Summary

This study based on the evaluation of *Cysticercus bovis* antigen for diagnosis of *Cysticercus bovis* in cattle and to detect the ratio of cross reactivity between *C. bovis* and sarcocystis species using ELISA and the immunoblot analysis.

For this purpose, eighty samples of blood and muscles of heart, tongue and masseter were collected for detection of *C. bovis* and oesophageal muscles for sarcocystis sp. *Cysticercus bovis* were detected macroscopically in 6.25% of cattle while sarcocystis sp. detected in 85% in the same examined cattle microscopically. By ELISA technique 5 cases of *Cysticercus bovis* naturally infected cattle reacted positive while negative cases were 58 and 17 cases as false positive. The sensitivity and specificity of *C. bovis* antigen for diagnosis of cysticercosis in cattle were 100% and 77.3%, respectively.

Immunoblot analysis of *Cysticercus bovis* antigen by using hyperimmune sera from immunized rabbits as positive control, revealed 5 polypeptides against *C. bovis* antigen ranging in their molecular weight of 215-12 kDa. In contrast only two bands were recognized when both naturally infected sera of *C. bovis* and sarcocystis sp. were used against *C. bovis* antigen with a common band of 115 kDa appeared between them. This may be cleared the ratio of cross reactivity in ELISA.

Introduction

Animal wealth, especially cattle is one of great economic importance, which is considered the main source of meat, milk and hides in Egypt. Such meat is the most common animal protein for human consumption, so it should be free...
from diseases particularly parasitic ones. Some species of protozoa and cestode parasites form intramuscular cysts within different tissues of cattle and cause economic loss in the carcasses and threaten human health.

*Taenia saginata* cysticercosis is one of the zoonotic parasites of great importance in public health. In abattoirs, infected meat with *Cysticercus bovis* is considered of down grade. It may be subjected to freezing in localized infection cases or total condemnation of carcasses and offal in generalized ones (*Wanzala et al., 2006*).

Cattle become infected by grazing on materials contaminated with *T. saginata* eggs, which can derived from human faeces directly or via sewage plants after flooding or sewage sediment distributed on pasture (*EFSA, 2006*). Eggs hatch in the intestine and the oncospheres, liberated from the eggs, penetrate the intestinal wall and circulate through the lymphatic and blood stream. Finally, the larvae settle down in muscle tissue, the predilection sites are the masseter muscle, tongue, heart and diaphragm. The mature cysticercus, firstly, are transparent, but with time, the irritated tissue reacts by forming cyst walls around the parasites and the immune system of the host might kill the cysticercus over time, forming caseous or calcified cyst (*McGavin et al., 2001*). Another cyst-forming parasite is sarcocystis, which is considered one of the most important tissue protozoa infected herbivorous animals which acting as an intermediate host. Cattle is infected after ingesting sporocysts, shed in the feces of carnivores which are definitive hosts. Sarcocystis infection was considered one of important causes limiting animal production (*Dubey et al., 1989*). Acute sarcocytosis might cause abortion and even death of the host (*Dubey and Bergeron, 1982*). Chronic or a symptomatic disease is the most common form encountered during sarcocystis spp. Infection may cause granulomatous myositis, reduced weight gain and poor milk production, thus affect significantly animal production. As well, the presence of sarcocystis infection in muscles produced a toxin called sarcocystin, which might affect on the ability of heart function and gastrointestinal tract (*Herbert and Smith, 1987*). During meat inspection, some sarcocystis spp. lead to condemnation of the infected carcasses, as sarcocystis infection renders meat unacceptable to the consumer and causes some health hazards. The diagnosis of sarcocystis infections depends on using compressorium to detect muscle microcyst or during histopathological examination, while the routine inspection proce-
dure for bovine cysticercosis depends on naked eye inspection of the slaughtered animal to detected macrocysts in the predilection sites. It has been shown that the currently established inspection methods are not suitable for sensitive detection of cysticercus metacestodes, which in many cases, were found in tissues other than the predilection sites (Wanzala et al., 2002). The enzyme-linked immunosorbent assay (ELISA) was considered one of the useful methods for serodiagnosis of bovine cysticercosis to indicate the spread of infection in outbreaks or high-infected areas (Harrison et al., 2005).

