Characterization of immunogenic protein fractions of Sheep Cysticercosis in Cairo, Egypt

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Characterization of immunogenic protein fractions of Sheep Cysticercosis in Cairo, Egypt

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ABSTRACT. Cysticercosis is a parasitic infection that causes severe economic and public health problems. The overall incidence of infection in sheep slaughtered in Cairo, Egypt during meat inspection was 31.22% and precisely 19.72% for *Cysticercus tenuicollis* and 11.50% for *C. ovis*. Sera collected from infected animals used to evaluate the diagnostic efficacy of the extracted antigens using ELISA. The sensitivity of the test was 100% for both *C. tenuicollis* and *C. ovis*, while the specificity was 80.26% and 87.03%, respectively. Using EITB the fractions of 36 KDa and 23 KDa appears to be specific for diagnosis of *C. tenuicollis*; while, 77 KDa and 73 KDa were specific for diagnosis of *C. ovis*. Moreover, several protein fractions which were detected in all antigens extracted didn’t react with its target sera but at the same time did react specifically versus sera from animals infected with another cysticerci. Those fractions are considered as common immunogenic fractions between different cysticerci, so strictly identified specific fractions must to be used for diagnosis of Cysticerci infection to avoid cross reactions with other cysticerci antibodies.

Keywords: Cysticerci - ELISA- EITB- Sheep, Egypt.
INTRODUCTION

Cysticercosis is a parasitic disease caused by metacestodes of Taenia species infecting human and carnivores all over the world, producing severe economic and public health problems related to its zoonotic nature (Ahmadi and Badi, 2011). The disease has the special economic significance of the veterinary level as it causes severe losses due to the condemnation of infected meat and organs (Goswami et al. 2013).

Until now, no routine technique is adopted for diagnosis of infection by cysticercosis in animals, animals other than postmortem meat inspection (PM) Abdel-Radi, (2014). A variety of serological techniques were applied for diagnosis of metacestode infection in different animals (Oliveira et al. 2010; Paulan et al. 2013 & Mousa et al. 2014); however, the sensitivity and specificity of these techniques were highly affected by the type of the tested sera as well as a degree of purity of the antigen especially with the complex nature of metacestodes antigen, which exhibits variable degrees of cross reactivity (Varela-Diaz et al. 1977). A combination of more than one serological test, using of the sera of known infection history and purified antigens was recommended to achieve a proper diagnosis of cysticercosis (Sultan et al. 2012).

The present study aimed to determine the incidence of infection by different Cysticerci in slaughtered sheep in Cairo governorate, Egypt via PM inspection. Sera collected from infected animals will be used to determine the diagnostic values of different Cysticerci antigen using ELISA. Moreover, these antigens were fractionated by SDS-PAGE and then the sensitivity and specificity of the obtained fractions were investigated aiming to spot some light on the level of cross reactions between ant-bodies of different infection versus the fractioned antigens using EITB technique.

MATERIALS AND METHODS

Inspected animals and collected samples:

Aiming to determine the incidence of infection by C. teniuicollis and C. ovis in Cairo governorate, a total of 426 sheep slaughtered at Cairo abattoirs (El-Basatin) during the period from January to December 2012 were exposed to postmortem (PM) inspection according to Abdel-Radi, (2014) The muscles and visceral organs, particularly the lung, liver, kidney, heart and spleen of each animal, were examined carefully for the presence of the previous cysts. All the detected Cysticerci were collected in separate identified suitable cellophane bags and transported to the laboratory in an ice box for further examination and antigen preparation. Rectal fecal and blood samples were collected one week before slaughtering and at the time of slaughtering from each inspected animal. After PM inspection cysticerci infected carcasses were identified. Fecal samples were examined for diagnosis of different internal parasitic stages. Using concentration flotation (by salt solution) and concentration sedimentation of water as described by Pritchard and Kruse (1982) Thin Giemsa stained blood smears were prepared and examined for detection of different blood parasites according to Garcia, (2007). Sera of animals proved to be free from enteric and blood parasites were selected and used for serological study.

