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Molecular characterisation and expression analysis of two heat-shock proteins in *Taenia multiceps*

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Abstract

Background: *Taenia multiceps* is a harmful tapeworm and its larval form (coenurus cerebralis) is the causative agent of coenurosis, a disease affecting the health of herbivores, resulting in great economic loss to animal husbandry. Heat-shock proteins (HSPs), expressed in all prokaryotes and eukaryotes, act as molecular chaperones and can affect pathogenicity.

Methods: Herein, cDNAs of *T. multiceps* genes *Tm*-HSP60 and *Tm*-p36 were cloned and molecularly characterised by bioinformatics analyses. The immunogenicity and immunoreactivity of recombinant *rTm*-HSP60 and *rTm*-p36 proteins were investigated by immunoblotting and indirect ELISA was established to evaluate their serodiagnostic potential. Tissue localisation and transcriptional level at different life stages of *T. multiceps* were determined by immunohistochemical and quantitative real-time PCR analyses.

Result: The 533 residue *rTm*-HSP60 and the 314 residue *rTm*-p36 proteins share typical highly conserved features of HSPs. *Tm*-p36 shares structural characteristics with metazoan small HSPs, with two N-terminal α -crystallin domains. Compared with *Tm*-p36, *Tm*-HSP60 displayed stronger immunogenicity, and the indirect ELISA based on *rTm*-HSP60 exhibited a sensitivity of 83.3% and a specificity of 87.5%, while *rTm*-p36 was not suitable to develop indirect ELISA. *Tm*-HSP60 was widely distributed in all stages of *T. multiceps*, albeit at relatively low levels, while *Tm*-p36 was specifically distributed in the protoscolex and oncosphere.

Conclusions: The sequence, structural and functional analyses of these two HSPs indicates that they may play important roles in the life-cycle of *T. multiceps* as molecular chaperones. *Tm*-HSP60 displayed stronger immunogenicity compare to *Tm*-p36, and has the potential for antibody detection. *Tm*-p36 was strongly associated with the activation of oncospheres and has potential interest for vaccination.

Keywords: *Taenia multiceps*, *Coenurus cerebralis*, Metacestode, Heat-shock protein, Immunohistochemistry, Quantitative real-time PCR, Indirect ELISA

Background

Taenia multiceps is a common taeniid cestode and multi-host parasite. Adult worms adhere to the small intestine of canids, while the larval stage (*coenurus cerebralis*) causes coenurosis, a disease affecting herbivores,

resulting in great economic loss to animal husbandry in Europe, the USA, Africa and Asia [1–4]. The larval parasites are localized in the central nervous system, as well as subcutaneous and muscle tissue of the intermediate host, most of which are herbivorous animals [5–7], but humans can also act as intermediate hosts if eggs are eaten by mistake [8].

Taenia multiceps has a complex life-cycle that involves different phases of development and migration within the host [9, 10]. To overcome possible obstacles, parasites

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have developed tolerance mechanisms to assist survival and proliferation. One mechanism counteracts stress *via* the expression of heat-shock proteins (HSPs) that act as important molecular chaperones [11]. HSPs ensure the correct folding, processing and functionality of their protein substrates. In addition to facilitating cytoprotection against stress, HSPs also participate in the differentiation and development of parasites, and may even serve as diagnostic or vaccine candidates [12].

HSPs in some cestodes have been characterised, such as HSP60 and HSP70 that are immunodominant antigens in *Echinococcus granulosus* and *T. multiceps* [13, 14], and p36, a unique type of small HSP (sHSP) in metazoans such as *Taenia saginata*, *Taenia solium* and *E. granulosus* [15–18]. However, previous research on HSPs in cestodes is limited to exploring immunogenicity, and the relevance to biological functions throughout other life-cycle stages is unknown.

Herein, we investigated HSP60 and p36 from *T. multiceps*, identified previously from transcriptomic analyses [19, 20]. We expressed the recombinant proteins using a prokaryotic system, and performed bioinformatics analyses, tissue localisation studies, and analysed their transcriptional level by quantitative real-time PCR (qPCR) to probe their possible biological functions. This is a preliminary exploration of the roles of these two proteins in *T. multiceps*, and the findings lay the foundation for further understanding of their functions.

Methods

Parasites and animals

Cysts of *T. multiceps* were obtained from naturally infected goats in Sichuan Province, China. Adult worms were obtained from intestine samples from four 2-month-old dogs at 30 days after infection with 20,000 protoscolices. Mature eggs were obtained from fresh gravid proglottids, and oncospheres were released from the proglottids using trypsin and used for RNA extraction. Nine-week-old female New Zealand white rabbits were obtained from Dashuo Experimental Animal Co., Ltd (Chendu, China).

Primers

The cDNA sequences of *Tm*-HSP60 and *Tm*-p36 were amplified using primers designed from Unigene 5021 and 18510 of the assembled *T. multiceps* transcriptome dataset, which is homologous to the genome sequences of *Echinococcus multilocularis* (GenBank: CDS35950.1) and *T. saginata* (GenBank: Q7YZT0.1). All primers (Table 1) were synthesised by Sangon (Shanghai, China).