MATERIALS AND METHODS

I. Collection of samples:
Muscles of heart (40), tongue (20) and masseter muscles (20) were collected for detection of *C. bovis* from 80 local cattle slaughtered at El-Bassatin abattoir. Eighty samples of oesophageal muscles were collected from the same animals for detection of sarcocystis cyst. Sera were collected form these animals and stored at -20°C till use.

II. Parasitological examination:
   a. Macроскопical examination for detection of *Cysticercus bovis* was applied.
   b. Микроскопical examination for detection of sarcocystis species was applied by using compression technique (Thornton and Gracey, 1976). One gram from each fresh sample was divided into small pieces of an oat-grain, and compressed between the two-glass plates of compressorium. The prepared samples were examined by using the low power of light microscope.

III. Preparation of *Cysticercus bovis* antigen:
Preparation of *Cysticercus bovis* antigen according to the technique described by Hillyer and Santiago de Weil (1977) as follows: The cysts were removed from the surrounding tissues without injury the cystic wall. The collected cysts were washed with water and re-washed three times in PBS pH 7.2, then homogenized by using homogenizer for 10 minutes followed by sonication for 5 times within 2 minutes intervals. The sonicated material was centrifuged at 14000 rpm for 45 minutes, at 4°C then the supernatant was collected and aliquated.

The protein concentration of the antigen was determined according to Lowry et al. (1951).

IV. Preparation of hyperimmune sera:
Hyperimmune sera against *Cysticercus bovis* antigen was produced in New Zealand white rabbits at the age of 2 months. Two
rabbits were primed subcutaneously with 200 µg protein of the prepared cysticercus antigen emulsified in Freund's complete adjuvant. They were boosted 14 and 28 days after priming with 150-µg protein emulsified in Freund's incomplete adjuvant. Rabbits were bled on day 35 post-priming and sera were collected and pooled (El-Shater and El-Kelesh, 2006).

V. Collection of naturally infected sera:
Blood samples were collected from 15 positive cases with sarcocystis species and 5 positive cases of *Cysticercus bovis*. Sera of each type were pooled for immunoblotting.

VI. Serodiagnostic techniques:-

a- Enzyme-linked immunosorbent assay (ELISA):
Specific antibody against *Cysticercus bovis* antigen was detected in cattle tested by ELISA (Iacona et al., 1980).

The ELISA plates were coated with 100 µl/well of *Cysticercus bovis* antigen at the concentration of 30 µg protein/ml of coating buffer and incubated overnight at 4°C. After washes with PBS-3% Tween, wells were blocked with blocking buffer (200 µl/well) and incubated at room temperature for 2 hours. After washing, 100 µl/well of 1:100 diluted serum samples from naturally *Cysticercus bovis* infected cattle and negative sera were added and the plates were incubated for 2 hours at 37°C with shaking. After washing with PBS-3% Tween, the plates were conjugated with 100 µl/well by anti-bovine IgG alkaline phosphatase (1:3000 Sigma) for one hour at 37°C with shaking. After washing P-nitrophenylphosphate substrate (1 tab./5 ml buffer) was added (Sigma chemicals). The optical density (O.D.) was measured at 405 nm against blank control well. The tested sera were considered to be positive when the absorbency values were as more than the cut off values (The cut off = double fold of the mean negative sera).

VII. Immunoblot technique:
Protein bands of *Cysticercus bovis* antigen were transferred from polyacrylamide gels to nitrocellulose membrane according to Towbin et al. (1979) technique. NC sheets were cut into 0.5 cm strips followed by blocking in 5% BSA in PBS for 2 h. on rocker platform. Rabbit and naturally infected sera of both *C. bovis* and sarcocystis sp. were diluted at 1:100 in 5% BSA/PBS were reacted with fractionated cysticercus antigen. NC strips for 2 h on rocker platform. Following washing, anti-rabbit IgG peroxidase diluted at 1:1000 in PBS (Bio-Rad) was added to NC strips for 1 h on rocker platform. The chromogen
AEC substrate (Sigma Comp.) was added to NC strips and allowed to develop for 10 min. The reaction was visualized by the naked eye.

**RESULTS**

Five cases of 80 fresh samples of heart, tongue and masseter muscles of slaughtered cattle were found to be positive for *Cysticercus bovis* with a prevalence of infection 6.25%, while sixty-eight of 80 fresh oesophageal muscle samples collected from the same animals were found to be positive by percentage of 85% for sarcocystis species using compression technique.

