaccines have been developed for cestode parasites, including *Taenia ovis*, *T. solium*, *T. saginata*, and *Echinococcus granulosus* (Lightowlers, 2006).These studies provide important information that could be used for the development of a genetically engineered vaccine against *T. pisiformis* larvae. Previous studies on *T. pisiformis* have mainly focused on observing its morphology (Shield et al., 1973), biological characteristics (Kyngdon et al., 2006; Zhou et al., 2008; Toral-Bastida et al., 2011), epidemiology (Rashed et al., 1991), and treatment (Rajasekariah et al., 1985). To date, very little is known about recombinant antigens conferring protective immunity against *T. pisiformis* infection.

The ubiquitin-dependent proteolytic system is essential for the maintenance of homeostasis in a broad range of eukaryotic proteins. It is a major pathway for protein degradation in eukaryotes, and plays a vital role in the control of numerous cellular processes (Finley et al., 1989). Ubiquitin is conjugated to the target protein via a cascade of enzymatic reactions, which involve an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2 or UBC2), and an ubiquitin-protein ligase (E3). E2 enzymes act via selective protein-protein interactions with the E1 and E3 enzymes and, in doing so, lead to different effects on downstream substrates that have either a single or chain of Ub/UBL molecules. In addition, E2 has recently proven to be a key mediator within the ubiquitin chain assembly (Ye and Rape, 2009).

In this study, we isolated the ubiquitin-conjugating enzyme gene of the *T. pisiformis* oncosphere based on the analysis of expressed sequence tags (Yang et al., 2012). The recombinant protein (*r*TpUbc2) was expressed, analyzed, and purified for the immunization of rabbits.

MATERIAL AND METHODS

Rabbits

Twenty-eight, 60-day-old female white New Zealand rabbits were purchased from the

Laboratory Animal Center of Sichuan Agricultural University (China). All animals from which specimens were collected were handled in accordance with the animal protection law of the People's Republic of China (a draft of the animal protection law in China was released on September 18, 2009). This study was approved by the National Institute of Animal Health Animal Care and Use Committee at Sichuan Agricultural University (approval number 2010-021).

Parasite eggs

Eggs of *T. pisiformis*, which originated from 5 different worms, were collected from 1 experimentally infected dog in the Department of Parasitology, College of Veterinary Medicine, Sichuan Agricultural University (China).

Total RNA isolation and amplification of TpUbc2

Total RNA was isolated from the active oncosphere of *T. pisiformis* using an RNA isolation kit (TaKaRa, Dalian, China), and first-strand cDNA synthesis was performed using a cDNA synthesis kit (TaKaRa) and an oligo (dT) 18 primer (TaKaRa). The resulting cDNA was used for amplification by polymerase chain reaction (PCR) using a sense primer (5'-ATGGCACTCAAAAGAATCCAGAAGG-3') and an antisense primer (5'-TCACATCGCGTACTTCTGTGTCCAT-3'). PCR products were separated by electrophoresis on agarose gel (1%), purified using a Gel Extraction Kit (TIANGEN, Beijing, China), following manufacturer protocols, and cloned into a pMD19-T vector (TaKaRa, Dalian, China), following manufacturer protocols. The resultant plasmid was transferred to *Escherichia coli* strain DH5α (TIANGEN), and sequenced.

DNA sequence analysis, B cell epitope prediction, and cross-reactivity prediction

An Open Reading Frame (ORF) Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) and the BLAST network server of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to analyze the ORF, nucleotide sequence, and deduced amino acid sequence of TpUbc2, to determine their similarities with previously reported sequences in the current database. Analysis of the signal sequence was performed using the SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). B cell epitopes were predicted using the web server of Immune Epitope Database Analysis (IEDA) (http://tools.immuneepitope.org/tools/bcell/iedb_input). Cross-reactivity with known allergens was predicted using the web server Structural Database of Allergenic Proteins (SDAP) (http://fermi.utmb.edu/SDAP/).