Antigen preparation

Two cysticerci antigens (Ag) were extracted from C. teniuicollis and C. ovis cysts. Viable non calcified cysts with cystic fluid and clear invigilated scolex free from surrounding tissue, Love and Hutchinson, (2003) were selected for antigen preparation. C. teniuicollis antigens were prepared from the whole cysts, according to Goswami et al. (2013) While, C. ovis crude Ag was prepared, according to (Melcher, 1943). The protein content of each antigen was measured according to Lowry et al. (1951). The antigens were allocated in 1ml vial and stored at -20°C until use.

Hyper-immune sera (HIS)

Rabbit hyper-immune sera (RHIS) were produced versus the 2 tested antigens according to (Fagbemi et al. 1995). Two white New Zealand rabbits (2 months old) per each antigen, were bled for negative control sera then injected with 1.2 mg protein for each antigen, mixed with an equal volume of mineral oil subcutaneously. After 3 weeks, 3 consecutive injections of 0.4 mg protein antigen in equal volume of oil were given intra-muscularly at biweekly intervals. Rabbits were bled from the ear vein for serum collection 10-14 days after the last injection. The collected sera were stored at –20°C until used.
Serodiagnostic studies
Indirect- enzyme linked immunosorbent assay (ELISA):

The test was performed as described by Iacona et al. (1980). The plate was coated with each antigen (C. tenuicollis and C. ovis) separately in (4 ug / ml in coating buffer) which adjusted after checkerboard titration. After overnight incubation at 4oC, the plate was washed, then blocked with 0.1% bovine serum albumin fraction V (Sigma), 100ul/well. Serum samples were used at 1:50 dilution. RHI sera was used as reference positive control. Horseradish peroxidase conjugated (HRPC) rabbit anti-sheep IgG, {Heavy and light chains (H & L)} (Sigma) as well as HRPC goat anti-rabbit IgG (whole molecule) (Sigma) was used in 1:2000 dilution. Ortho-phenylenediamidine-OPD was added at a concentration of 340 ug/ ml substrate buffer. Absorbency was read by 490nm using full automated Titerteckmultiskan ELISA reader. The cut off points were set as 2SD above the mean of control negative samples. After optimization of the test by checker board titration, the test was used to determine the value of different Cysticerci antigens in capturing the specific and cross reacted anti-bodies in naturally infected sheep sera aiming to spot some light on the specificity and sensitivity of them in the diagnosis of infection in field collected serum sample.

Electrophoretic fractionation of antigens and western blot analysis:

For identification of immunogenic proteins, poly-peptide profiles of cystic fluid antigen and whole cyst lysate antigen preparations of each C. tenuicollis and C. ovis were fractionated using SDS-PAGE (Genei Bangalore, India) containing 10% gel under protein denaturing condition according to (Laemmli, 1970). Standard molecular weight marker protein (Lonza, USA) was run simultaneously. Two separate gels for each sample ran in same conditions using the same antigen samples prepared from each Cysticerci. After completion of running of the SDS-PAGE, one gel along with molecular weight markers was shifted to stain with Coomassie Brilliant Blue. The remaining one was washed with transfer buffer and Cysticerci proteins resolved by SDS-PAGE were electrophoretically transferred (Genei, Bangalore) from the gel to a nitrocellulose membrane (NC) as per the standard method according to (Towbin et al. 1979) and probed by western blotting using laboratory prepared RHIS in comparison with sera of naturally infected animals and a negative control one. C. tenuicollis and C. ovis protein fractions that reacted versus reference positive sera and at the same time did not react versus the negative control were considered as specific protein fractions.

RESULTS

Incidence of Cysticerci infection in the inspected sheep.

The total incidence of infection with the different cysticerci was 133 (31.22%) from 426 inspected slaughter sheep during one year at Cairo abattoir. It was found that 84 (19.72%) for C. tenuicollis and 49 (11.50%) for C. ovis in the different infected organs (Fig. 1-2).