The ELISA using the antigen from *Cysticercus bovis* cysts showed that in positive five cases for *Cysticercus bovis* were found to be highly positive O.D. reading over 0.500 while 17 cases considered as false +ve (suspected cases of sarcocystis spp.) which the O.D. reading was over 0.200 .

Fifty-eight cases were found to be negative cases. A very high correlation was observed between the parasitological results and that obtained from ELISA which used antigen prepared from cysticercus of cattle as the sensitivity was 100%, while the specificity was 77.3%.

Table (1): Incidence of *Cysticercus bovis* and sarcocystis species in slaughtered cattle by using parasitological examination.

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Type of collected muscles</th>
<th>Total No. of cases</th>
<th>% of +ve infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac M.</td>
<td>Maseter M.</td>
<td>Tongue M.</td>
<td>Oesophageal M.</td>
</tr>
<tr>
<td>No.</td>
<td>+ve</td>
<td>-ve</td>
<td>No.</td>
</tr>
<tr>
<td><em>C. bovis</em></td>
<td>40</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Sarcocystis spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (2): Serodiagnosis of *Cysticercus bovis* of naturally infected cattle by ELISA using *C. bovis* antigen.

<table>
<thead>
<tr>
<th>Total No. of cases</th>
<th>+ve cases</th>
<th>-ve cases</th>
<th>False +ve (Suspected cases of sarcocystis spp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>5</td>
<td>58</td>
<td>17</td>
</tr>
</tbody>
</table>
Table (3): Determination of sensitivity and specificity of *C. bovis* antigen for diagnosis of cysticercosis in cattle (number of animals = 80).

<table>
<thead>
<tr>
<th>Types of examination</th>
<th>+ve cases</th>
<th>-ve cases</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parsitological examina-</td>
<td>5</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serological examination</td>
<td>5</td>
<td>58</td>
<td>100%</td>
<td>77.3%</td>
</tr>
</tbody>
</table>

Immunoblot analysis using hyperimmune sera against *Cysticercus bovis* antigen from rabbits as a positive control were recognized 5 polypeptides bands against *C. bovis* antigen ranging in their molecular weight of 215-12 kDa. In contrast only two polypeptides were recognized with *C. bovis* naturally infected sera with molecular weight 115 -25 kDa. While, two bands (115-26.5) was recognized when used naturally infected sera with sarcocystis sp. against *C. bovis* antigen. The band of 115 kDa was appeared with *C. bovis* and sarcocystis sp. naturally infected sera, this may be cleared the ratio of cross reactivity in ELISA.
Fig. (1): Characterization of *Cysticercus bovis* antigen by SDS-PAGE and immunoblot.
Marker: Prestained wide range molecular weight markers (Bio Lab. Comp.).
Lane 1: Immunoblot analysis of *C. bovis* antigen using hyperimmune sera.
Lane 2: Immunoblot analysis of *C. bovis* antigen using naturally infected sera with *C. bovis*.
Lane 3: Immunoblot analysis of *C. bovis* antigen using naturally infected sera with sarcocystis species.

**DISCUSSION**

*Cysticercus bovis* in cattle is a world wide infection, Taeniasis was common in regions where people were accustomed to consume insufficiently cooked or smoked meat, or where poor sanitary conditions were obvious and favored the dispersal of faeces in fields, or during sufficient meat inspection for the elimination of infested meat form the market (Thornton and Gracey, 1974). On the other hand, the sarcocystis spp. infection was considered one of the causes of severe and even
fatal disease in cattle and the source of infection was carnivorous animals (El-Shater and El-Kelesh, 2007).

In the present study, the incidence of *C. bovis* among 80 cattle slaughtered in El-Bassatin abattoir was 6.25%. This result was relatively in agreement with those reported in Egypt by Awad (1981), 7.3%, Maha (1994), 7.8% and Abo El-Nor (2007) who recorded that the percentage of infection with *Cysticercus bovis* in cattle was 10.7%. In this study, the percentage in cattle 6.28% was lower than those reported by Bundza *et al.* (1988) in Canada (37%), and Okafor (1988)s in Nigeria (26.14%). On the other hand, other workers reported lower incidence. In Egypt, El-Saieh (1982) reported an incidence rate of (3.27%), in Cuba Corodoves *et al.* (1985) recorded a rate of (0.8%). This variation in rate of infection in cattle might be related to the environmental and sanitary conditions at countries under studies.

