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Research Article

Protein profile of the cysts of *Taenia hydatigena*, *Taenia saginata*, *Echinococcus granulosus* and *Taenia ovis*

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Abstract. An accurate species identification in *Taenia* infestations is essential to understand the epidemiology and transmission dynamics of different *Taenia* species and points the way for effective prevention and control of taeniid species as well as development of vaccines and diagnostic tools. Antigenic and morphologic similarities between different cestodes do exist. These similarities could be the cause of overestimated seroprevalence of many of these cestodes. The proteins of the cysts of *Taenia hydatigena*, *Taenia saginata*, *Echinococcus granulosus* and *Taenia ovis* were extracted and separated with SDS-PAGE. Because the cysts of *T. hydatigena* were used as a model organism for other cestodes, the proteins of different compartments of the cysts were also extracted separately. Results showed similarities between the extracted proteins from different cestodes with *T. hydatigena*, so serological tests based on *T. hydatigena* cysts' antigens might serve as a diagnostic tool to distinguish between these important parasites.

Key Words: Protein profile, *Taenia hydatigena*, *Taenia saginata*, *Echinococcus granulosus*, *Taenia ovis*

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Introduction

Members of the family Taeniidae are the most important cyclophyllidean tapeworms. Different species of the genera *Echinococcus* and *Taenia* are responsible for major medical and economic losses in humans and animals (Budke et al 2006; Rostami et al 2013). In addition, one of the most prevalent *Taenia* species in livestock is the bladder worm *T. hydatigena* (Rostami et al 2013). The metacestode of *T. hydatigena* is known as thin-necked bladder worm or *Cysticercus tenuicollis*, matures over a period of five to eight weeks to become infectious for the definitive host. *C. tenuicollis* prevalence ranges from 2-79% in sheep, 5-55% in goats, 0-27% in cattle and 7% in pigs (Braae et al 2015; Oryan et al 2012; Oğuz & Değer 2013; Scala et al 2015; Sissay et al 2008).

Accurate serological diagnosis of cestodes' infestations is problematic due to the serological cross-reactivity with several other taeniid cestodes (Yong & Heath 1979; Yong et al 1984; Lightowlers & Gottstein 1995). Due to this serological cross reactivity, the development of practical screening tests for detecting different cestodes' infestations requires intensive study of the different antigens of these cestodes.

As many morphologic and antigenic similarities between different cestodes exist (Miquel et al 2015), *T. hydatigena* was used as a model organism for *T. saginata*, *E. granulosus* and other cestodes (Buttar et al 2013; Mcmanus 2014). Crude protein of *T. hydatigena* cysts' fluid was used as ELISA antigen for the detection of antibodies against *T. saginata* cysticercosis (Bøgh et al 1995; Kamanga-Sollo et al 1987; Rhoads et al 1991), *E. granulosus* hydatidosis (Carmena et al 2006), and *T. solium* cysticercosis (Kara et al 2003). Moreover, immune-mediated cross-protection in the intermediate host has been documented for the infestations with *T. ovis*, *T. saginata*, *T. solium* and *E. granulosus*, whose metacestode establishment can be inhibited by pre-exposure to *T. hydatigena* (Conlan et al 2012; Roberts et al 1987), although cross-protection is always less than that conferred by the homologous species (Flisser et al 1979). In addition, *T. ovis* provides a model system for the development of live vectors for delivery of anti-parasite vaccines to domestic animals (Lightowlers & Rickard 1993). The success with the *T. ovis* recombinant vaccine has shown the potential for developing practical vaccines to assist in control of the important zoonotic species of taeniid cestodes (Rickard 1991).

The present study aimed to clarify and compare the different extracted and separated proteins of the cysts of *T. hydatigena* (as a whole cyst and from different compartments of the cysts), *T. saginata*, E. granulosus and *T. ovis*.