The diagnostic value of different antigens was evaluated using ELISA versus control and natural infected sera. Optimization of ELISA using checker board titration revealed that 2μg antigen/well, with
1:100 serum dilution and 1:2000 conjugate, appear as the best condition for application of the assay under the condition of the present study.

The examined number of collected sera (n = 62) for C. tenuicollis & (n = 30) for C. ovis from naturally infected sheep at PM inspection (cyst/animal). These sera reexamined by using ELISA which, aiming to determine the level of sensitivity and specificity of each of the two antigens in capturing of its specific antibodies in the sera of natural infected and control animals. The data revealed absolute sensitivity for each of the tested antigens (100%) in capturing its target antibodies in sera of infected animals. Serum samples gave a positive reading when tested at 1:100 serum dilution, Table (1).

For estimating the degree of specificity, each type of antigen was exposed to sera of animals infected by the other types of Cysticerci as well as its original HIS obtained from rabbits vaccinated by the same antigen. The data in table (1) revealed the level of cross reactions as sera containing anti-C. tenuicollis Ab cross reaction with C. ovis and this fact is decreasing the specificity of the assay of 87.09%. Also, sera containing anti-C. ovis Ab crossed reacted with the C. tenuicollis showing a decrease in specificity of up to 73.33%.

From the previous results, anti-C. tenuicollis Ab in infected sera was considered the less cross reacted Ab with tested antigen, followed by that of C. ovis. At the same time no cross reactions could be detected in treatment of any of the two antigens versus control RHIS or negative sera of sheep.

**Specific and cross reacted Cysticerci protein fractions:**

In order to further investigate the level of cross reactions between the different tested Cysticerci antigens SDS-PAGE was applied and the specificity and sensitivity of each fraction was determined after transferring them to NC membrane and treating of them versus specific and nonspecific antibodies in sera of file collected naturally infected sheep as demonstrated in Table (2) & Fig. (3).

The data revealed that 4 protein bands corresponding to molecular weights (MW) of (97, 63, 36 & 23kDa) were reacting specifically after treatment of C. tenuicollis fractioned antigen versus C. tenuicollis infected sheep sera. At the same time two protein bands (at 78KDa & 17KDa) were reacting specifically after treatment of corresponding NC strip versus the sera of C. ovis infected animals.

By the same approach, NC strips caring C. ovis fractioned antigens, showing 4 bands at MW of (97KDa, 77KDa, 73KDa & 65kDa) which reacted specifically versus sera of sheep infected with C. ovis. At the same time, the fractions at the level of (48KDa & 26KDa) reacted specifically when treated a corresponding NC strip by the sera of sheep infected with C. tenuicollis. At the same time 2 other bands

<p>| Table 1. Sensitivity and specificity of the two antigens in capturing the specific and cross reacted anti-bodies in naturally infected sheep sera |</p>
<table>
<thead>
<tr>
<th>Antigen ELISA Plate</th>
<th>C.tenuicollis (n= 62)</th>
<th>C.ovis (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity test</td>
<td>C.tenuicollis</td>
<td>62 (100%)</td>
</tr>
<tr>
<td></td>
<td>C.ovis</td>
<td>-</td>
</tr>
<tr>
<td>Specific RHIS for each Ag</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Specificity test</td>
<td>C.tenuicollis</td>
<td>8 (87.09%)</td>
</tr>
<tr>
<td></td>
<td>C.ovis</td>
<td>-</td>
</tr>
<tr>
<td>Negative rabbit or animal sera</td>
<td>-</td>
<td>--</td>
</tr>
</tbody>
</table>

-(n) = The number of testing serum samples collected from naturally infected sheep.

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on the level of (135 KDa & 52KDa) were detected at treatment of similar NC strip by C. ovis non-infected sheep sera, Table (2) & Fig. (3).

DISCUSSION

Incidence of infection by different Cysticerci in slaughtered animal still has special importance as it gives a clear picture about the role of slaughtered animals as a source of infection by different Taenia species infecting dogs and human for special area. This in fact points out the importance of application of proper meat inspection and condemnation of the infected parts as well as prevents the arrival of stray dogs to animal farms.