Bioinformatics analyses

Bioinformatics analyses were performed using ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) to predict open reading frames (ORFs), the TMHMM Sever 2.0 (<http://www.cbs.dtu.dk/services/TMHMM-2.0>) to identify transmembrane regions, and half-life and instability indices were predicted with the ExPASy Proteomics Server (<http://web.expasy.org/protparam/>). Signal peptides were predicted using the SignalP server (<http://www.cbs.dtu.dk/Services/SignalP/>), subcellular localisation and B-cell epitopes were predicted by BaCelLo (<http://gpcr.biocomp.unibo.it/bacello/pred.htm>) and Antibody Epitope Prediction (<http://tools.immuneepitope.org/bcell/>), and protein tertiary structures were predicted using SWISS-MODEL (<http://swissmodel.expasy.org/>). Multiple sequence alignment was performed with Clustal X software version 1.83.

Cloning, expression and purification of recombinant proteins

TRIzol reagent (Tiangen, Beijing, China) was used to extract total RNA from adult worms, protoscolices and oncospheres. cDNA was reverse-transcribed using a RevertAid First Strand cDNA Synthesis Kit following the manufacturer's instructions (Thermo Fisher Scientific, Vilnius, Lithuania). Target fragments were amplified, ligated into the pET32a(+) vector (TaKaRa, Dalian, China) and transformed into BL21 (DE3) competent cells. Expression of the recombinant proteins was induced by 1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) and purification was achieved using Ni²⁺ affinity chromatography (Bio-Rad, Hercules, CA, USA).

Table 1 Primers used for amplification and qPCR. All primers used for amplification included *Bam*HI or *Eco*RI restriction enzyme sites (underlined)

Primer	Sequence (5'–3')
<i>Tm</i> -HSP60F ^a	CGGGATCCATGCTTGTGGTGTGCGAT
<i>Tm</i> -HSP60R ^a	CGGAATTCACATCATAACCGCCC
<i>Tm</i> -p36F ^a	CGGGATCCATGTCATCTTTCCG
<i>Tm</i> -p36R ^a	CGGAATTCATTTAAAGAGAGGGCG
<i>Tm</i> -HSP60F ^b	TGCGTGATATGGCTATTGCGTCTG
<i>Tm</i> -HSP60R ^b	GAGCGTGTGCTCTTGGTGATG
<i>Tm</i> -p36F ^b	CGAGAGTGTGATGAAGGAGATGAGTG
<i>Tm</i> -p36R ^b	ACTTGTCTTGTCCGCCTTGATGG
actinF	CTAAGGCCGAACCGTGAGAAGATGAC
actinR	GGCATGAGGCAAGGCGTAACC

^a For amplification

^b For qPCR

Sera

Corresponding sera of *T. multiceps* (24 samples), *Taenia hydatigena* (12 samples), *Fasciola hepatica* (12 samples) and *Haemonchus contortus* (12 samples) were isolated from naturally infected goats in Sichuan Province and sera of *E. granulosus* (12 samples), *Moniezia expansa* (12 samples) were isolated from naturally infected sheep. Serum samples from cestode-free goats at autopsy were used as negative control.

Four rabbits were used to prepare polyclonal antibodies of *Tm*-HSP60 and *Tm*-p36, and rabbit serum samples were obtained before immunisation as a negative control. The first immunisation was a subcutaneous injection with 100 µg recombinant protein mixed with same volume of Freund's complete adjuvant (Sigma-Aldrich, St Louis, MO, USA). The second and third immunisations were injections with 100 µg recombinant protein mixed with same volume of Freund's incomplete adjuvant. The immunisation interval was 1 week. Two weeks after the final immunisation, serum samples were collected and the serum titre was determined by enzyme-linked immunosorbent assay (ELISA). Immunoglobulin G (IgG) was further isolated from the serum using a Protein G-Sepharose column (Bio-Rad, Richmond, USA).

Western blotting analysis

A Mammalian Protein Extraction Kit (Solarbio, Beijing, China) was used to extract crude proteins from adult worms. Proteins were separated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane using an electrophoretic transfer cell (Bio-Rad, Hercules) for 30 min. After washing with TRIS-buffered saline containing Tween-20 (TBST), membranes were incubated with 5% (w/v) skimmed milk at 37 °C for 2 h and incubated at 4 °C with polyclonal antibodies (1:200 v/v dilution) or serum from infected goats (1:200 v/v dilution) for 12 h, then with a 1:1,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG or rabbit anti-goat IgG (Boster, Wuhan, China) for 2 h. Signals were measured using an Enhanced HRP-DAB Chromogenic Substrate Kit (Tiangen).

Immunolocalisation of *Tm*-HSP60 and *Tm*-p36 at different life stages of *T. multiceps*

Samples (adult worms and protoscolices) were washed with phosphate-buffered saline (PBS), fixed in 4% (w/v) paraformaldehyde, embedded, and sliced into 5 µm thick sections. After dewaxing and dehydration, sections were treated with 0.01 M citrate buffer and incubated at 37 °C with 5% (w/v) skimmed milk for 4 h, then incubated with polyclonal antibodies or negative control rabbit serum (1:200 v/v) overnight at 4 °C. After washing, sections

were incubated at 37 °C with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (1:200 v/v) for 1 h in the dark and signals were monitored using a fluorescence microscope (Nikon, Tokyo, Japan).

Transcriptional profiles of *Tm*-HSP60 and *Tm*-p36 at different life stages of *T. multiceps*

For qPCR, 20 µl reactions were performed following the manual accompanying SsoAdvanced Universal SYBR Green (Bio-Rad, Hercules) with 2 µl of cDNA obtained from different proglottid and life-cycle stages (adult worms, protoscolices and oncospheres) as templates. The program involved an initial denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 56 °C for 31 s and 95 °C for 15 s, and a final extension step at 65 °C for 1 min and 95 °C for 15 s. Transcriptional levels of *Tm*-HSP60 and *Tm*-p36 were estimated by the $2^{-\Delta\Delta C_t}$ method and a housekeeping gene actin was used as an internal control for normalization (Table 1).