Expression and purification of the recombinant TpUbc2 fusion protein

The coding region of TpUbc2 was amplified by PCR using a sense primer (5'-CGC GGATCCATGGCACTCAAAAGAATCC-3') containing a *Bam*HI site and an antisense primer (5'-CCCAAGCTTTCACATCGCGTACTTCTGT-3') containing a *Hin*dIII site. The PCR fragments were digested with *Bam*HI and *Hin*dIII (TaKaRa) and ligated into the *Bam*HI and *Hin*dIII sites of the plasmid expression vector pET32a (+) (TaKaRa). *E. coli* strain BL21 (DE3)

cells were transformed with the recombinant constructs using standard methods (Sambrook, 2001). Transformant colonies were evaluated by DNA restriction analysis of the plasmid. *E. coli* strain BL21 (DE3) (TIANGEN) harboring the recombinant plasmid was propagated in lysogeny broth medium (50 mg ampicillin, 5 g Bacto yeast extract, and 10 g NaCl per liter of distilled water, pH 7.0), and expression was induced with 1 mM IPTG (isopropyl β-D-1-] thiogalactopyranoside) at 37°C for 3 h. Purification of recombinant TpUbc2 was carried out following previously described methods (Chen et al., 2003).

Immunoblot analysis

SDS-PAGE analysis was performed using 14% polyacrylamide gels with a recombinant protein electrophoresis system. Electrophoresis was carried out at a constant voltage of 160 V for 180 min. Following electrophoresis, the proteins were transferred to nitrocellulose membranes (Millipore, Dermstadt, Germany), and the membranes were blocked with 1% bovine serum albumin (BSA)-PBS-Tween (0.05% PBST) for 2 h at room temperature. Serum from rabbits infected with *T. pisiformis* at a dilution of 1:500 was used to detect the presence of *r*TpUbc2. After the membranes were washed 5 times with PBST, horseradish peroxidase-conjugated goat anti-rabbit IgG (AMRESCO, TX, USA) was used as a secondary antibody at a dilution of 1:8000. After the membranes were again washed 5 times with PBST, the signal was visualized with 3,3',5,5'-tetramethyl benzidine (TMB; TIANGEN) following the manufacturer protocol.

Challenge infection and sampling

The rabbits were randomly divided into 4 groups of 7 rabbits. Specifically: 1) group A was immunized with 0.9% sodium chloride solution (NS) as the control; 2) group B was immunized with 100 μg QuilA (0.01 g/mL; International Laboratory, USA) in 0.9% sodium chloride solution, as the adjuvant control; 3) and 4) groups C and D were immunized with 100 μg fresh *r*TpUbc2 (histidine fusion protein) mixed with 100 μg QuilA. The antigen was reconstituted in sterile deionized water immediately before the vaccination of the rabbits. All animals were immunized twice by subcutaneous immunization at 1-week intervals. Two weeks after the 2nd vaccination, each rabbit received an oral challenge infection with 5000 viable eggs. Blood samples were collected from auricular veins of all rabbits, and were taken 1) immediately before vaccination and then 2) once a week following the vaccination and challenge, until week 7 post-challenge. The rabbits were sacrificed 50 days after infection. All cysticerci in the peritoneal cavity (greater omentum, mesentery, liver capsule, and mesorectum) were counted. The percentage reduction was calculated as: reduction rate (%) = [1- (mean number of cysticerci with vaccination / mean number of cysticerci for NS) x 100%].