Materials and methods

Sample collection

T. hydatigena cysts (n=10) were collected from sheep slaughtered in an abattoir at the northern part of Palestine. Cysts were sent to the laboratories of An-Najah National University,

Palestine, for DNA isolation and subsequent species confirmation as described by Adwan et al 2013 and Jayousi 2014. *T. saginata* cysts (n=10) were collected from cattle slaughtered at an abattoir in northern Germany. The cysts were collected during routine meat inspection procedures. Each sample was kept in a special container, refrigerated at 4°C, and transferred within 24 h to the laboratories of the Veterinary Institute, Hanover, Lower Saxony State Office for Consumer Protection and Food Safety, for further examination. The cysts were examined macroscopically and classified accordingly as viable or degenerated (Abuseir et al 2006; Minozzo et al 2002). Crude antigen preparation and protein extraction were performed from mature *T. saginata* cysts only. The cysts' DNA was isolated and subsequent species confirmation was done with PCR using two sets of primers as described by Gonzales et al 2000, 2002a,b and Abuseir et al 2006. *E. granulosus* cysts (n=10) and *T. ovis* cysts (n=10) were collected from sheep slaughtered in an abattoir at the northern part of Palestine. Hydatid fluid was aspirated from the *E. granulosus* cysts and examined for the presence of protoscolices. Sterile cysts were excluded from subsequent protein extraction. *E. granulosus* cysts were sent to the laboratories of An-Najah National University, Palestine, for DNA isolation and subsequent species confirmation described by Adwan et al 2013. Also crude protein extraction was done for the viable cysts of *T. ovis* only.

Extraction of cyst proteins

Crude protein extraction was performed from *T. saginata* cysts, *E. granulosus* cysts, *T. ovis* cysts, and from whole *T. hydatigena* cysts as well as its different cysts' compartments (outer membrane, outer fluid, inner membrane including the scolex and inner fluid) as shown in Fig. 1.

Whole cysts of different examined cestodes and membrane compartments of *T. hydatigena* were chopped with a scissor and then homogenized at 4 °C in 1/5 wt/vol PBS (pH 7.4) containing protease inhibitors (10 mM PMSF and 2.5 mM Leupeptin). First, the chopped cysts and membranes were disrupted in a tissue lyser (TissueLyser, Qiagen) with three stainless steel beads (shaking speed: 30/s for 3 min). Further homogenization was done with a Bandelin Sonophus ultrasound HD 2070 at 40 % power for 1 min. Afterwards the samples were stirred at 4 °C for 2 h and then centrifuged at 15,000 × g for 1h. The soluble supernatant was collected and protein concentration was determined by Bradford assay (Bradford 1976). *T. hydatigena* cysts' fluids were processed directly without any homogenization.

SDS-PAGE was performed on 6, 8, 10, 12, and 15 percentage gels. 30-40 µg of whole cyst or cyst compartment protein was loaded. Prestained protein standards used were from Fermentas Life Science (PageRuler™ Plus Prestained Protein Ladder) and Roth (Roti®-Mark 10-150 Plus).

Results

The protein bands of *T. hydatigena* whole cysts, *T. saginata*, *E. granulosus* and *T. ovis* cysts are shown in Fig. 2 and summarized in Table 1.

T. hydatigena cysts:

The protein bands of *T. hydatigena* cysts were twelve visible protein bands ranging from 290 to 12 KDa. These were as follows: 290, 270, 260, 150, 130, 80, 67, 55, 35, 23, 14 and 12 kDa.

T. saginata cysts:

The SDS-PAGE profile followed by Coomassie Blue staining of *T. saginata* cyst antigens showed ten visible protein bands ranging from 260 to 14 kDa.

These bands were approximately 260, 150, 130, 67 (a major band), 60, 55, 50, 23, 18, and 14 kDa.

E. granulosus:

The visible protein bands of *E. granulosus* resulting from cyst material were eleven visible protein bands ranging from 260 to 23 kDa and were as follows: 260, 250, 150, 130, 120, 80, 67, 60, 55, 35, and 23 kDa.

