1 *Teladorsagia circumcincta* 1,6 biphosphate aldolase: molecular and biochemical
2 characterisation, structure analysis and recognition by immune hosts

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ABSTRACT

A 1095 bp full length cDNA encoding *Teladorsagia circumcincta* aldolase (*TciALDO*) was cloned, expressed in *Escherichia coli*, the recombinant protein purified and its kinetic properties determined. A phylogenetic tree was constructed using helminth aldolase sequences. The predicted protein consisted of 365 amino acids and was present as a single band of about 44 kDa on SDS-PAGE. Multiple alignments of the protein sequence of *TciALDO* with homologues from other helminths showed that the greatest similarity (93%) to the aldolases of *Haemonchus contortus* and *Dictyocaulus viviparus*, 82-86% similarity to the other nematode sequences and 68-71% similarity to cestode and trematode enzymes. Substrate binding sites and conserved regions were identified and were completely conserved in other homologues. At 25 °C, the optimum pH for *TciALDO* activity was pH 7.5, the $V_{\max}$ was 432 ± 23 nmoles.min⁻¹.mg⁻¹ protein and the apparent K_m for the substrate fructose 1,6-bisphosphate was 0.24 ± 0.01 μ M (mean $\pm$ SEM, n = 3). Antibodies in both serum and saliva from field-immune, but not nematode-naïve, sheep recognised recombinant *TciALDO* in enzyme-linked immunosorbent assays. The recognition of the recombinant protein by antibodies generated by exposure of sheep to native aldolase indicates similar antigenicity of the two proteins.

Keywords: Aldolase; Cloning; Expression; kinetic properties; *Teladorsagia circumcincta*

1 **1. Introduction**

2 Fructose 1,6-bisphosphate aldolase (FBA) (EC 4.1.2.13) catalyses the reversible
3 reaction that splits fructose 1,6-bisphosphate into the triose phosphates
4 dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P). The
5 forward reaction occurs during glycolysis and the reverse reaction forms fructose 1,6-
6 bisphosphate during gluconeogenesis. FBA enzymes belong to two classes depending
7 on the mechanism of the reaction: class I, which form covalent Schiff-base conjugates
8 with a conserved lysine, are present mainly in higher eukaryotes and a few bacteria,
9 whereas class II require a divalent metal ion as cofactor for enzymatic activity and are
10 found principally in bacteria, algae and fungi (Grazi et al., 1962; Marsh and Lebherz,
11 1992; Gefflaut et al., 1996; Sánchez et al., 2002; Tittmann). Thus, class I and II
12 enzymes can be distinguished by inhibition of the latter by ethylene diamine tetraacetic
13 acid (EDTA). There are three isoforms of vertebrate FBA: aldolase A which is
14 principally expressed in muscle, aldolase B in liver and aldolase C in brain (Lebherz
15 and Rutter, 1969).

16 The genes encoding FBAs have been sequenced from the free-living nematode
17 *Caenorhabditis elegans* (Inoue et al., 1997), the animal-parasitic *Haemonchus*
18 *contortus* (Yan et al., 2013), the plant-parasitic *Heterodera glycines* and *Globodera*
19 *rostochiensis* (Kovaleva et al., 2005), as well as other helminths, including *Schistosoma*
20 *mansoni* (El-Dabaa et al., 1998) *Echinococcus granulosus* (Lorenzatto et al., 2012),
21 *Clonorchis sinensis* (Li et al., 2014), *Schistosoma japonicum* (Hu et al., 2015) and
22 *Opisthorchis viverrini* (Prompipak et al., 2017). As nematode FBAs were shown to
23 have some structural properties similar to vertebrate FBA A, but catalytic properties
24 more like those of FBA C, aldolases were suggested to be the products of primordial

1 genes from which vertebrate FBA genes have evolved (Reznick and Gershon, 1977a).
 2 Subsequent genetic studies have shown that in *C. elegans* there are two isozymes
 3 encoded by different genes, one of which has similar kinetic properties to vertebrate
 4 aldolase C and the other broader substrate specificity in addition to fructose 1,6-
 5 biphosphate (Inoue et al., 1997), which could explain the earlier conclusions about
 6 nematode aldolases being evolved by duplication and diversification.

7 The kinetic properties of FBA enzymes are generally similar, such as the typical
 8 temperature and pH optima of 40°C and pH 7.5 respectively of the *H. contortus* enzyme
 9 (Yan et al., 2013). The reported K_m of purified nematode aldolases varied between
 10 species and even within studies, e.g. the aldolase in homogenates of *H. glycines* had a
 11 lower activity than either the *C. elegans* or *Panagrellus redivivus* enzymes (Reznick
 12 and Gershon, 1977b) because of there might be a mixture of different isozymes.
 13 Enzyme activity declined with age in the free-living *Turbatrix aceti* (Zeelon et al.,
 14 1973). Parasitic helminths may have more active enzymes than their hosts, as seen for
 15 *S. japonicum* FBA, which had a lower apparent K_m of 0.06 μ M and higher activity than
 16 that of human FBA A (Hu et al., 2015).