ELISA

To evaluate the serodiagnostic potential of recombinant proteins, an indirect ELISA was performed following the standard checkerboard titration procedures [21]. Briefly, proteins were diluted into six concentration gradients (ranging from 75 to 2 µg/ml) in 0.1 M carbonate buffer (pH 9.6) and coated with 100 µl per well in polystyrene 96-well microtiter plates. After incubation at 4 °C for 12 h, plates were washed three times using phosphate-buffered saline-Tween-20 (PBST) then blocked with 5% (w/v) skim milk at 37 °C for 1.5 h. After washing three times, plates were incubated with 100 µl serum samples diluted in PBS (concentration ranging from 1:20 to 1:640) at 37 °C for 1 h. Following washing, 100 µl of HRP-labeled rabbit anti-goat or sheep IgG (Boster) diluted 1:2000 by PBS were added and incubated at 37 °C for 1 h. The substrate TMB (Tiangen) was used to react and stopped with 2 M H₂SO₄. The optical density at 450 nm (OD₄₅₀) was recorded using a microplate reader (Thermo Scientific, Pittsburgh, PA, USA). We chose the optimal working conditions which gave the highest P/N value, and the cut-off value was determined by 24 negative goat serum samples, which was calculated as the mean OD₄₅₀ plus three standard deviations (SD).

To further investigate the feasibility of the indirect ELISA, 24 serum samples from goats infected with *T. multiceps* were evaluated and the sensitivity was calculated as ELISA positive × 100/true *T. multiceps*-positive. Meanwhile, a total of 60 serum samples (12 samples for each parasite) derived from sheep infected with *E. granulosus* and *M. expansa*, and goats infected with *T. hydatigena*, *F. hepatica* and *H. contortus* were used to evaluate

the cross-reactivity. Furthermore, an additional 24 negative serum samples were evaluated to determine the specificity of the indirect ELISA: ELISA negative × 100/true *T. multiceps*-negative.

Statistical analyses

All data are presented as the mean ± SD. Statistical analyses were performed using Student’s t-tests for comparison between life stages, and Mann-Whitney U-tests for different serum groups. All statistical tests were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered to be significant.

Results

Bioinformatics analyses

The 1602 nucleotide ORF in the cDNA sequence of *Tm*-HSP60 (GenBank: MH595536) encodes a 533 amino acid (aa) polypeptide, while the 945 nucleotide ORF in the cDNA sequence of *Tm*-p36 (GenBank: MH595535) encodes a 314 residue protein. No signal peptide was predicted for either protein, and neither appears to possess transmembrane regions. The predicted subcellular localisation of *Tm*-HSP60 is mitochondria, compared with nuclei for *Tm*-p36.

Tm-HSP60 is highly conserved, sharing 99.44, 96.62 and 80% sequence identity with orthologs in *T. solium*

(geneDB ID: TsM_000546800), *E. granulosus* (GenBank: CDS 22737.1) and *Schistosoma mansoni* (GenBank: XP 018645622.1; Fig. 1a). *Tm*-HSP60 shares the structural and functional characteristics of the molecular chaperone superfamily and is similar to HSP60 in other organisms. The structure includes typical ATP/Mg²⁺ binding sites, protein hinge regions, and oligomerisation shared with homologous chaperone proteins (Fig. 1b).

The *Tm*-p36 sequence is most similar to orthologs in *T. saginata* (GenBank: CAD80255.1), *T. solium* (GenBank: CAD36617.1), *E. granulosus* (GenBank: EUB62057.1) and *S. mansoni* (GenBank: XP 018649073.1), sharing 97.45, 96.18, 86.94 and 27.09% sequence identity, respectively (Fig. 2a). The tertiary structure of *Tm*-p36 was predicted to include two N-terminal α-crystallin domains composed of 12 β-sheets (Fig. 2b).

Cloning, expression and purification of r*Tm*-HSP60 and r*Tm*-p36

Tm-HSP60 and *Tm*-p36 genes were amplified from adult worms, expressed in a prokaryotic expression system, and the resulting fusion proteins yielded single bands of ~76 kDa and ~54 kDa (including the ~18 kDa epitope tag fusion peptide from pET-32a) following separation by 12% SDS-PAGE (Fig. 3).