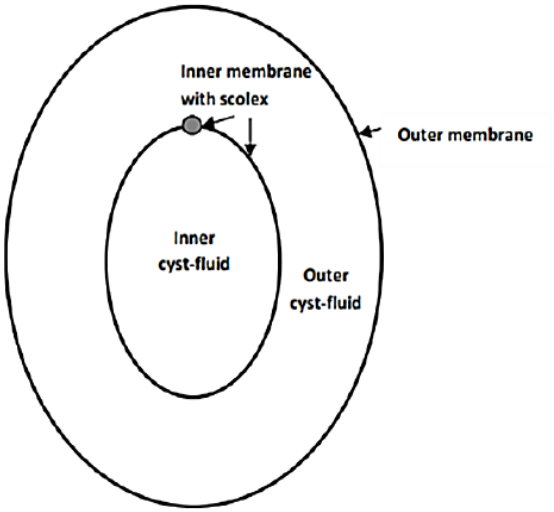

Fig. 1. Scheme of *T. hydatigena* cysts' compartments.

Table 1. Summary of the protein bands of different cestodes separated with SDS-PAGE

T. hydatigena-**cysts**	*T. saginata*-**cysts**	*E. granulosus*-**cysts**	*T. ovis*-**cysts**
290	------	------	------
270	------	------	------
260	260	260	------
------	------	250	------
150	150	150	------
130	130	130	------
------	------	120	------
80	------	80	------
67	67	67	67
------	60	60	60
55	55	55	55
------	50	------	50
35	------	35	35
23	23	23	23
------	18	------	18
14	14	------	14
12	------	------	------

T. ovis

The SDS-PAGE profile followed by Coomassie Blue staining of *T. ovis* cyst antigens showed eight visible protein bands ranging from 67 to 14 kDa. These bands were approximately 67 (a major band), 60, 55, 50, 35, 23, 18, and 14 kDa.

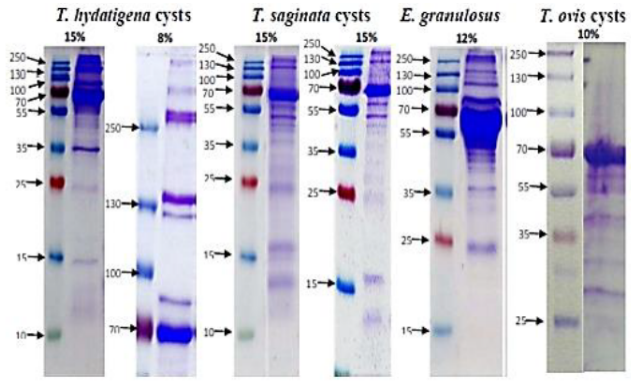

Fig. 2. Protein profile of different cestodes separated with 8-15% SDS-PAGE.

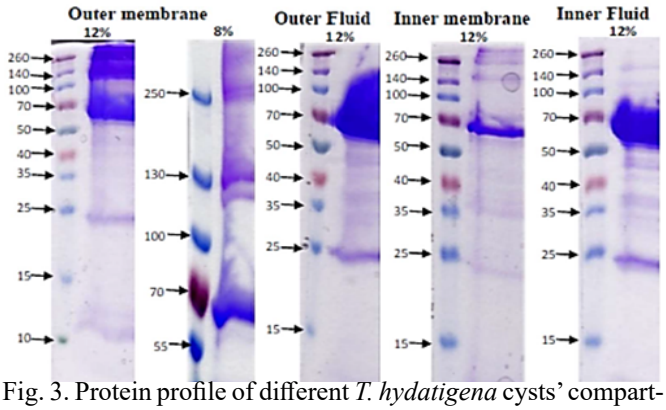

Fig. 3. Protein profile of different *T. hydatigena* cysts' compartments separated with 8-12% SDS-PAGE.

Table 2. Summary of the protein bands of *T. hydatigena* cysts' compartments separated with SDS-PAGE.