Enzyme-linked immunosorbent assay (ELISA)

Serum samples were collected from immunized and control rabbits to assess antibody responses. Serum levels of antigen-specific IgG and IgA antibody were measured by an ELISA. The recombinant proteins were bound to 96-well microtiter plates by incubating 2 μL/mL *r*TpUbc2 in 0.1 M carbonate buffer (10 mM Na_2CO_3, 30 mM $NaHCO_3$, pH 9.6) for 16

h at 4°C, after which they were washed 5 times with 0.05% PBST. Wells were blocked with 100 µL PBST containing 5% BSA for 1 h at 37°C. After the wells were washed 5 times with PBST, 100 µL rabbit serum (diluted 1:500) was added and incubated at 37°C for 1 h. Then the plates were washed 6 times in PBST, and probed with 1:10,000 peroxidase-conjugated goat anti-rabbit IgG (AMRESCO) or 1:10,000 goat anti-rabbit IgA (AMRESCO) and diluted in 1% sodium caseinate-PBST. The plates were incubated for 1 h at 37°C, followed by a further 6 washes with PBST. Then 100 µL TMB substrate (45 mM dibasic sodium phosphate, 0.22 mM citric acid, 0.42 mM TMB, and 30% H_2O_2; TIANGEN) was added to each well. Both the test sera and conjugates were diluted in PBS, pH 7.5. The reaction was stopped after 30 min by the addition of 100 µL 1% SDS, and the optical densities were read at 450 nm (Dynatech MR 500, Deckendorf, Germany). A positive control, a negative control, and a blank control were always included on each plate.

Statistical analysis

Data were processed in Excel 2010. The number of cysticerci between experimental groups was statistically compared using the non-parametric Mann-Whitney test. The two-side P values <0.05 were considered as statistically significant. The antibody data between experimental groups were compared by analysis of variance (ANOVA), The Dunnett test, and least significant difference (LSD). Differences with P values <0.05 were considered as statistically significant. Statistical analyses were performed using SPSS version 8.0 for windows.

RESULTS

TpUbc2 cDNA cloning and structural analysis

A cDNA sequence was obtained with an ORF of 444 bp (GenBank accession No. KC182511) to encode a protein of 147-amino acid residues. The molecular mass of the peptide was calculated as 16.63 kDa and the isoelectric point as 6.77. No signal peptide or transmembrane domains were evident in the protein, indicating that the peptide chain is not anchored to a cellular membrane. The alignment of *T. pisiformis* TpUbc2 with other ubiquitin-conjugating enzyme proteins is shown in Figure 1. The amino acid sequence of TpUbc2 is rich in Pro (10.20%), Asp (9.52%), and Leu (8.84%) amino acids, and highly homologous to *Litopenaeus vannamei*, *Oreochromis niloticus*, *Oikopleura dioica*, and *Danio rerio* ubiquitin-conjugating enzyme proteins with identities of 88, 88, 87, and 86%, respectively.

B cell epitope prediction

Identification of epitopes within proteins may be useful for diagnostic purposes and in the development of peptide vaccines. B cell epitope prediction is based on experimental antigenic determinant data and the physicochemical properties of amino acid residues. These parameters are able to predict antigenic determinants with an accuracy of approximately 75% (Kolaskar and Tongaonkar, 1990). Based on the Kolaskar and Tongaonkar antigenicity methodology, 8 possible B cell epitopes were predicted in the TpUbc2 protein in *T. pisiformis* (Table 1).

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Taenia_pisiformis       MALKRIQKELSDLGRDPPAQCSAGPVGDDLFHWQATIMGPSDSPFEGGVFFLDIHFPTDY
Litopenaeus vannamei    MALKRINKELQDLGRDPPAQCSAGPVGDDLFHWQATIMGPTDSPFQGGVFFLNIHFPTDY
Oreochromis niloticus   MALKRIHKELNDLARDPPAQCSAGPVGDDMFHWQATIMGPSDSPYQGGVFFLTIHFPTDY
Oikopleura dioica       MALKRINKELQDIGRDPPAQCSAGPVGDDLFHWQATIMGPSDSPYQGGVFFLNIHFPNDY
Danio rerio             MALKRIHKELHDLGRDPPAQCSAGPVGDDMFHWQATIMGPNDSPYQGGVFFLTIHFPTDY
                        ****** *** *. ***************.********** ***..****** ****.**