17 Aldolase, like many other glycolytic enzymes, has both intra- and extra-
 18 cellular further activities in parasites in addition to its enzymatic function (Gómez-
 19 Arreaza et al., 2014); these include plasminogen binding (Ramajo-Hernández et al.,
 20 2007) and immunomodulation (Marques et al., 2008). It is released into the
 21 extracellular environment and can be detected in excretory/secretory (ES) products
 22 (Ramajo-Hernández et al., 2007; Guillou et al., 2007; Morassutti et al., 2012) and has
 23 been also located in the tegument of adult *Schistosoma bovis* (Ramajo-Hernández et al.,
 24 2007) and *S. mansoni* (El-Dabaa et al., 1998). FBA is antigenic: the protein from

1 *Dirofilaria immitis* is recognised by the sera of infected dogs (Sassi et al., 2014); the
2 serum of *Onchocerca volvulus* infected and putatively immune humans recognised
3 aldolase (McCarthy et al., 2002), *Angiostrongylus cantonensis* (Morassutti et al., 2012)
4 or *S. japonicum* (Peng et al., 2009) and the *Teladorsagia circumcincta* L3 protein
5 reacted with ovine IgA in the gastric lymph of previously-infected sheep (Ellis et al.,
6 2014).

7 In the present study, the cDNA encoding *T. circumcincta* aldolase (*TciALDO*)
8 was cloned, expressed in *Escherichia coli*, the recombinant protein was purified and
9 some kinetic properties determined. Enzyme-linked immunosorbent assays (ELISAs)
10 were performed to determine if the enzyme was recognised by saliva and serum from
11 sheep previously exposed to nematode parasites in the field. To gain insight into the
12 substrate specificity of *TciALDO*, we modelled the crystal and tertiary structures using
13 the full-length amino acid sequence of *TciALDO*.

14 **2. Material and methods**

15 All chemicals were purchased from the Sigma Chemical Co. (Mo, USA)
16 unless stated otherwise. Use of experimental animals for culturing and harvesting adult
17 worms for RNA extraction has been approved by the AgResearch Grasslands Animal
18 Ethics Committee (protocol #13052).

19 *2.1. Parasites*

20 Pure cultures of *T. circumcincta* were maintained in the laboratory by regular
21 passage through sheep. Adult worms were recovered from the abomasa of infected
22 sheep as described previously (Umair et al., 2013). Briefly, abomasal contents were
23 mixed 2:1 with 3% agar and the solidified agar blocks incubated at 37 °C in a saline

1 bath. Clumps of parasites were collected from the saline soon after emergence and
2 frozen in Eppendorff tubes at -80 °C for molecular biology procedures.

3 2.2. RNA isolation and cDNA synthesis

4 Adult *T. circumcincta* (50-100 µl packed volume) in 1 ml Trizol (Life
5 Technologies) were ground to a fine powder in a mortar under liquid N₂ and RNA
6 extracted according to the manufacturer's instructions. The quality and concentration of
7 the RNA was assessed and first strand cDNA synthesised from 1 µg using the iScript
8 Select cDNA Synthesis Kit (Bio-Rad) and a 1:1 mixture of Oligo (dT)₂₀ and hexamer
9 primers.

10 2.3. Cloning and expression of *T. circumcincta* recombinant TciALDO in *E. coli*

11 A partial *T. circumcincta* ALDO sequence TDC00486 (NEMBASE)
12 containing the 5' end was used and the 3' of *TciALDO* cDNA was obtained by 3'
13 RACE (SMARTer RACE cDNA Amplification Kit, Clontech) using *T. circumcincta*
14 adult RNA, as outlined by the manufacturer. The full length *TciALDO* cDNA was
15 amplified from this cDNA in a PCR containing the oligonucleotide primers Tci
16 aldo_FL-F1 (5'- CACCATGGCTTCCTACTCGCAGTA-3') and Tci aldo_FL-R1 (5'-
17 TCAATAGGCATGATTAGCCAC-3'). The full-length gene was then transformed into
18 TOP10 cells and subsequently cloned into the expression vector Champion pET100
19 Directional TOPO (ThermoFisher Scientific) and transformed into *E. coli* One shot
20 BL21 (DE3) according to the manufacturer's instruction. The construction was
21 sequenced to check its integrity.

22 *E. coli* strain BL21 (DE3) transformed with pET 100 using a NH₂ tag
23 *TciALDO* was grown in 10 ml Luria Broth (LB) supplemented with 100 µg/ml

1 ampicillin for 16 h at 30 °C and 250 rpm. The culture was diluted 20-fold in LB with
2 100 µg/ml ampicillin and 1% glucose and grown to OD₆₀₀ of 0.6-0.8 at 30 °C and 250
3 rpm. Isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to a final
4 concentration of 1 mM and the culture grown at 30°C and 250 rpm for an additional 16
5 h. Bacteria were harvested by centrifugation and the soluble extract was obtained by
6 incubating harvested cells in CelLyticB (5ml/g wet cell paste), 0.2 mg/ml Lysozyme,
7 50U/ml Benzonase and protease inhibitors at room temperature for 10 min. The crude
8 lysate was centrifuged at 15,000xg at 4 °C for 20 min to remove all the cell debris and
9 the supernatant collected and filtered through a 0.45 µm filter.