Outer membrane	Outer fluid	Inner membrane with the scolex	Inner fluid
290	------	------	------
270	------	------	------
260	------	260	------
150	------	150	150
130	------	130	130
80	------	------	------
67	67	67	67
55	55	55	55
------	35	35	35
23	23	23	23
14	------	------	------
12	------	------	------

The protein bands of the different compartments of *T. hydatigena* cysts are shown in Fig. 3 and summarized in Table 2.

The protein bands of *T. hydatigena* cysts' outer membrane ranged from 290 to 12 kDa. These were as follows: 290, 270, 260, 150, 130, 80, 67, 55, 23, 14 and 12kDa.

The protein bands of *T. hydatigena* cysts' outer fluid ranged from 67 to 23kDa. These were as follows: 67 (a major band), 55, 35, and 23kDa.

The protein bands of *T. hydatigena* cysts' inner membrane, including the Scolex, ranged from 260 to 23KDa. These were as follows: 260, 150, 130, 67, 55, 35 and 23kDa.

The protein bands of *T. hydatigena* cysts' inner fluid ranged from 150 to 23kDa. These were as follows: 150, 130, 67 (major band), 55, 35 and 23kDa.

Discussion

Cross-reactivity is a widely spread trait among phylogenetically related and unrelated parasites. It emerges from the wide existence of common antigens, which suggest that antigenic continuity is the rule rather than the exception, as the origin of these parasites is similar at the beginning of creation (El-Moghazy & Abdel-Rahman 2012).

This study clarified the similarities between the extracted proteins of different cestodes that might be helpful in serodiagnosis of such helminthes, as the antigenic similarities among cestodes often prevent accurate serological diagnosis in livestock due to serological cross-reactivity with other *Taenia* or *Echinococcus* species (Conlan et al 2012; Dorny et al 2004; Deckers & Dorny 2010; Mcmanus 2014). Many similar antigens between these cestodes were immunogenic with Immunoblot (Abuseir et al 2013). In addition, accurate determination of the antigens in these helminthes will surely improve the diagnostic techniques, which is the first step to control it. The host-parasite interaction during the course of the infection should also be studied.

Regardless of the disadvantages of these similarities in precise immunodiagnosis, wide similarities in the antigenic structure among parasites often allow the use of a diagnostic antigen from one species potentially to protect from another, as the use of a cross reactive antigen might be helpful in some cases to protect against multiple infestations (El-Moghazy & Abdel-Rahman 2012).

Some protein-bands in the tested cestodes appeared in huge quantities and appeared very clear but others with very low quantities appeared faint. Notably, the 67-, 55-, and 23-kDa proteins were present in all tested cestode-species and in all compartments of *T. hydatigena* cysts. These results when compared with other similar studies (Indira et al 2008; Arunkumar et al 2014) were similar in some aspects and different in others. This can be attributed to the fact that very high molecular weight protein complexes in some cestodes may dissociate under reducing conditions to smaller size, two or more subunits (Mcmanus 2014). This may also explain the presence of some smaller proteins in some samples appearing in SDS-PAGE, while with other samples these small proteins were absent or very faint. In addition, different locations of specific proteins are involved in the development of some cestodes' infestations in the animal body (Miquel et al 2015).

These findings might be helpful for future work on development of a recombinant vaccine, but it should also be tested intensively with Western Blot and other tests in terms of sensitivity and specificity.

It should also be emphasized that the future application of taeniid cestode vaccines will be limited unless the mechanism by which the vaccine is administered is made simpler and cheaper. High levels of immunity against a challenge infection with taeniid cestode eggs can be stimulated by immunization with extracts of the parasites, particularly with extracts of the oncosphere life-cycle stage (Lightowlers & Rickard 1993). A major obstacle in developing commercial vaccines against cestodes has been the difficulty in obtaining adequate supplies of these antigens (Rickard 1991). So similar proteins from different

tested cestodes extracted from one available cestode, could offer a feasible way of developing commercial vaccines against many of them.

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