Taenia pisiformis       PFKPPKITFTTRIYHPNINSNGNICLDILRNQWSPALTISKVLLSICSLLTDPNPDDPLS
Litopenaeus vannamei    PFKPPKVAFTTRIYHPNINSNGSICLDILRTQWSPALTISKVLLSICSLLTDPNPDDPLV
Oreochromis niloticus   PFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTISKVLLSICSLLCDPNPDDPLV
Oikopleura dioica       PFKPPKVSFITRIYHPNINSNGSICLDILRSQWSPALTISKVLLSICSLLTDPNPDDPLV
Danio rerio             PFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTISKVLLSICSLLCDPNPDDPLV
                        ******..* ************ ******* ******************.********

Taenia pisiformis       PDIARTYKTDRQKYDKTAKEWTQKYAM
Litopenaeus vannamei    PEIARQYKTDREKYNKLAQEWTHKYAQ
Oreochromis niloticus   PEIARIYKTDSQKYTKMAKEWTQKYAM
Oikopleura dioica       PEIARVYKTDRDKYQKLAREWTQKYAM
Danio rerio             PEIARIYKTDREKYNRIAREWTQKYAM
                        *.*** **** ** . *.***.***
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Figure 1. Comparison of TpUbc2 with the ubiquitin-conjugating enzyme E2 amino acid sequences of other species. GenBank accession numbers: *Litopenaeus vannamei*, ABI98679; *Oreochromis niloticus*, XP003442904; *Oikopleura dioica*, CBY19808; *Danio rerio*, NP957253.

Table 1. B cell epitope predictions in *Taenia pisiformis* TpUbc2.

No.	Start position	End position	Peptide	Peptide length	Value
1	4	11	KRIQKELS	8	1.056
2	18	25	PAQCSAGP	8	1.065
3	46	67	EGGVFFLDIHFPTDYPFKPPKI	22	1.069
4	71	77	TRIYHPN	7	1.132
5	83	89	NICLDIL	7	1.156
6	94	112	SPALTISKVLLSICSLLTD	19	1.161

Prediction of cross-reactivity

A FASTA alignment was used to determine whether the TpUbc2 protein is potentially cross-reactive based on the Food and Agricultural Organization (FAO)/World Health Organization (WHO) allergenicity rules. The results showed that TpUbc2 was related to 8 allergenic proteins from 7 species (Table 2). The most homologous protein to TpUbc2 in the SDAP was found to be Asc s 1.0101 (*Ascaris suum)* isolated from pig ascarids ($E < 0.01$; Table 2).

Expression of recombinant TpUbc2

The putative TpUbc2 cDNA ORF was amplified by PCR and cloned into the expression vector pET32a (+). SDS-PAGE analysis showed that the molecular mass of the expressed protein was approximately 36.63 kDa, and contained the predicted 16.63-kDa TpUbc2 protein

and an additional peptide expressed from the pET-32a (+) vector (20 kDa) (Figure 2). Recombinant TpUbc2 was confirmed to be a soluble protein.

Table 2. Predicted cross-reactivity between TpUbc2 and known allergens in SDAP.

No.	Allergen	Accession No.	Species	Sequence length	Bit score[a]	E score
1	Asc s 1.0101	AF051702	*Ascaris suum*	767	54.9	3.0e-09
2	Ves v 6.0101	G8IIT0	*Vespula vulgaris*	1312	41.2	4.1e-05
3	Sola t 4	CAA45723	*Solanum tuberosum*	165	38.1	3.4e-04
4	Ber e 2	AAO38859	*Bertholletia excelsa*	343	37.0	7.6e-04
5	Fag e 1	Q9XFM4	*Fagopyrum esculentum*	401	36.5	1.1e-03
6	Ses i 6.0101	Q9XHP0	*Sesamum indicum*	353	35.9	1.7e-03
7	Sola t 4	P30941	*Solanum tuberosum*	168	34.1	5.6e-03
8	Sch c 1.0101	D8Q9M3	*Schizophyllum commune*	468	33.6	7.9e-03

[a]The bit score is equivalent to the bit score reported by BLAST. A 1-bit increase in score corresponds to a 2-fold reduction in expectation, and a 10-bit increase implies 1000-fold lower expectation. Sequences with E values <0.01 are almost always homologous. SDAP = Structural Database of Allergenic Proteins.