10 2.4. Purification

11 Purified recombinant poly-histidine protein was obtained by FPLC under native
12 conditions using a Ni-NTA column (Qiagen), and a Biologic DUO-FLOW BIO-RAD
13 chromatography system (Bio-Rad, USA). Sodium bi-phosphate buffer was used as an
14 equilibration buffer, sodium bi-phosphate containing 20 mM imidazole as the wash
15 buffer and sodium bi-phosphate containing 500 mM imidazole as elution buffer. The
16 protein was dialysed overnight following the elution and the concentration was
17 determined by the Nanodrop (Thermofisher Scientific) using the A280 nm assay with
18 extinction coefficient (34755 M⁻¹cm⁻¹) and molecular weight (43.8 KDa).

19 2.5. Bioinformatics

20 Alignment of protein sequences was performed using the Muscle alignment
21 option in Geneious Prime (Biomatters Ltd) with the Blosum 62 similarity matrix used
22 to determine similarity to *H. contortus* and other helminth aldolases. The predicted

1 tetramer structure of *Tci*ALDO was constructed using SWISS-MODEL, a fully
2 automated protein structure homology-modelling server with default parameters.

3 2.6. *Gel electrophoresis*

4 SDS-PAGE was performed using NuPAGE Novex 4-12% Bis-Tris gels
5 according to the instructions of the manufacturer (Invitrogen). Gels were stained with
6 SimplyBlue safe stain (Life technologies). A western blot was also performed using a
7 monoclonal anti-poly histidine-peroxidase antibody. Blots were blocked, incubated
8 overnight in 1:2000 antibody in buffer (4% skim milk powder in Tris-buffered saline
9 and 0.1% Tween-20) at room temperature and developed to detect His-tagged
10 recombinant protein.

11 2.7. *Tci*ALDO activity (*E.C. 4.2.1.11*)

12 The enzyme activity of *Tci*ALDO was measured at 30 °C in a coupled assay
13 with reversible conversion of fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate
14 and dihydroxyacetone phosphate using Sigma aldolase kit (Catalogue # MAK223). The
15 NADH production was measured colorimetrically at 450 nm. The final reaction
16 mixture (100 ul) contained assay buffer, enzyme mix, enzyme developer, recombinant
17 protein (50 µg) and the substrate. NADH standards and the blank were set up as
18 described by the manufacturer.

19 (1) The optimum pH was determined with a substrate concentration of 0.5 mM
20 fructose 1,6-bisphosphate with pH range 6 to 9. Subsequent assays were carried out
21 at pH 7.5.

1 (2) The apparent K_m for fructose 1,6-bisphosphate was determined in reaction
2 mixtures containing 0-5 mM fructose 1,6-bisphosphate.

3 (3) The effects of EDTA as potential activators/inhibitors on recombinant
4 *TciALDO* with substrate concentrations of 0.5 mM fructose 1,6-bisphosphate and
5 10 mM EDTA.

6 2.8. ELISA

7 Serum and saliva samples taken from parasite-naïve and parasite-exposed
8 sheep were tested for the presence of antibodies that react with the recombinant
9 *TciALDO* by ELISA. Serum and saliva samples were collected from 18 male Romney
10 lambs 6-7 months old and previously exposed to multiple species of parasites including
11 *H. contortus* and *T. circumcincta*. Lambs had developed immunity against *T.*
12 *circumcincta* infection and pooled serum and saliva samples were used for ELISA.
13 Plates were coated with 5 µg/ml *TciALDO* onto ELISA plates (Maxisorp, Thermofisher
14 Scientific) overnight at 4 °C. Plates were washed and free binding sites were then
15 blocked with Superblock (Thermofisher Scientific) at room temperature for 30 min and
16 then incubated with serial dilutions (200- to 12800-fold for serum) or (20- to 320-fold
17 for saliva) in ELISA buffer for 2h at room temperature. Bound serum immunoglobulins
18 were then detected with rabbit anti-sheep IgG-HRP diluted 1:4000 by incubation at
19 37°C for 2h and colour development with 3,3',5,5' tetramethylbenzidine (TMB). Saliva
20 IgA was detected with rabbit anti-sheep IgA-HRP diluted, incubated and colour was
21 developed as described for serum IgG.

22 2.9. Protein modelling and structural analysis of *TciALDO*

1 The structural model of *Tci*ALDO was constructed by submitting the amino
2 acid sequence obtained as described above, to the I-TASSER server (Yang et al., 2015).
3 From the best five models obtained, a model was selected with a C-score of -0.11, a
4 TM value of 0.70 ± 0.12 . TM-score is a metric for measuring the similarity of two
5 protein structures, or a global fold similarity between the generated model and the
6 structure it was based on. Scores higher than 0.5 assumes the parent structure and
7 modelled protein share the same fold while below 0.17 suggests a random nature to the
8 produced model (Zhang and Skolnick, 2004). C-score is a confidence score for
9 estimating the quality of predicted models by I-TASSER. It is calculated based on the
10 significance of threading template alignments and the convergence parameters of the
11 structure assembly simulations. C-score is typically in the range of -5 to 2, where a C-
12 score of higher value signifies a model with a high confidence and vice-versa. The
13 structural model with highest C-score was further validated using Procheck (Laskowski
14 et al., 1996) and ProSA-web (Wiederstein and Sippl, 2007). The substrate Binding
15 domain was identified and active site residues were deduced and pictured using PyMol
16 (Schrodinger, 2010).

