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جامعـــة النجـــاح
الوطنيـــة
كلية الزراعة والطب البيطري
دائرة الطب البيطري

17.01.2017

Professor Lucy Robertson
Chair of the COST Action
FA1408, (A European Network for Foodborne Parasites (Euro-FBP))

Subject: Summarized Scientific Report of a Short Term Scientific Mission (STSM).

Dear Professor Robertson,

Referring to the approved STSM (Reference code: COST-STSM-ECOST-STSM-FA1408-181216-081487) from the period between 18.12.2016 to 10.01.2017 at the Institute for Parasitology at the University of Veterinary medicine, Hannover, Germany.

STSM Topic: Differential serodiagnosis of *Taenia saginata*, *Echinococcus granulosus*, *Taenia hydatigena*, and *Taenia ovis* infestations in the intermediate host

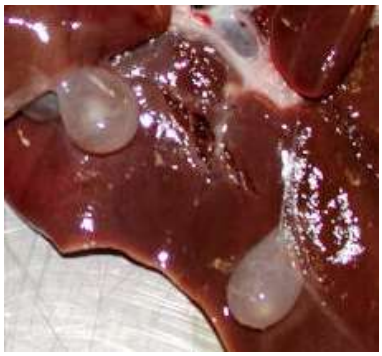
The purpose of the STSM was to compare the extracted and separated proteins of different cestodes against different known positive sera with Western Blot.

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. One of the most prevalent *Taenia* species in livestock is the bladder worm *Taenia hydatigena*.

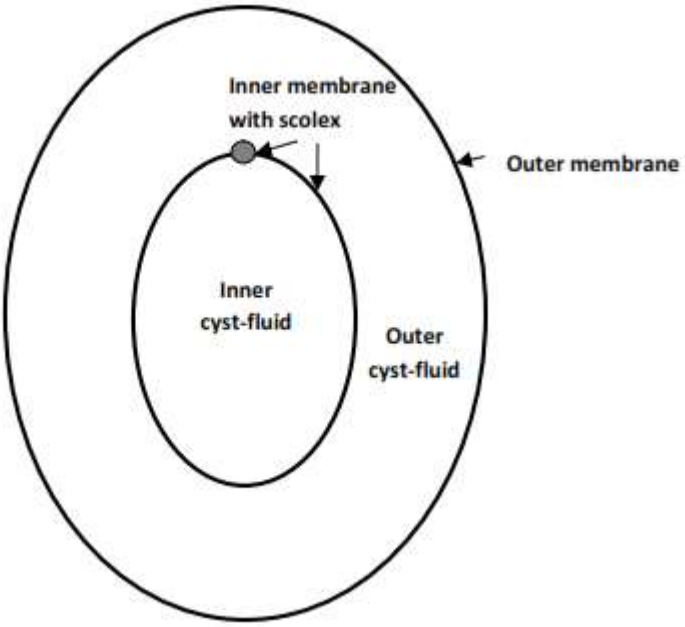
As many morphologic and antigenic similarities between different cestodes exist, *T. hydatigena* was used as a model organism for *T. saginata*, *E. granulosus* and other cestodes.

For example, *T. hydatigena* cysts (*C. tenuicollis*) were used as ELISA antigen for the detection of antibodies against *T. saginata* cysticercosis. Moreover, immune-mediated cross-protection in the intermediate host has been documented for the ovine infections with *T. ovis*, *T. saginata*, *T. solium* and *E. granulosus*, whose metacestode establishment can be inhibited by pre-exposure to *T. hydatigena*, although cross-protection was always less than that conferred by the homologous species. On the other hand, antigenic similarity among cestodes often prevents accurate serological diagnosis in livestock due to serological cross-reactivity with other *Taenia* or *Echinococcus* species. The frequent existence of multiple infections with different taeniid species, antigenic cross-reactivity and the low level of specific antibodies restrict improvement of diagnosis tools to differentiate between cestode-infections in natural intermediate hosts. The present study aimed to develop a serological tool to differentiate between *T. hydatigena*, *T. saginata*, *E. granulosus* and *T. ovis* infections in livestock.



Extraction of cyst proteins

Proteins were extracted from *T. saginata*, *E. granulosus*, *T. hydatigena*, and *T. ovis* cysts. The proteins were also extracted separately from different compartments of *T. hydatigena* cysts (outer membrane, outer fluid, inner membrane and inner fluid) as shown below. All these extracted proteins were then separated using SDS-PAGE and were tested with Western Blot against known positive sera and meat juice (when the sera were not available) from naturally and experimentally infested animals with the similar cysts of these cestodes.



Whole cysts and membrane compartments 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 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 1 h. The soluble supernatant was collected and protein concentration was determined by Bradford assay (1976).