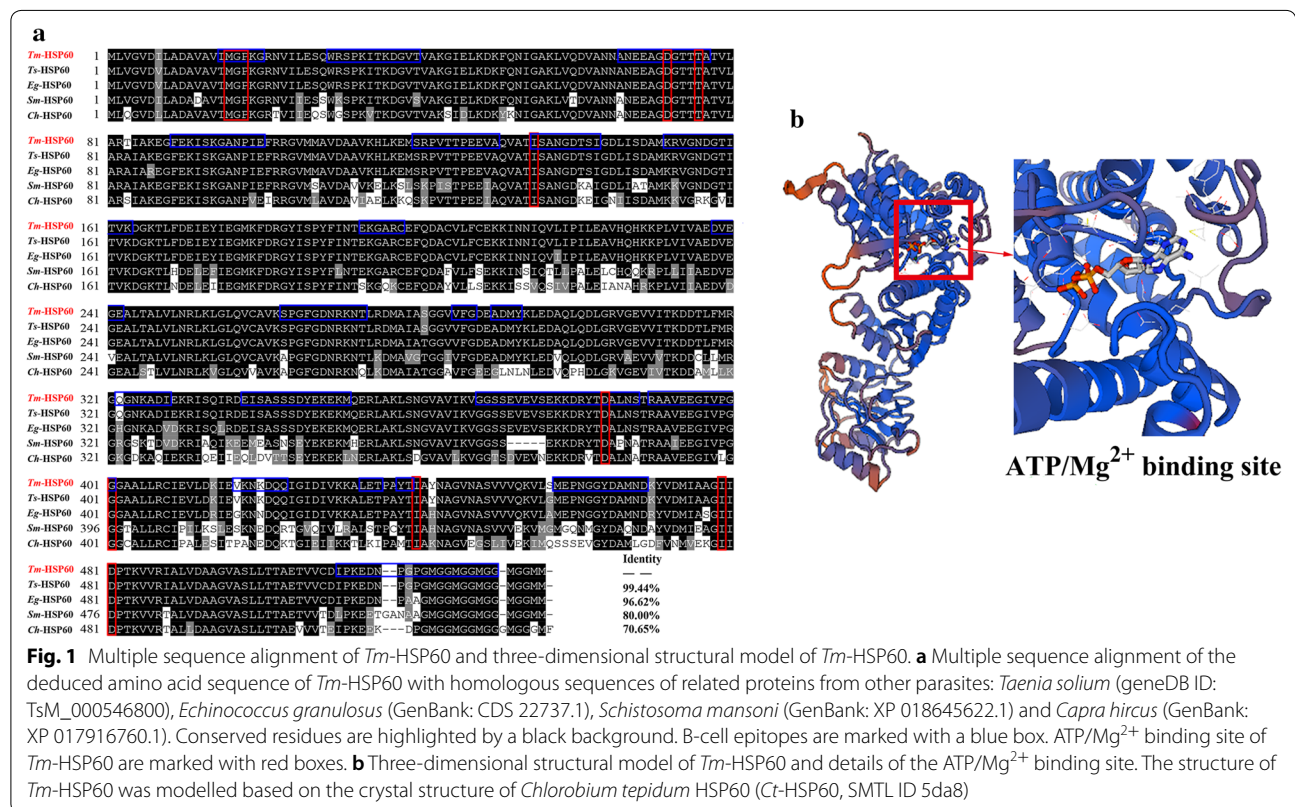


Fig. 1 Multiple sequence alignment of *Tm*-HSP60 and three-dimensional structural model of *Tm*-HSP60. **a** Multiple sequence alignment of the deduced amino acid sequence of *Tm*-HSP60 with homologous sequences of related proteins from other parasites: *Taenia solium* (geneDB ID: TsM_000546800), *Echinococcus granulosus* (GenBank: CDS 22737.1), *Schistosoma mansoni* (GenBank: XP 018645622.1) and *Capra hircus* (GenBank: XP 017916760.1). Conserved residues are highlighted by a black background. B-cell epitopes are marked with a blue box. ATP/Mg²⁺ binding site of *Tm*-HSP60 are marked with red boxes. **b** Three-dimensional structural model of *Tm*-HSP60 and details of the ATP/Mg²⁺ binding site. The structure of *Tm*-HSP60 was modelled based on the crystal structure of *Chlorobium tepidum* HSP60 (*Ct*-HSP60, SMTL ID 5da8)

Western blotting

The r*Tm*-HSP60 protein was reacted with polyclonal antibodies against r*Tm*-HSP60 and subjected to reaction with sera from goats infected with coenurus cerebralis; the results indicated it has some immunogenicity. The r*Tm*-p36 protein was recognised by polyclonal antibodies against r*Tm*-p36, but reaction with sera from infected goats was weak (see Additional file 1: Figure S1), and none in the negative controls. The native *Tm*-HSP60 and *Tm*-p36 proteins in adult worms were identified using polyclonal antibodies, yielding single bands of ~60 kDa and ~36 kDa (Fig. 3).

Immunolocalisation of *Tm*-HSP60 and *Tm*-p36 at different life stages of *T. multiceps*

Localisation of *Tm*-HSP60 and *Tm*-p36 in different stages of proglottid development was performed by immunofluorescence analysis using rabbit antibodies. A small amount of *Tm*-Hsp60 was found to be present in all stages of development of adult *T. multiceps*, mainly in the parenchymatous zone of the scolex, immature and mature proglottids, and also on the small hooks of the protoscolex, but there was no signal in gravid proglottids. *Tm*-p36 was found to be mainly distributed in the parenchyma and tegument of proglottids, with a small amount in the eggs of gravid proglottids, but much larger amounts in the body cavity of the protoscolex (Fig. 4).

Transcriptional profiles of *Tm*-HSP60 and *Tm*-p36 at different stages of proglottid development and life stages of *T. multiceps*

Transcriptional levels of *Tm*-HSP60 in different proglottid tissues were ordered immature proglottid > scolex > mature proglottid > gravid proglottid. Transcriptional profiles of *Tm*-HSP60 at different life stages were ordered oncosphere > protoscolex (Fig. 5a). By comparison, the levels of *Tm*-p36 in different proglottid tissues were ordered scolex > gravid proglottid > mature proglottid > immature proglottid, and at different life stages the order was oncosphere > protoscolex. Interestingly, the relative transcriptional level of *Tm*-p36 in oncosphere was consistently higher than in other life stages (Fig. 5b).