17

18 2.10. Data analysis

19 Replicate data are presented as mean $\pm$ SEM. Graph Prism v5 was used to plot
20 kinetic data and estimate K_m and V_{max} . The kinetic data were analysed using the non-
21 linear fit function of Graph Prism and the best fit shown to be a one-site binding
22 hyperbola.

23

24 3. Results

1 3.1. *TciALDO* gene sequence

2 The 1,095 bp full length *T. circumcincta* cDNA sequence, amplified from adult *T.*
3 *circumcincta* cDNA, has been deposited in Genbank as Accession No **KX452943**. The
4 predicted protein consisted of 365 amino acids (Fig. 1). A multiple alignment, using
5 Alignment Geneious Prime, of the protein sequences of *TciALDO* with homologues
6 from *H. contortus*, *C. elegans*, *Caenorhabditis briggsae*, *Ancylostoma ceylanicum*, *C.*
7 *sinensis*, *E. granulosus*, *Necator americanus*, *S. japonicum*, *O. viverrini* and *H. glycines*
8 is shown in Fig. 1. Substrate binding sites and conserved regions in other homologues
9 were identified and shown to be completely conserved in *TciALDO*.

10 To identify the active site as well as infer both functional and structural
11 characteristics, we modelled the 3D structure of *TciALDO* using the I-TASSER server
12 (Fig. 2). The C-score of the best five models were less than -2.9, expected TM Score
13 was < 0.7 and normalized z-scores were less than 7.93. the ITASSER modelled protein
14 produced was similar to the parent molecule of with a C-score of -0.11 and a TM value
15 of 0.70 all within acceptable ranges.

16 Shown in Fig. 2 is the protein structure for *TciALDO*, superimposed best
17 structural model corresponding to the monomer of 3TU9 (Mabiala-Bassiloua et al.,
18 2011), as well as the 1,6-fructose diphosphate ligand (2FP) binding site and catalytic
19 and active site residues that fall within 4 Å of the substrate (Ala-68, Ser-75, Ser-72,
20 Glu-71, Asp-70, Lys-144, Lys-183, Arg-185, Glu-224, Lys-266, Leu-308, Gly- 310,
21 Ser-309, Tyr-339, Arg-341 and Gly-340). The lysine at position 230 is the residue
22 where Schiff base intermediates are formed.

23 3.2. Recombinant protein expression

1 A number of varying conditions were used in the trial expression and based on
2 which maximal production of functional recombinant ALDO was obtained in the *E.*
3 *coli* strain BL21 (DE3) when expression was induced with 1 mM IPTG at 30 °C for 16
4 h. The purified N-terminal His recombinant *Tci*ALDO protein appeared as a single
5 band of about 44 kDa on SDS-PAGE (Fig. 3). The presence of a His-Tagged
6 recombinant protein as well as a high purity of the recombinant protein were
7 confirmed by Western blotting.

8 3.3. Enzyme assays

9 The optimum pH for recombinant *Tci*ALDO activity at 25°C was 7.5 (Fig. 4).
10 The apparent K_m for fructose 1,6-bisphosphate was $0.24 \pm 0.01 \mu\text{M}$ and the V_{max} was
11 432 nmoles $\text{min}^{-1} \text{mg}^{-1}$ protein (mean $\pm$ SEM, n=3) (Fig. 5). The Hill Coefficient was
12 calculated to be 1.70.

13 3.4. Host recognition

14 Recombinant *Tci*ALDO was recognised in an ELISA by antibodies in both
15 serum and saliva which had been collected from sheep exposed to nematodes in the
16 field (Fig. 6). There was no antibody detection when serum or saliva samples from
17 parasite-naïve animals was used.

18 4. Discussion

19 A 1095 bp full length cDNA sequence encoding *T. circumcincta* aldolase
20 (*Tci*ALDO) was amplified from adult *T. circumcincta* cDNA, cloned and expressed in
21 *E. coli*. The 365 amino acid *Tci*ALDO protein expressed in *E. coli* was typical of

aldolase monomers of many species and had 77-94% similarity to the aldolase of other nematodes and 62-64 % similarity to that of cestodes and trematodes (Fig. 1).

The 3D-structure as well as binding and catalytic sites have been determined for a wide range of FBAs and are known to be highly conserved (Fig. 2) (Gefflaut et al., 1996; Dalby et al., 1999; Roslan et al., 2017). This was also true for other helminth homologues (Fig. 1), although there were minor differences in the trematode and cestode aldolase sequences, in which serine was replaced by threonine at amino acid 38.