SDS-PAGE and immunoblot

SDS-PAGE and immunoblot were performed as described by Abuseir et al. (2013). In short, 30-40 µg of whole cyst or cyst compartment protein was loaded on 6, 8, 10, 12, and 15 % gels. Prestained protein standards were from Fermentas Life Science (PageRuler™ Plus Prestained Protein Ladder) and Roth (Roti®-Mark 10-150 Plus). After separation, proteins were transferred to a nitrocellulose membrane using the horizontal semi-dry technique.

T. hydatigena, *T. saginata* and *E. granulosus* positive sera were diluted 1:50 and meat juice 1:20 in TBS-Tween 0.05 %. Antigen detection was visualized with a monoclonal alkaline phosphatase conjugated anti-bovine or anti-sheep IgG antibody (Sigma) and BCIP/NBT as substrate.

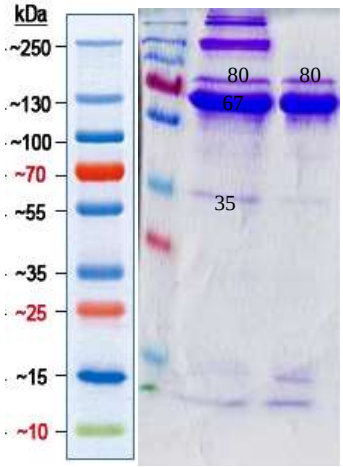
Results of SDS-PAGE

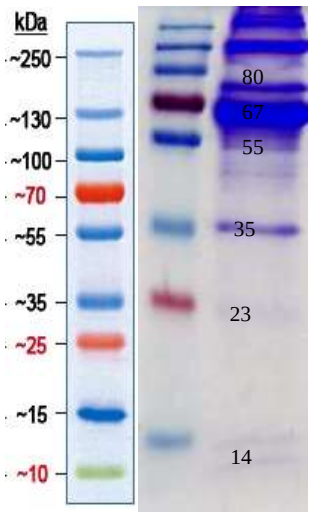
The protein bands of *T. hydatigena* whole cysts and cyst compartments ranged from 290 to 12 kDa. Most protein bands (n =12) appeared from whole cyst protein and the least (n = 4) from outer fluid.

SDS-PAGE of *T. hydatigena* cysts:

Crude protein of cysts showed 12 visible protein bands, ranging from 290 kDa to14 kDa:

290 kDa
270 kDa
260 kDa
150 kDa
130 kDa
80 kDa: very clear.
67 kDa: major band
55 KDa
35 kDa: very clear.
23 kDa
14 kDa
12 kDa





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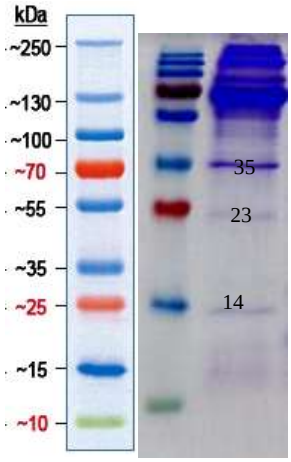

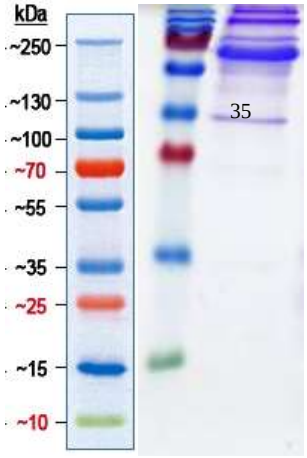

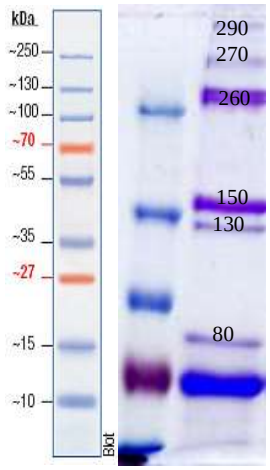

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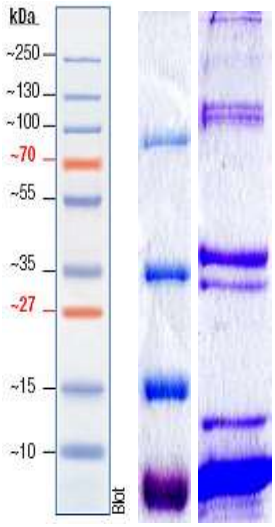


Summary of the protein-bands separated in SDS-PAGE:

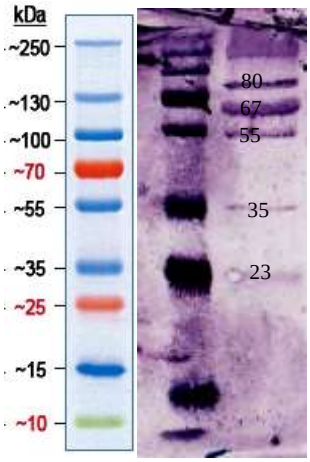
***T. saginata* cysts**	***E. granulosus***	***T. hydatigena* cysts**
		290
		270
260		
	250	
150		
130		
	120	
	80	
67		
60		
55		
50		
	35	
23		
18		
14		14

Results of Immunoblot

The results obtained showed many similar proteins and cross-antigenic reaction between the proteins isolated from the above-mentioned cestodes.