Serodiagnostic potential of r*Tm*-HSP60

The r*Tm*-HSP60 has definite potential for serological diagnosis through the established indirect ELISA. From standard checkerboard titration procedures, the optimum coating concentration of r*Tm*-HSP60 was found to be 0.2 µg/well and the optimum dilution ratio of serum was 1:160, with the highest P/N value of 2.255 (see Additional file 2: Table S1). Under optimal conditions, the average OD₄₅₀ value of 24 negative serum samples was 0.211 with a SD of 0.035. Thus, the cut-off value was determined to be 0.316. However, as the highest P/N value of r*Tm*-p36 was only 1.5 (see Additional file 3:

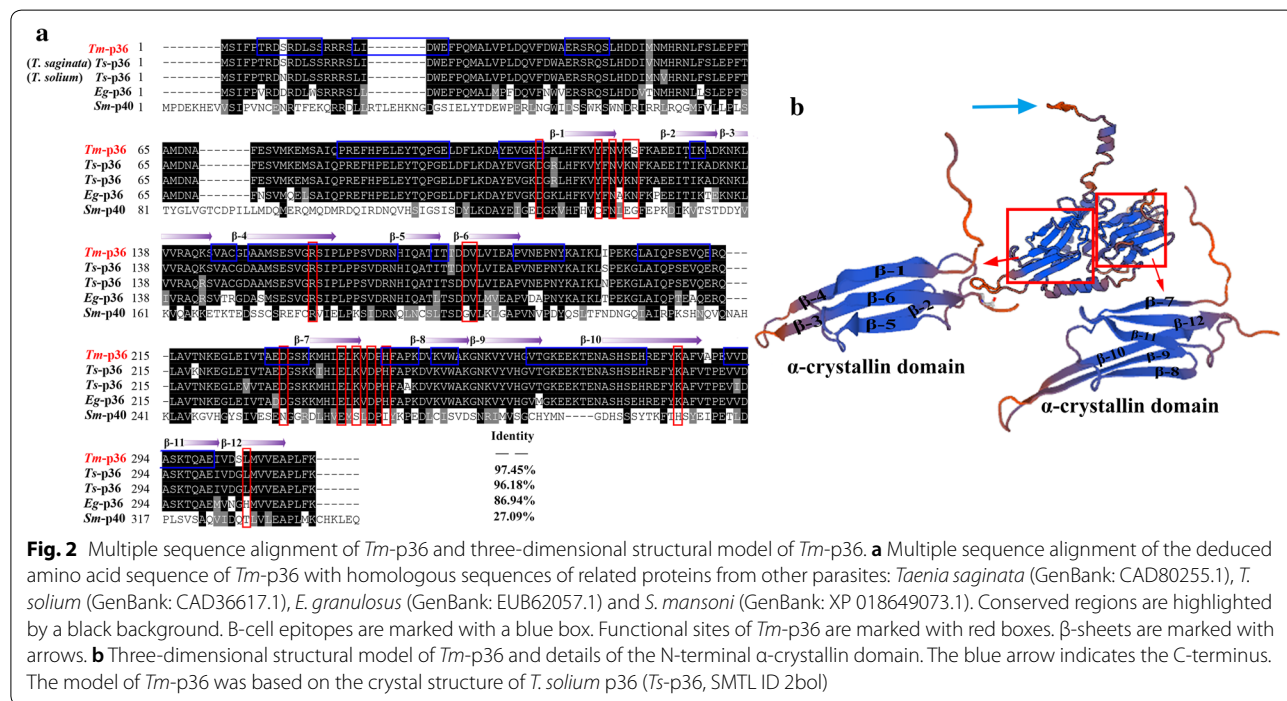


Fig. 2 Multiple sequence alignment of *Tm*-p36 and three-dimensional structural model of *Tm*-p36. **a** Multiple sequence alignment of the deduced amino acid sequence of *Tm*-p36 with homologous sequences of related proteins from other parasites: *Taenia saginata* (GenBank: CAD80255.1), *T. solium* (GenBank: CAD36617.1), *E. granulosus* (GenBank: EUB62057.1) and *S. mansoni* (GenBank: XP 018649073.1). Conserved regions are highlighted by a black background. B-cell epitopes are marked with a blue box. Functional sites of *Tm*-p36 are marked with red boxes. β-sheets are marked with arrows. **b** Three-dimensional structural model of *Tm*-p36 and details of the N-terminal α-crystallin domain. The blue arrow indicates the C-terminus. The model of *Tm*-p36 was based on the crystal structure of *T. solium* p36 (*Ts*-p36, SMTL ID 2bol)