The kinetic properties of the recombinant *Tci*ALDO were generally similar to those of enzymes of other species. The optimum pH for *Tci*ALDO activity at 25 °C was pH 7.5 (Fig. 4), similar to that for the aldolase of *H. contortus* (Yan et al., 2013). The enzyme was very active at 25 °C ($V_{\max}$ 432 ± 23 nmoles.min⁻¹.mg protein⁻¹), which is below its optimal temperature, assuming that it is similar to the 40-45°C of the closely related aldolases of *H. contortus* (Yan et al., 2013) and *C. sinensis* (Li et al., 2014). *Tci*ALDO activity was unchanged by the addition of 10 mM EDTA, indicating that the enzyme was class I and not class II aldolase, which is strongly inhibited by EDTA (Grazi et al., 1962; Gefflaut et al., 1996). The apparent K_m of *Tci*ALDO for the substrate fructose 1,6-bisphosphate was 0.2 ± 0.01 µM (Fig. 5). This is higher than the 0.06 µM reported for recombinant *S. japonicum* aldolase (Hu et al., 2015), but lower than the very variable values reported for the partially purified *Ascaris suum* (Kochman and Kwiatkowska, 1972) or *H. contortus* aldolases (Rhodes, 1972). Kinetic properties may be more accurately reflected by recombinant enzymes than purified proteins and suggest that parasitic helminths may have more active enzymes than their hosts (Hu et al., 2015).

Native *T. circumcincta* aldolase is antigenic and antibodies in both serum and saliva from field-immune, but not nematode-naïve, sheep recognised recombinant *Tci*ALDO in an ELISA (Fig. 6). Ovine IgA in the gastric lymph of previously-infected sheep reacted with aldolase in L3 *T. circumcincta* (Ellis et al., 2014). The antigenicity of aldolase has been exploited in detecting previous exposure of water buffaloes to *S. japonicum* (Peng et al., 2008), dogs to *D. immitis* (Sassi et al., 2014) or humans to *O. volvulus* (McCarthy et al., 2002), *A. cantonensis* (Morassutti et al., 2012) or *S. japonicum* (Peng et al., 2009). Aldolase, like many other glycolytic enzymes, has both intra- and extra-cellular activities in pathogens in addition to its enzymatic function (Gómez-Arreaza et al., 2014), which have been suggested to facilitate their establishment in the host. These appear to be essential to successful establishment of many pathogens, including helminths. This is supported by the protection against infection induced by vaccination of mice with the aldolase of *S. mansoni* (Marques et al., 2008) or fish with the aldolase of several pathogenic bacteria (Sun et al., 2015).

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Figure Legends

Fig. 1. Multiple sequence alignment of aldolases from *T. circumcincta* (GI: **KX452943**), *H. contortus* (GI: **ADT61995**), *Caenorhabditis elegans* (GI: **CAB03291**), *Caenorhabditis elegans* aldolase 2 (GI: **CCD65997**), *Caenorhabditis briggsae* (GI: **XP002643138**), *Ancylostoma ceylanicum* (GI: **EPB73313**), *Clonorchis sinensis* (GI: **GAA50927**), *Echinococcus granulosus* (GI: **EUB64508**), *Necator americanus* (GI: **XP013291330**), *Schistosoma japonicum* (GI: **CAX78614**), *Opisthorchis viverrini* (GI: **OON18662**) and *Heterodera glycines* (GI: **AAG47838**), homologues. Amino acid residues indicated in the marked box are essential to the aldolase activity.

Fig. 2. (A) The predicted tertiary structure of *Tci*ALDO monomer (B) Superposition of the predicted tertiary structure of *Tci*ALDO from *T. circumcincta* (orange) and 3TU9 (purple). 3TU9 is the repository code for the coordinates of rabbit muscle aldolase stored at the protein data bank (<https://www.rcsb.org/structure/3TU9>), and was the template for our model (C) Location of the active site within *Tci*ALDO. (D) The active site of *Tci*ALDO (in green) within 4Å of the superimposed 2FP (in blue). 2FP is abbreviation for “1,6-fructose diphosphate” is also stored at the protein data bank (<https://www.rcsb.org/ligand/2FP>), and was the ligand modelled for our structure.

Fig. 3. SDS-PAGE of recombinant *Tci*ALDO. Lane1: standards; lane 2: unbound; lane 3: wash 1; lane 4: wash 2; lane 5: elution 1; lane 6: elution 2; lane 7: standards.

Fig. 4. Effects of pH on the activity of recombinant *Tci*ALDO at 25 °C (mean ± SEM, *n* = 3). Enzyme activity was estimated from the rate of NADH production, which was measured colorimetrically at 450 nm.

1 **Fig. 5.** Effects of varying substrate concentration on the activity of recombinant
 2 *Tci*ALDO at 25 °C (mean ± SEM, $n = 3$). Enzyme activity was estimated from the rate
 3 of NADH production, which was measured colorimetrically at 450 nm.

4 **Fig. 6.** Recognition of *Tci*ALDO by serially diluted serum (IgG) (top) or saliva (IgA)
 5 (bottom) (●) from parasite exposed animals, but not by serum or saliva (■) from
 6 parasite-naïve animals.