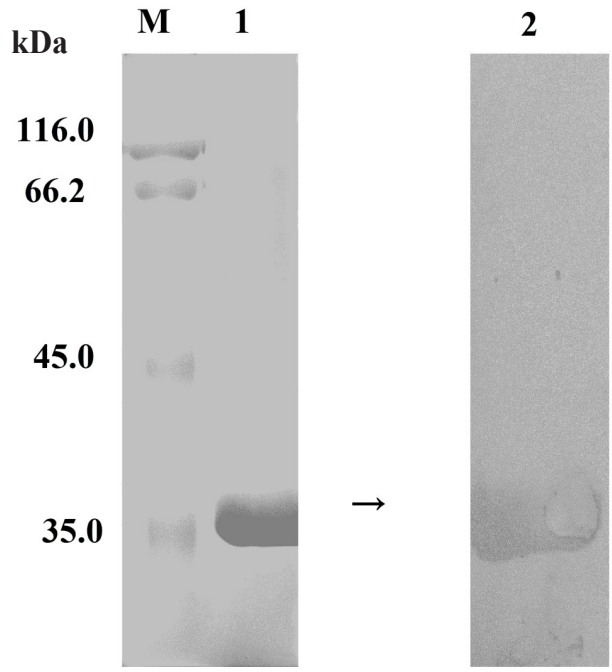

Figure 2. Western blot analyses of recombinant proteins. *Lane M* = protein marker; *lane 1* = purified recombinant proteins; *lane 2* = Western blot results.

Western blot analysis

Western blot analysis showed that the purified recombinant protein (*r*TpUbc2) reacted with sera from rabbits inoculated with *T. pisiformis* viable eggs (Figure 2), while no reaction was observed using the negative control serum (data not shown).

Protection against the *T. pisiformis* egg challenge

The protective effect against *T. pisiformis* larvae in rabbits inoculated with recombinant TpUbc2 was identified by counting the number of cysticerci 50 days after challenge with *T. pisiformis* eggs. The number of cysticerci in the vaccinated groups was reduced by 79.3% (42.6/206.0) and 90.8% (19.0/206.0), compared with the control (NS group). Table 3 shows the numbers of peritoneal cysticerci found in the NS group, QuilA alone group, and *r*TpUbc2-QuilA immunized rabbit group.

Table 3. Reduction in the number of *Taenia pisiformis* cysticerci in rabbits immunized with the *r*TpUbc2 vaccine.

Vaccine group	Mean number of cysticerci (means ± SD)	Reduction (%)
A (Control)	206.0 ± 157.0[a]	
B (QuilA)	108.7 ± 137.8[a]	
C (*r*TpUbc+QuilA)	42.6 ± 28.5[b]	79.3
D (*r*TpUbc+QuilA repeat)	19.0 ± 13.5[b]	90.8

Reduction rate (%) was calculated by the following formula: [1- (mean number of cysticerci with vaccination / mean number of cysticerci for control) x 100%], compared to control group. Different superscript letters indicate the statistically significant differences ($P < 0.05$) by the non-parametric Mann-Whitney test.

Serological analyses of rabbit sera from samples taken throughout the vaccine study indicate that specific immune responses to the recombinant antigens were produced in the vaccinated animals, with a clear increase in the OD value of IgG being observed after the 1st and 2nd immunizations (Figure 3). These levels were significantly higher compared to the levels observed in the QuilA and non-vaccinated groups ($P < 0.01$). The specific IgG antibody levels in all of the rabbits in the vaccinated groups reached a peak at week 4 after the 1st vaccination (OD_{450} = 1.92 and 1.88 in groups C and D, respectively). The IgG levels remained high for the remainder of the experimental period (7 weeks post-challenge). In the QuilA and non-vaccinated groups, the OD_{450} values of specific IgG remained low throughout the experiment.