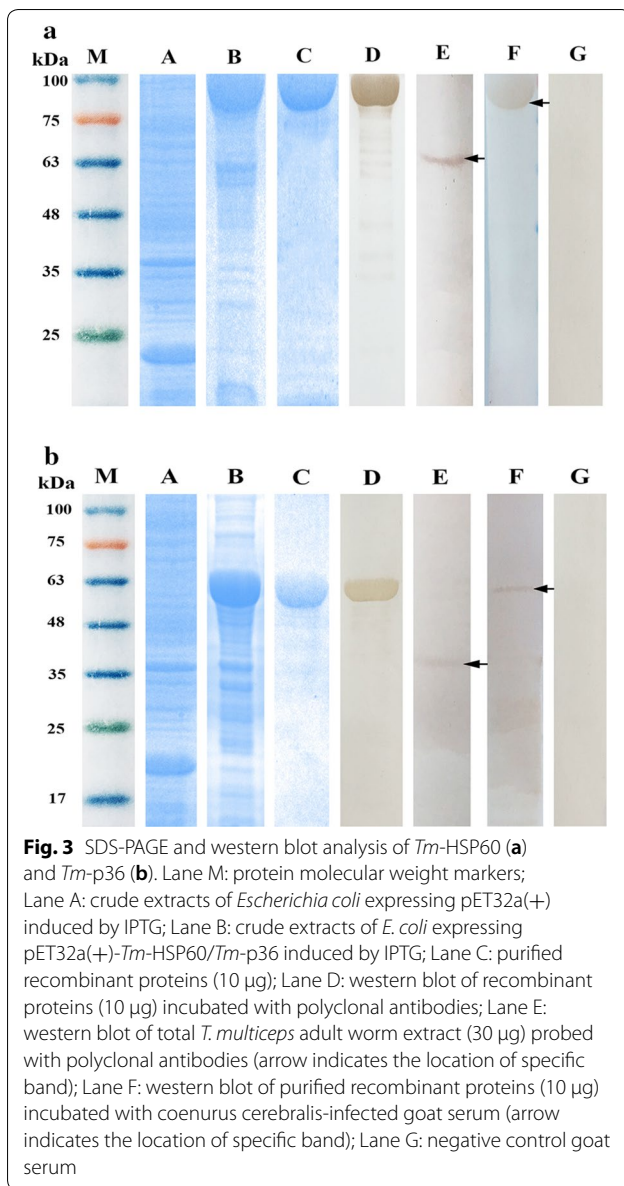


Table S2), we stopped the further exploration of the serodiagnostic potential of *Tm*-p36.

The sensitivity of indirect ELISA identified by positive serum samples was 83.3% (20/24), and the specificity by negative serum samples was 87.5% (21/24). No cross-reaction between r*Tm*-HSP60 and antibodies of *T. hydatigena* ($n = 12$), *F. hepatica* ($n = 12$) and *H. contortus* ($n = 12$) was obvious, but cross-reactivity was observed

with five serum samples of *E. granulosus* ($n = 12$) and five of *M. expansa* ($n = 12$) (Fig. 6).

Discussion

HSPs are a family of proteins expressed in all prokaryotes and eukaryotes during growth and development, and expression is stimulated by various physical, chemical and pathological factors. These non-secretory proteins are among the most highly conserved among development-related proteins and are also the most important molecular chaperones [22]. The main biological function of HSPs is to facilitate the folding, translocation, renaturation and degradation of their protein substrates in order to protect cells from harmful factors, both internally and externally in all organisms, ranging from mammals to microbes, consistent with their important roles in all species [23].

Traditionally, HSPs can be divided into eight groups according to their molecular weight, including sHSPs that have a molecular weight ranging between 12–43 kDa [24]. Like other HSPs, sHSPs are involved in many cellular processes, and most are characterised by a conserved C-terminal α -crystallin domain of 80–100 residues [25]. They usually form large homo- or heteromeric complexes to achieve chaperone-like and other activities [26].

In parasites, HSPs play an extremely important role in life processes, many of which pass through multiple life-cycle stages, during which parasites must face a variety of environmental challenges such as severe hypoxia, high redox conditions, and temperature changes [27]. Adult worms of *T. multiceps* adhere to the small intestine of their hosts, while metacestodes are present in the central nervous system and subcutaneous or muscular tissues of the intermediate hosts and oncospheres invade intermediate hosts through the intestine; all these processes occur in harsh conditions [28, 29]. In response to these harsh living conditions, parasite HSPs may play an important role. In addition to their molecular chaperone activities, HSPs are also involved in the growth and development of parasites and can be recognised by the host immune system as an important antigen during invasion [30–33].

Tm-HSP60 shares high homology with the GroEL molecular chaperone in *Escherichia coli*. The ATP/Mg²⁺ binding site is highly similar to that in HSPs in mammals and bacteria, and these sites are known to be important for molecular chaperone activity [34, 35]. The expression of *Tm*-HSP60 at all life stages indicates that *Tm*-HSP60