	1	60
<i>Teladorsagia circumcincta</i> aldolase	---MASYSQPPPKEKEDELRGIANAIIVAPGKGILAA DESTG SMDKKMKGIGTENTEEQR	
<i>Haemonchus contortus</i> aldolase	---MASHSQYLTKQEDELRGIANAIIVAPGKGILAA DESTG SMDKKMQNIGTENTEEQR	
<i>Caenorhabditis elegans</i> aldolase 1	---MASYSQFLTKAQEDELRSIANAIIVTPGKGILAA DESTG SMDKRLNSIGLENTEENR	
<i>Caenorhabditis elegans</i> aldolase 2	MATVGGAFKDSLTAQKDELHQIALKIVQDGKGILAA DESTG TIGKRLDAINLENNETNR	
<i>Caenorhabditis briggsae</i> aldolase	---MASYSQYLTKAQEDELRSIANAIIVAPGKGILAA DESTG SMEKRLTSIGLENNEENR	
<i>Drosophila melanogaster</i> aldolase	MTTYFNYPSKELQDELREIAQKIVAPGKGILAA DES GPTMGKRLQDIGVENTEDNR	
<i>Ancylostoma ceylanicum</i> aldolase	---MASYSQFLPKKEKEDELRGIANAIIVSAGKGILAA DESTG SMEKRLKSIGLENTEENR	
<i>Clonorchis sinensis</i> aldolase	----MSLPKYLQPEQEARLKAIANQIVQPGKGILAA DESTG TIGNRFKKIGVENSEENR	
<i>Echinococcus granulosus</i> aldolase	---MARFVPYLCAEKMKELRENASAIIVAPGKGLLA DEST STIGKRFAAINLENTEENR	
<i>Necator americanus</i> aldolase	---MASYSQYLTKKEKEDELRGIANAIIVASGKGILAA DESTG SMEKRLKSIGLENTEENR	
<i>Schistosoma japonicum</i> aldolase	---MPRFPPYLTEAQEDDLRQIAQAICAPGKGILAA DEST ATMGKRLQQIGVENNEENR	
<i>Opisthorchis viverrini</i> aldolase	MPAVMPRFTEYLPPEGKLELRDIANAIVAPGKGILAA DES VPTLGKRLKAINVENSEENR	
<i>Heterodera glycines</i> aldolase	MAEVGNSYRPLSEEKQNELRAIAQKILQPGKGILAA DESTG SIGKRFDITIGLENTENNR	
		120
<i>Teladorsagia circumcincta</i> aldolase	RKYRQLLFTASPEMSKHISGVIMFHETFYQKCDDGTRFVDALKKQGIIPGI K VDKGVVPM	
<i>Haemonchus contortus</i> aldolase	RKYRQLLFTASPEMSKHISGVIMFHETFYQKTDDGTRFVDVLKKQGVIPGI K VDKGVVPM	
<i>Caenorhabditis elegans</i> aldolase 1	RKYRQLLFTAGADLNKYISGVIMFHETFYQKTDDGKPFALLQEQQIIPGI K VDKGVVPM	
<i>Caenorhabditis elegans</i> aldolase 2	QKYRQLLFTT-PNLNQHISGVILYEETFHQSTDKGEKFTDLLIKQGIIVPGI K LDLGVVPL	
<i>Caenorhabditis briggsae</i> aldolase	RKYRQLLFTAGADLNKYISGVIMFHETFYQKTDDGKPFALLQEQQIIPGI K VDKGVVPL	
<i>Drosophila melanogaster</i> aldolase	RAYRQLLFSTDPKLAENISGVILFHETLYQKADDGTPFAEILKKKGIIILGI K VDKGVVPL	
<i>Ancylostoma ceylanicum</i> aldolase	RKYRQLLFTGNPDLGKHISGVIMFHETFYQKADDGTRFVDALKKQGIIPGI K VDKGVVPM	
<i>Clonorchis sinensis</i> aldolase	RHYRQLLFTTDPCLANSISGVILFHETFYQVADDGVRLVDHLKRKGILPGI K LDKGVVPL	
<i>Echinococcus granulosus</i> aldolase	RAYRELLFTTDPEFAKHISGVILFHETVYQKTKDGKPFVELLRERGVVPGI K VLDLGVVPL	
<i>Necator americanus</i> aldolase	RKYRQLLFTGNPDLGKHISGVIMFHETFYQKADDGTRFVDVLKKQGIIPGI K VDKGVVPM	
<i>Schistosoma japonicum</i> aldolase	RLYRQLLFSADHKLAQNISGVILFEETLHQKSDDGKTLPTLLAERHIIIPGI K VDKGVVPL	
<i>Opisthorchis viverrini</i> aldolase	RAYRQLLFSADPVLAKNISGVILYHETLYQKTDDGMPLVRLQLDRGIIIPGI K VDKGVVSL	
<i>Heterodera glycines</i> aldolase	RRYRQLLFTT-PSFGQNLISGVILFDETFRQSTDNGVRFVDVIKKAGAVAGI K VDTGVVPL	