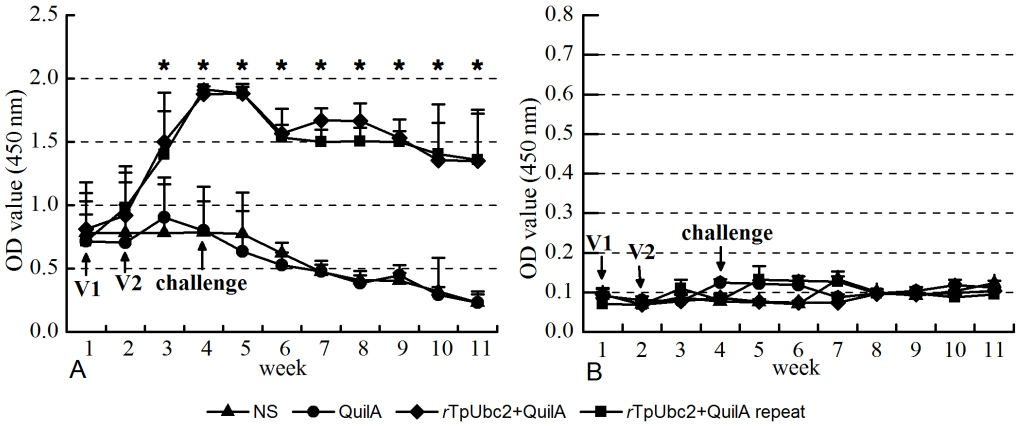

Figure 3. Specific IgG (**A**) and IgA (**B**) antibody levels in the sera of rabbits immunized with *Taenia pisiformis*. *r*TpUbc2 detected by ELISA. Rabbits were vaccinated twice with *r*TpUbc2, QuilA, and saline and challenged with 5000 *T. pisiformis* eggs. The results are reported as means ± SD. OD values were determined as absorbance at 450 nm. V1 = first vaccination; V2 = second vaccination. *$P < 0.01$ compared to NS (control group).

Due to the lack of an effective secondary anti-rabbit IgA antibody reaction, we chose to measure the levels of total IgA. Compared to the pre-immune serum, the total IgA serum levels did not change after 2 immunizations in any of the groups. At 4 weeks after challenge, the IgA levels of the vaccinated group, unvaccinated group, and QuilA group remained at a low level (OD_{450} < 0.2). No statistically significant difference was observed between the *r*TpUbc2 group and the control group.

DISCUSSION

In this study, we described the molecular characterization of TpUbc2 from the *T. pisiformis* oncosphere, and evaluated its ability to induce protection against infection in rabbits. We successfully cloned and expressed the ubiquitin-conjugating enzyme gene TpUbc2, with the TpUbc2 protein showing a high degree of homology to the Asc s 1.0101 allergen of *A. suum*, the Vesv 6.0101 allergen of *Vespula vulgaris*, and the Sola t 4 allergen of *Solanum tuberosum*. The prediction of cross-reactivity indicated a highly significant sequence match between TpUbc2 and the *A. suum* allergens. Thus, TpUbc2 is not a specific allergen for *T. pisiformis* larvae, and may have a high level of cross-reactivity with the Ubc2 of other nematodes and plants.

Although purified native antigens could theoretically be used in cysticercosis vaccination trials, the lack of an *in vitro* culture protocol means that it is difficult to obtain large quantities of native material. In previous studies, most researchers have focused on other methods of vaccine production to control cysticercosis, such as recombinant vaccines (Gauci et al., 2012; Jayashi et al., 2012), synthesized peptide vaccines (Huerta et al., 2001; De Aluja et al., 2005; Cruz-Revilla et al., 2006; Gazarian et al., 2012), and phage vaccines (Betancourt et al., 2012). Recently, the efficacy of the oral *vs* injectable S3Pva-synthetic and S3Pvac-phage vaccines from *Taenia solium* were compared. The data showed that oral S3Pvac-papaya significantly reduced the expected number of hepatic lesions and peritoneal cysticerci to a similar extent to that of the injectable vaccines (Betancourt et al., 2012). In the current study, the reduction in the recovery of larvae induced by the TpUbc2 recombinant vaccine was 79.3 and 90.8%. These results support the use of *r*TpUbc2 as a potential candidate to develop a vaccine against *T. pisiformis* larvae.