During the present study, incidence of C. tenuicollis was 19.72% in examining sheep. The same result was previously recorded by El-Massry (1988) The authors recorded an incidence reached to 19.5% in the same localities of our study. While, the incidence was slightly lower than that recorded in Ethiopia by (Endale et al. 2013) as it reached 22%. On the other hand, El-Dakhly et al. (2012) recorded a very low percentage (1.3%) in middle Egypt. Moreover, the recorded incidence in our study was in the range previously mentioned by Abdel-Maogood, 2005 & Sissay et al. 2008 as they determine a percentage of 5.66% in Egypt & 26.0% in eastern Ethiopia, respectively.

In the author’s opinion and agreement with El-Massry (1988) incidence of cysticercosis recorded among the sheep in the present study may be related to the high infection rate of dogs with the Taenia worm in the study sites. This can supported by some habit of the people in Egypt, as they usually slaughter sheep at home and throw offal’s in garbage which usually eaten easy by the present stray dogs.

In the author’s opinion, the difference in the incidence rate recorded in the present study in comparison with the other similar one may be related to different grazing systems, eating habits, accuracy of PM inspection protocol, the hygienic and control measures applied in each area.

The second part of the present study was directed to use sera collected from infected animals in evaluating the diagnostic values of different Cysticerci antigen from the aspect of their ability to capture of its specific antibodies (Ab) using ELISA. Each of testing antigens (C. tenuicollis and C. ovis) revealed absolute sensitivity (100%) in capturing of its target Ab in the tested sera obtained from animals infected by each cyst alone. This result came in agreement with similar previous studies on diagnosis of C. tenuicollis and C. ovis (Abdel-Maogood, 2005 and Radwan, 2008). This result disagreed with that recorded by Sultan et al. (2012) who recorded sensitivity rate for ELISA in diagnosis of C. tenuicollis, which reaches up 90%. These variations might be related to the degree of purification of the used antigen as well as the standardization of the assay and the reference control sera.

Figure 3. Specific and non-specific protein fractions of different cysticerci antigen on NC treated versus positive and negative sera.

A) C. tenuicollis
1- NC strip is treated with C. tenuicollis +ve serum.
2- NC strip is treated with C. tenuicollis -ve serum.
3- NC strip is treated with C. ovis. +ve serum

B) C. ovis
4- NC strip is treated with +ve serum of C. ovis.
5- NC strip is treated with -ve serum of C. ovis.
6- NC strip is treated with +ve serum of C. tenuicollis.
Concerning the specificity of each antigen in capturing of antibodies (Abs) present in the sera of animals infected by the other two types of Cysticerci, the obtained results clear different degrees of decrease in specifying as sera containing anti-C. tenuicollis Ab was cross react with C. ovis Ag is decreasing the specificity of the test to 87.09%. Moreover, sera containing anti-C. ovis Ab crossed reacted with the antigen of C. tenuicollis showing decrease in specificity reached to 73.33%. This result is supported by (Varela-Diaz et al., 1977) who mentioned that metacestodes have a complex nature of antigen and exhibits cross reactions among themselves. Also, Kandil et al., (2004) found that a high level of non-specific cross reactions to occur when crude somatic extracts or cyst fluid preparations of Taeniid cestodes are used for the immunodiagnosis of larval cestodes infections. But this result disagrees with Abdel-Maogood, (2005), who recorded that the specificity of C. tenuicollis and C. ovis was 50% and 54.38%. (Sultan et al. 2012) who found that the specificity of non-purified crude antigens derived from the whole cyst of C. tenuicollis was 60%, so the purification of antigens is required.

From the previous results, anti-C. tenuicollis Ab in infected sera considered the less cross reacted Ab with other tested antigens; this may be related to the habitat. The nature of these larvae as they usually present hanged in the peritoneal cavity non-embedded in tissue as the condition of C. ovis metacestode.