180

<i>Teladorsagia circumcincta</i> aldolase	AGTVGEGTTQGMDDLNARCAQYKKDGAQFAK WRC VHKISATTPSHMALVEIAEVLARYAS
<i>Haemonchus contortus</i> aldolase	AGTVGEGTTQGMDDLNARCAQYKKDGAQFAK WRC VHKISATTPSHMALVEIAEVLARYAS
<i>Caenorhabditis elegans</i> aldolase 1	AGTIGEGTTQGLDDLNARCAQYKKDGAQFAK WRC VHKISSTTPSVTALKEIASNLARYAS
<i>Caenorhabditis elegans</i> aldolase 2	AGTIGEGTTQGLDKLAERAAAFKKGCGFAK WRC VLNIGTHTPSHLGMLLENANVLARYAS
<i>Caenorhabditis briggsae</i> aldolase	AGTIGEGTTQGLDDLNARCAQYKKDGAQFAK WRC VHKISSTTPSVTALKEVAQVLARYAS
<i>Drosophila melanogaster</i> aldolase	FGSEDEVTTQGLDDLAARCAQYKKDGCDFAK WRC VLKIGKNTPSYQSILENANVLARYAS
<i>Ancylostoma ceylanicum</i> aldolase	AGTVGEATTQGLDDLNRCAQYKKDGAQFAK WRC VHKISTTPSHTALVEIAQVLARYAS
<i>Clonorchis sinensis</i> aldolase	AGTLNECTTQGLDGLAERCAQYRKDGAEFAK WRC VLKITSHTPSYLAMMENANVLARYAS
<i>Echinococcus granulosus</i> aldolase	GGTADECTTQGLDNLAQRCAYYKDGCRFAK WRC VLKISSHNPSYLAMLENANVLARYAS
<i>Necator americanus</i> aldolase	AGTVGEATTQGLDDLNRCAQYKKDGAQFAK WRC VHKISVTTPSHTALVEIAQVLARYAS
<i>Schistosoma japonicum</i> aldolase	AGTDNETTTQGLDDLASRCAYRKLGCRFAK WRC VLKISSHTPSYQAMLENANVLARYAS
<i>Opisthorchis viverrini</i> aldolase	PGTPEECTTQGLDGLSDRCAQYKKDGCDFAK WRC VLKISDRTPSYLAMKENANVLARYAV
<i>Heterodera glycines</i> aldolase	AGTLDEGTTQGLDKLSDRCAEYKKGCGDFAK WRC VLNIGAHKPSHLALMENANVLARYAS

240

<i>Teladorsagia circumcincta</i> aldolase	ICQQNGLVPIVE PE ILPDGEHDIDRCRKITETVLSYCYRALNDHHVYLEGTLL KPNM VTA
<i>Haemonchus contortus</i> aldolase	ICQQNGLVPIVE PE ILPDGEHDIHRCQKITETVLSYCYRALNDHHVYLEGTLL KPNM VTA
<i>Caenorhabditis elegans</i> aldolase 1	ICQQNGLVPIVE PE ILPDGEHCLARGQKITETVLSYVYHALNEHHVYLEGTLL KPNM VTS
<i>Caenorhabditis elegans</i> aldolase 2	ICQANGLVPIVE PE EVLCDEGEHDLARAQKVTEQVLAFFVYKALADHHVYLEGTLL KPNM VTP
<i>Caenorhabditis briggsae</i> aldolase	ICQQNGLVPIVE PE ILPDGEHDLARGQKITETVLSYVYHALNEHHVYLEGTLL KPNM VTS
<i>Drosophila melanogaster</i> aldolase	ICQSQRIVPIVE PE EVLPDGDHDLARAQKVTEQVLAFFVYKALADHHVYLEGTLL KPNM VTA
<i>Ancylostoma ceylanicum</i> aldolase	ICQQNGLVPIVE PE ILPDGEHDIARCQKITETVLSYCYRALNDHHVYLEGTLL KPNM VTA
<i>Clonorchis sinensis</i> aldolase	ICQQNGLVPIVE PE EVLPDGDHDLATAQKVTEHVLSTYKALMDHHVYLEGTLL KPNM VTA
<i>Echinococcus granulosus</i> aldolase	ICQQNGLVPIVE PE EVLPDGDHDLATAQKVTEQVLSFVYKALADHHVYLEGTLL KPNM VTC
<i>Necator americanus</i> aldolase	ICQQNGLVPIVE PE ILPDGEHDIARCQKITETVLSYCYRALNDHHVYLEGTLL KPNM VTA
<i>Schistosoma japonicum</i> aldolase	ICQQNGLVPIVE PE EVLPDGDHDLATAQKVTEEVLSFVYKALADHHVYLEGTLL KPNM VTA
<i>Opisthorchis viverrini</i> aldolase	ICQQNGIVPIVE PE EVLDQGNHDLATAQKVTEEVLSFVYKALADHHVYLEGTLL KPNM VTA
<i>Heterodera glycines</i> aldolase	ICQQNGLVPIVE PE EVLCDEGHSLERCQKVTELTAYTYKALADHHVYLEGTLL KPNM VTP