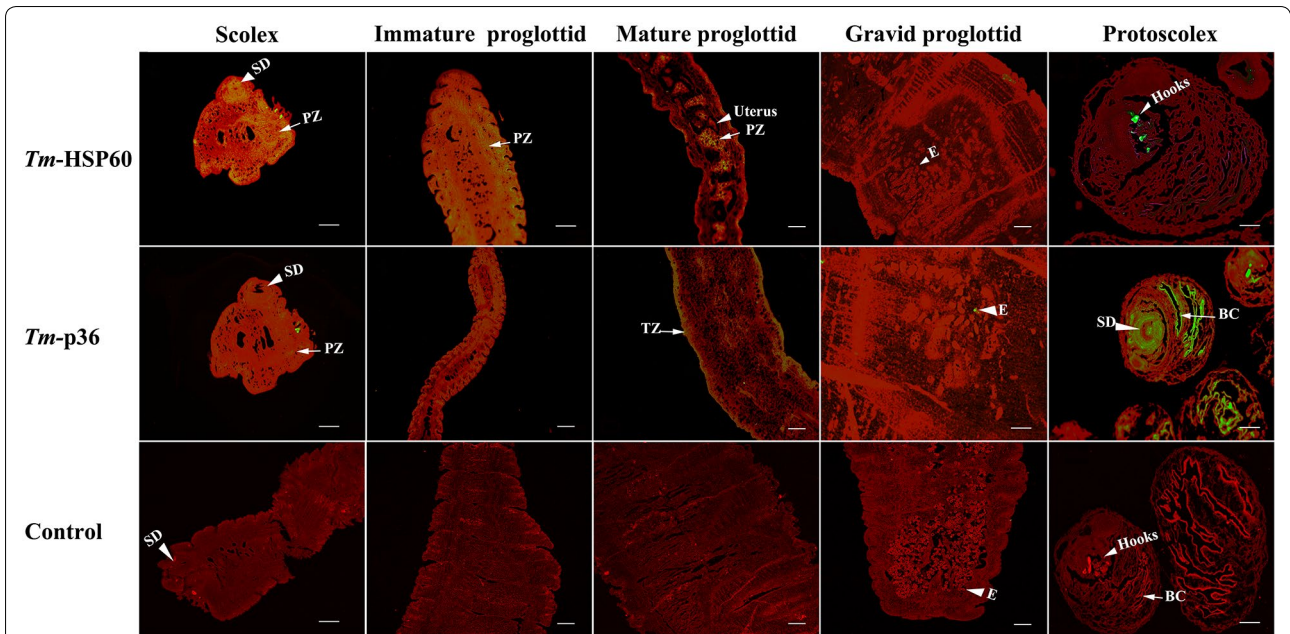


Fig. 4 Immunohistochemical localisation of *Tm*-HSP60 and *Tm*-p36 in different proglottid tissues and different life stages of *T. multiceps*. Images of scolex, protoscolex, immature of *Tm*-HSP60 and gravid proglottid of *Tm*-p36; images of control in mature proglottids are magnified at $\times 400$, and other images are magnified at $\times 200$. Tissues and organs are marked with arrows. Scale-bars: 200 μm . Abbreviations: SD, sucking disc; PZ, parenchymatous zone; E, egg; TZ, tegument zone; BC, body cavity

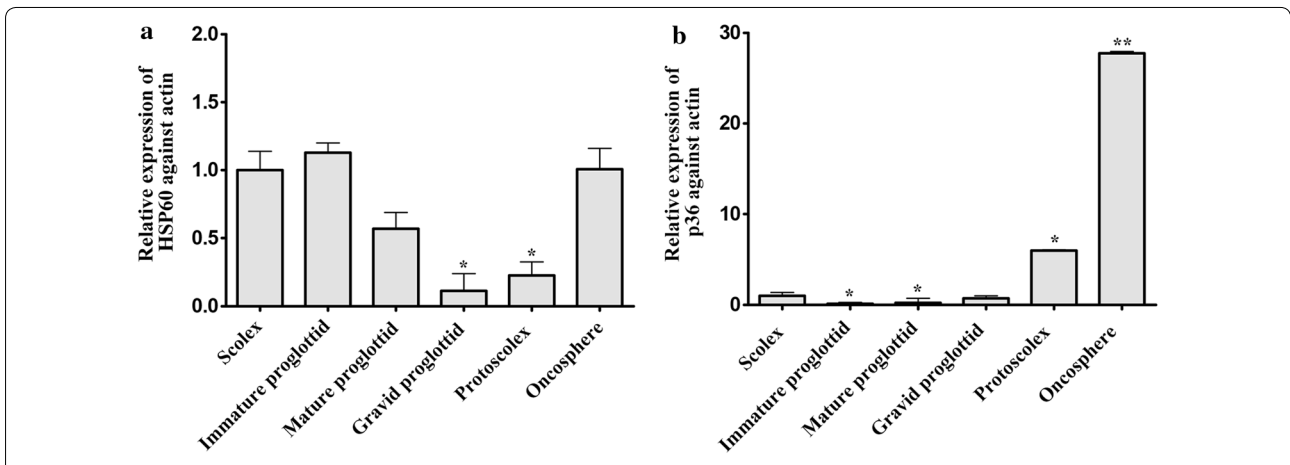
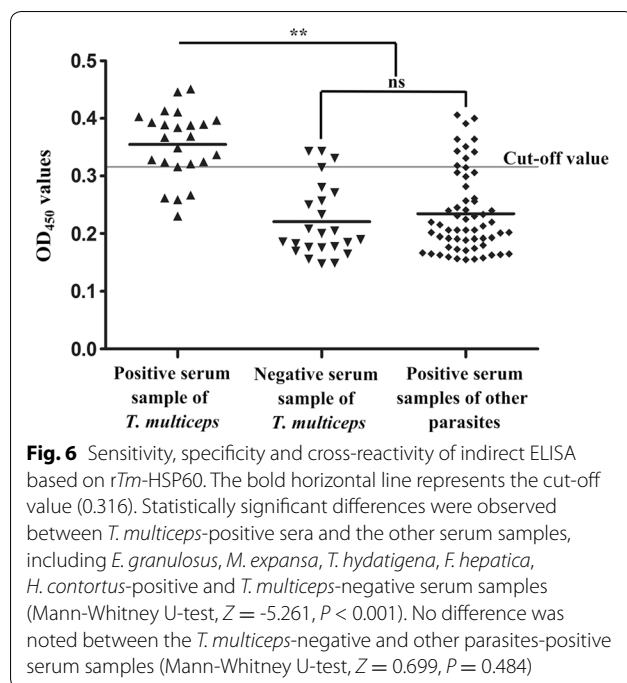