The last part of this study was directed to investigate the presence of common or cross reacted protein fractions in each of the evaluated antigen responsible for the demonstrated degree of cross reactions between them. For this reason the antigens were fractionated by SDS-PAGE, then the sensitivity and specificity of the obtained fractions were investigated using EITB technique. The data revealed that the fractions at the MW level of 36KDa & 23KDa were found to be specific for diagnosis of C. tenuicollis. While that at 77 &73 KDa were specific for diagnosis of C. ovis. This result was in agreement with (Goswami et al. 2011) who recorded that 36.2 &23 kDa protein fractions are recognized as immunodominant polypeptides and could be explored for serodiagnosis of C. tenuicollis infection. On the other hand, this result disagrees with that reported by Abdel-Maogood (2005) who found that the specific epitope for diagnosis of C. ovis infection in sheep was 26.494 KDa, While 74.224, 66.725, 55.567 & 21.224 KDa were the specific for diagnosis C. tenuicollis in sheep.

Concerning the level of cross reactions between different antigenic fractions, the data revealed that two protein bands corresponding to MW of 78 &17 KDa were present in the fractioned C. tenuicollis Ag and react specifically versus sera of C. ovis infected animals. These data mean the presence of some common fractions between C. tenuicollis antigens and anti-bodies of other Cysticerci, rather than

### Table 2. Protein bands detected on NC strips containing different fractioned antigens after treatment by positive and negative sera

<table>
<thead>
<tr>
<th>Tested sera</th>
<th>Protein bands detected on NC strips containing different fractioned antigens after treatment by its positive sera and other sera MW (KDa)</th>
<th>Non specific bands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. tenuicollis</strong> +ve</td>
<td>97,63,36 &amp; 23</td>
<td>48 &amp; 26</td>
</tr>
<tr>
<td><strong>C. ovis</strong> +ve</td>
<td>78 &amp; 17</td>
<td>97,77, 73 &amp; 65</td>
</tr>
<tr>
<td><strong>C. tenuicollis</strong> –ve</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>C. ovis</strong> –ve</td>
<td>-</td>
<td>135 &amp; 52</td>
</tr>
</tbody>
</table>

+ve= positive       -ve = negative
Non Specific Bands = common bands between C. tenuicollis and C. ovis
presence of unapparent infection by C. tenuicollis in the examined sera. In the same time, the fractions at the level of (48 & 26 KDa) were reacting specifically when treat NC strips carrying C. ovis fractioned Ags by the sera of sheep infected with C. tenuicollis. These protein fractions considered to be common immunogenic fractions between these Cysticerci as it captures the Ab of other cyst and in the same time did not react versus its own Ab. The specific fractions obtained in the present study were in agreement with that obtained by Abdel-Maogood (2005) who found that C. tenuicollis antigen gave bands ranging between 88.298-16.16 kDa and C. ovis Ag gave bands ranging between 94.806-15.894 kDa. Moreover, Goswami et al. (2013) who recorded that the band at 63 kDa MW considered to be specific for diagnosis of C. tenuicollis infection. This was disagreeing with Kandil et al. (2003).

The concerning presence of cross reacted fractions between different Cysticerci Ag, the obtained data were in agreement with Lawson, (1994) who recorded that C. tenuicollis and C. ovis shares some antigenic properties that could result in partial cross-protection in the intermediate host under natural conditions. While, Kandil et al. (2004) reported that the common band between C. tenuicollis and C. ovis antigens were at the level of 45 kDa. In the authors opinion, the difference in the size of the specific fractions described by other author’s may be related to some genetic variations in the parasites between different localities, age of the parasite as well as to the condition of the test and the method of antigens preparation and purification as described by Kandil et al. (2003).

CONCLUSION

The present results revealed that the sensitivity of C. tenuicollis and C. ovis was 100% for each one. While, the specificity was 80.26% and 87.03%, respectively by using EILSA. The EITB analysis cleared that 36 and 23 KDa protein bands were found to be specific bands for diagnosis of C. tenuicollis. While 77 and 73 KDa protein bands were found to be specific bands for diagnosis of C. ovis.

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