Immunization with *r*TpUbc2 formulated with QuilA elicited a specific immune response and protection against challenge with *T. pisiformis* eggs, as evidenced by a significant reduction in cysticerci compared to the non-vaccinated control groups. This result indicates that *r*TpUbc2 is able to eliminate *T. pisiformis* larvae through an immunological reaction. The levels of TpUbc2-specific serum IgG antibody in vaccinated rabbits, as measured by an ELISA, indicated that rabbits vaccinated with *r*TpUbc2-QuilA displayed a significantly higher level of specific IgG compared to the control groups. The results also indicated that *r*TpUbc2 could be used for the serodiagnosis of *T. pisiformis* larvae. However, more research is required to substantiate this hypothesis.

The effectiveness of the infection of *T. pisiformis* eggs in rabbits is variable. Craig and Zumbuehl (1988) found 155 cysts after infection with 1500 parasite eggs. However, in other studies by the same author, only 57 cysts were found after infection with 2000 eggs (Craig, 1984). Another group reported that an average of 8 cysticerci was recovered after challenge with 3300 eggs (Gemmell, 1965; Betancourt et al., 2012). We found an average of 206 cysticerci after infection with 5000 eggs. This variability might be related to the genetic make-up of the rabbits being tested. In addition, the relative infectivity of the particular strains used in the experiments should also be considered.

The oncosphere has been found to be highly susceptible to attack by the host's immune system (Jabbar et al., 2010). Studies using oncosphere antigens to elicit protective immunity against cysticercosis have been reported by several research groups (Jabbar et al., 2010; Gauci et al., 2012; Jayashi et al., 2012). The conjugation of ubiquitin to a target protein is a cascade enzymatic reaction involving an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2 or UBC), and an ubiquitin-protein ligase (E3). E2 is reacted with activated ubiquitin from E1, and donates ubiquitin monomers or multiubiquitin chains either directly or with the assistance of ubiquitin-protein ligase (E3) to an amino group of a lysine residue. The E2 is an important enzyme that is involved in the ubiquitin-proteasome system at all stages of the cestode lifecycle. In addition, the ubiquitin-proteasome system is also an essential mechanism for protein degradation, cell cycle progression, inflammatory response, transcriptional regulation, and signal transduction in eukaryotes.

The ubiquitin-conjugating (E2) isoenzyme family is characterized by the presence of a highly conserved UBC domain (Hofmann and Pickart, 2001). In humans, 35 active E2 enzymes have been identified to date, while other eukaryotic genomes harbor 16 to 35 E2 family members. The function of E2 has been extensively studied in yeast, and it appears to have distinct roles in a variety of cell events (Hofmann and Pickart, 2001). Homologous sequences to yeast E2 enzyme genes have also been characterized from other species, e.g., *Caenorhabditis elegans* (Zhen et al., 1993), *Drosophila melanogaster* (Koken et al., 1991), and many plants. Song et al. (2004) cloned and expressed the GST-tagged E2 gene of *Clonorchis sinensis*. The authors also confirmed that this expressed protein was functionally active. To date, the only UBC enzyme genes and proteins reported in Taeniidae studies are limited to bioinformatic analysis and the analysis of preliminary immunoreactivity in *Taenia asiatica* (Liao et al., 2009). The current study is the first to use a recombinant Ubc2 product as a potential vaccine based on a parasitic antigen.

The immune response elicited against the recombinant TpUbc2 cestode antigen might be related to the ubiquitin-proteasome protein degradation system; however, further research is required to determine all the factors that are involved.

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