Fig. 5 Relative expression patterns of *Tm*-HSP60 (a) and *Tm*-p36 (b). Data are presented as the mean and standard deviation (SD) of triplicate experiments. Statistically significant differences between scolex (controls) and other samples were determined using Student’s t-tests (* $P < 0.05$, ** $P < 0.01$)

possibly plays an important role throughout the growth and development of *T. multiceps*. However, subtle differences were apparent, since *Tm*-HSP60 was mainly distributed in immature stages (scolex, immature proglottid and oncosphere). It may be involved in the processing of target proteins to protect the body at all stages of growth in response to environmental changes. In a previous study, *Tm*-HSP70 was used as an important diagnostic

antigen in goats with coenurosis [14]. Similarly, *Tm*-HSP60 also displayed immunogenicity in the present work. Corresponding antibodies were detected from the serum of infected goats, indicated that this protein was recognised by the host as an important antigen during invasion. Therefore, we established an indirect ELISA to assess the serological diagnostic potential of r*Tm*-HSP60. A definite sensitivity (83.3%) and specificity (87.5%) was



observed. There was no cross-reaction with other species except for cestodes which is most likely due to high affinity. Non-homologous short fragments containing more antigenic epitopes may improve this situation. Feasible results suggested that *Tm*-HSP60 can be used as a diagnostic tool for coenurosis in the future.

Like metazoan α -crystallin-type sHSPs, *Tm*-p36 has two N-terminal α -crystallin domains, which is essential for sHSPs to perform their chaperone-related functions [36, 37]. As the name suggests, sHSPs have a relatively low molecular weight, but multiple proteins can be assembled into large homo- or heteromeric complexes, consisting of 9–50 subunits with overall molecular weights of 800 kDa or more to form a functional unit [38–40]. The transcriptional level of *Tm*-p36 in adults was low, but higher in protoscolices and oncospheres, especially in the latter, which is consistent with findings in other cestodes [15–18]. The protoscolex is the larval stage of *T. multiceps* (*coenurus cerebralis*), and the oncosphere is the stage that invades the intermediate host [41]. The specific distribution also indicates that *Tm*-p36 plays an important role in the life-cycle of *T. multiceps*, especially in growth and development of the protoscolices and oncospheres which face harsh living conditions such as extremely high acidity and an anoxic environment in the host immune system. In adult cestodes, oncospheres are also present in the eggs of gravid proglottids, but the results of immunohistochemistry and qPCR analyses revealed low *Tm*-p36 expression level in the gravid proglottids. These results indicate that *Tm*-p36 is closely related to the activation

of oncospheres during host invasion. However, *Tm*-p36 does not have good immunoreactivity from the results of western blotting and indirect ELISA. It may reveal that the short-term high expression of *Tm*-p36 is not recognized by the host immune system to produce the corresponding antibody for detection.

Some studies have shown that sHSPs of the parasite is overexpressed at a certain life-cycle stage or under stress, and most of them act as immune system stimulator antigen in host-parasite relationship [42–44]. Previous studies have also suggested that the major egg antigen of *S. mansoni*, also known as *Sm*-p40, is an immune system stimulator associated with granuloma formation inhibition and has been widely studied as a vaccine candidate in schistosomiasis [45–47]. Considering the similar situation, *Tm*-p36 could also serve as an important vaccine or therapeutic target for inhibiting activation or preventing invasion of oncospheres.

Conclusions

In conclusion, we performed sequence, structure, tissue distribution and transcriptional profile analyses of two HSPs in *T. multiceps* and found that *Tm*-HSP60 is widely distributed in all life-cycle stages, and compared with *Tm*-p36, *Tm*-HSP60 displayed stronger immunogenicity. Thus, *Tm*-HSP60 is of interest for antibody detection, while *Tm*-p36 was most strongly associated with oncospheres and protoscolices, and is of potential interest for vaccination for controlling *coenurus cerebralis* in the future.

Additional files

Additional file 1: Figure S1. Western blot of *rTm*-p36 with six *coenurus cerebralis*-positive sera. Lane M, protein molecular weight markers; Lane C, western blot of *rTm*-p36 (10 μ g) with negative control goat serum.

Additional file 2: Table S1. Determination of the optimal *rTm*-HSP60 coating concentration and serum dilution for indirect ELISA.

Additional file 3: Table S2. Determination of the optimal *rTm*-p36 coating concentration and serum dilution for indirect ELISA.

Abbreviations

HSPs: heat-shock proteins; sHSPs: small heat-shock proteins; qPCR: quantitative real-time PCR; IPTG: 1 mM isopropyl β -D-1-thiogalactopyranoside; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; TBST: TRIS-buffered saline containing Tween-20; PBS: phosphate-buffered saline; *rTm*-HSP60: recombinant *T. multiceps* HSP60; *rTm*-p36: recombinant *T. multiceps* p36.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Authors' contributions

YCL and GYY conceived and designed the study. YCL and CG performed the experiments. YCL and XWD analysed the data and drafted the manuscript. XBG and WML conceived the study and collected experimental materials. YX and XRP collected and analysed the raw data. GYY assumed overall responsibility for this study, participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animals involved in this work were purchased from Dashuo Experimental Animal Co., Ltd (Chendu). All animals were maintained in accordance with the guidance of the Animal Protection Law of the People's Republic of China, issued on 18 September 2009. All procedures were approved by the Institute of Health Animal Care and Use Committee of Sichuan Agricultural University of China (approval no. 2013-028).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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