***T. hydatigena*-cysts' proteins on nitrocellulose paper**
Against positive *T. saginata* Serum:

270
260
150
130
80
67
55
35
23



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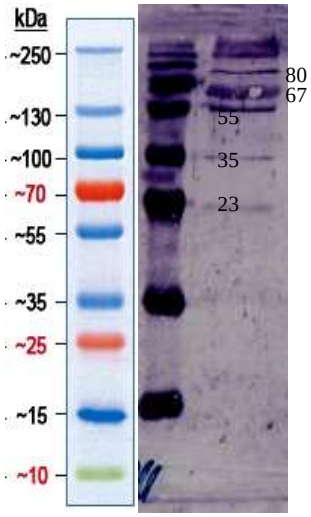

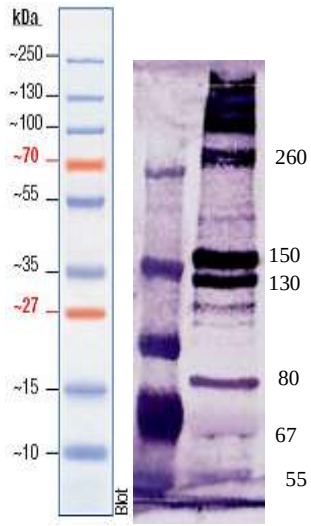

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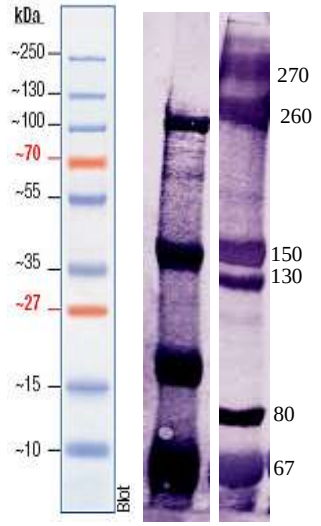

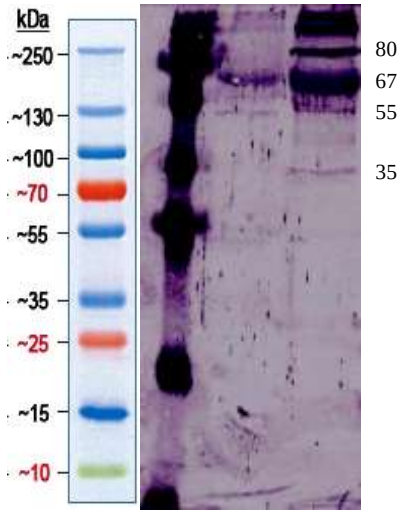

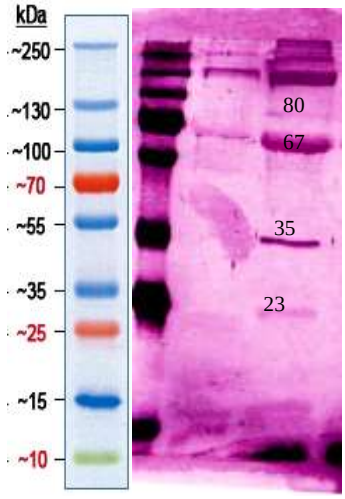

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The Immunoblot was also tested with negative known sera, and it showed high background with false positive results. During this STSM, many attempts were done to improve the results and optimize the immunoblot, to exclude the false positive results, and to decrease/eliminate the high background that were in the immunoblot.

The results are now being interpreted and the colleagues in Germany will make more tests to try to achieve optimal results and to exclude false positive results. A scientific publication will be written at the end of these trials to show the results.

During this STSM, the running cooperative-project "Molecular Characterization of *Taenia saginata* in Germany" was finalized, and a manuscript is being written to be applied for publication.

All this work was in cooperation with the colleagues at the host institution (Prof. Dr. Christina Strube) and the Veterinary Institute in Hannover (Dr. Uschi Nagel-Kohl).

For further information, please do not hesitate to contact me.

Best Regards

Sincerely yours

Dr. med.vet. Sameh Abuseir