300

<i>Teladorsagia circumcincta</i> aldolase	GQAFKGGKKPSHDEIALATITALQRSVPAAVPGVVFL LSGG QSEEDATLNLNAMNKLDTKKP
<i>Haemonchus contortus</i> aldolase	GQAFKGTKPSHDEIALATVTALQRAVPAAVPGVVFL LSGG QSEEDATLNLNAMNKLQTKKP
<i>Caenorhabditis elegans</i> aldolase 1	GQSFTGEKPSNADIGLATVTALQRGVPSAVPGVVFL LSGG QSEEDATLNLNAINQVSGKKP
<i>Caenorhabditis elegans</i> aldolase 2	GQS-SASKASHEAIGLATVTALRRGVPAAVPGITFL LSGG QSELDATANLNAINSVQLGKP
<i>Caenorhabditis briggsae</i> aldolase	GQSFTGEKPSNADIGLATVTALQRGVPSAVPGVVFL LSGG QSEEDATKNLNAINQVTGKKP
<i>Drosophila melanogaster</i> aldolase	GQS--AKNTPEEIALATVQALRRTPAAVTGVTFL LSGG QSEEEATVNLNAINNVPLIRP
<i>Ancylostoma ceylanicum</i> aldolase	GQGFKGKKPSPDDVGKATVTALQRSVPSAVPGVV-----AGRRP
<i>Clonorchis sinensis</i> aldolase	GQSCT-QRYTPEEIGLATVTALQRTVPVSVPGITFL LSGG QSEEEAAILNSAINCVEGHKP
<i>Echinococcus granulosus</i> aldolase	GQSCT-KKYSVEDNARATVEALQRTVPVAVPGIVFL LSGG QSELDATRNLNAINKYPGKKP
<i>Necator americanus</i> aldolase	GQGFKGKPKSNDVGLATVTALQRSVPAAVPGVVFL LSGG QSEEEATLNLNAMNKVAGKRP
<i>Schistosoma japonicum</i> aldolase	GQACK-KAYTPQENALATVRALQRTVPPAVPGITFL LSGG QSELDATKNLNEINKIPGPKP
<i>Opisthorchis viverrini</i> aldolase	GQSHS-HKYSVEQNAVATVQALQRTVPPAVPGIVFL LSGG MSEVEATQNLNAINAAPGRKP
<i>Heterodera glycines</i> aldolase	GQSHS-PKATPDQIGLATVTALRRSVPIAVPGIVFL LSGG QSEIEATRNLNAINSVPGPKP

360

<i>Teladorsagia circumcincta</i> aldolase	WALTF SYGRAL QASCMKSWGGKDENVKDAQAVFMQRAQANSALALGKYSGDPNADKAASQ
<i>Haemonchus contortus</i> aldolase	WALTF SYGRAL QASAMAKWSGKDENVPAKAVFMQRAQANSALALGKYSGDPNADKAASQ
<i>Caenorhabditis elegans</i> aldolase 1	WALTF SYGRAL QASCLAKWAGKDENIAAAQEVLLHRAQVNSLASVGKYTGDAADAAASQ
<i>Caenorhabditis elegans</i> aldolase 2	WKLTF SYGRAL QASVLKAWGGKDENIAAAQKTLHRSKANGDASLGKYAGEDAA-GAAAE
<i>Caenorhabditis briggsae</i> aldolase	WALTF SYGRAL QASCLAKWAGKDENIAAAQEVLLHRAQVNSLASVGKYTGDAADAAASQ
<i>Drosophila melanogaster</i> aldolase	WALTF SYGRAL QASVLRWAGKKENIAAGQNELLKRAKANGDAAQGGKYVA--GSAGAGSG
<i>Ancylostoma ceylanicum</i> aldolase	WVLT SYGRAL QASTLAKWAGKDENIKAAQAILLKRAQANSALASVGKYTGDPNADAAASK
<i>Clonorchis sinensis</i> aldolase	WRLTF SYGRAL QASVLAAWQGKPENVHKGQEELLKRAKVCGLASMGKYKGGQTG-AAGDQ
<i>Echinococcus granulosus</i> aldolase	WALS FSGRAL QASAIAAWQGKPENVKVGQAEFLQLAKANGAASLGKFEGELKT-AAGQK
<i>Necator americanus</i> aldolase	WVLT SYGRAL QASCLAKWGGKDENIKAAQAVLLHRAQNSALALGKYTGDPNADAAASK
<i>Schistosoma japonicum</i> aldolase	WALTF FSGRAL QASVLAATWQKKENVHAAQEELLKLAKANGAAAVGKFEGDMGT-ALGDK
<i>Opisthorchis viverrini</i> aldolase	WALTF FSGRAL QASVIQTWDGKKANVVSAAQQELLKLAQANGAAALGKYEGNLQT-AAGGQ
<i>Heterodera glycines</i> aldolase	WTL FSGRAL QSSVIKKWNGKDENLGDAQKVLHRAKANGDAQQGGKYTGEEAA-ASAAE

	Identity (%)
<i>Teladorsagia circumcincta</i> aldolase	SLFVANHAY
<i>Haemonchus contortus</i> aldolase	SLFVANHAY 94
<i>Caenorhabditis elegans</i> aldolase 1	SLFVANHSY 78
<i>Caenorhabditis elegans</i> aldolase 2	SLFVAKHSY 61
<i>Caenorhabditis briggsae</i> aldolase	SLFVANHAY 77
<i>Drosophila melanogaster</i> aldolase	SLFVANHAY 79
<i>Ancylostoma ceylanicum</i> aldolase	SLFVANHAY 65
<i>Clonorchis sinensis</i> aldolase	TLFVPSHAY 62
<i>Echinococcus granulosus</i> aldolase	SLFVANHAY 64
<i>Necator americanus</i> aldolase	SLFVANHAY 86
<i>Schistosoma japonicum</i> aldolase	SLFVANHAY 62
<i>Opisthorchis viverrini</i> aldolase	SLFSANHAY 63
<i>Heterodera glycines</i> aldolase	SLFVAKHAY 63

D