In the same time, the present study recorded that out of 80 oesophageal muscle samples of cattle, 68 (85%) were found to be positive for sarcocystis spp. cysts using compression technique. This incidence was in agreement with that recorded by El-Saieh (1998) who found that the incidence of sarcocystis infection in adult was 75.12% in Qena Governorate. Also, Hassanien (1992) recorded that 78.85% of examined cattle were infected with sarcocystis spp. in Kalubiya Governorate. The result reported during this study also was in parallel with El-Shater and El-Kelesh (2007) who found that the incidence rate of infection with sarcocystis spp. in cattle was 81.66%. On the other hand, Mohamed (1996) detected low incidence rate (30%) of sarcocystis infection among the examine cattle in Assiut Governorate. This great variation of the incidence rates of sarcocystis infections may be attributed to the difference in localities and methods used for diagnosis. Oesophageal muscles were subjected to be examine during this study, as they were considered the heaviest infected site with sarcocystis in cattle (El-Saieh, 1998).

The ELISA results showed that, anti-cysticercus bovis antibodies were detected using *Cysticercus bovis* antigen in the five cases that were positive in the examined fresh muscle samples of cattle. These facts indicated that, *Cysticercus bovis* antigen can be effectively used for diagnosis *Cysticercus bovis* infections in cattle. The seventeen cases that reacted with *Cysticercus bovis* antibodies with O.D. reading lower than the five positive cases may be due to that the animals were during the early stages of infection and the
cysts had not been composed yet or the examined animals were suspected to be infected with other tissue parasite as sarcocystis, as 85% of the examined samples during this study were proved to be infected with sarcocystis by parasitological examination. In the present study, the sensitivity of \textit{C. bovis} antigen was 100%. This rate of percentage was nearly similar with that mentioned by Draelents \textit{et al.} (1995), (87.5%); Van Kerckoven \textit{et al.} (1998), (92%) and Abo El-Nor (2007) who recorded a sensitivity rate of (100%). On the contrary, Geerts \textit{et al.} (1981) mentioned that the sensitivity of \textit{C. bovis} was (37.5%). The percentage of specificity in this study was 77.3%, this result may be attributed to that \textit{C. bovis} antigen was crossly reacted with other common cattle parasite (Kamanga \textit{et al.}, 1991 and Mousa, 1992).

Immunoblot technique revealed 5 polypeptides of cysticercus antigen recognized by hyperimmune sera against cysticercus antigen. As well as both naturally infected sera of \textit{Cysticercus bovis} and sarcocystis sp. were reacted with \textit{Cysticercus bovis} antigen with a common band between the both naturally infected sera indicated that this cross reaction between \textit{C. bovis} and sarcocystis sp. was referred to the presence of common antibodies.

Although, Bogh \textit{et al.} (1995) demonstrated that there was no cross reaction took place between hydrophobic antigens (low molecular weight) from \textit{T. hydatigena} cyst fluid with \textit{Sarcocystis cruzi}, they confirmed the results in the presents study, as 115 kDa (high molecular weight) was cross reacted \textit{C. bovis} antigen with sarcocystis. Presumably, purification of antigen would overcome the cross reactions associated with crude antigens. This results was supported by Maha (1994) who reported a cross reaction between \textit{C. bovis} and \textit{F. gigantica} crude antigen and Omnia \textit{et al.} (2004) who found strong cross reactivity between fractionated and crude antigens of \textit{C. bovis}, \textit{C. ovis} and \textit{C. taenuicolis}.

From this result, more accurate diagnosis for \textit{Cysticercus bovis} and sarcocystis sp. infections may be obtained from the immunoblot technique by using specific band for each parasite. In conclusion, polypeptides 215, 33, 32 and 12 kDa could be specific for diagnosis of \textit{Cysticercus bovis} and 12 kDa band was the immunodiagnostic band.

These findings were in agreement with those obtained by Bogh \textit{et al.} (1995) who recorded that low molecular weight 10-18 kDa of hydrophobic fractions of \textit{T. hydatigena} cyst fluid reacted with sera from infected calves with \textit{Taenia saginata} for 60 days using im-
munoblot technique. Also, Kamanga et al. (1987) demonstrated low molecular weight (10 kDa) of fractions isolated from *T. hydatigena* cyst fluid were the immune diagnostic antigens.

**REFERENCES**

Abo El-Nor, A.A. (2007): "Some studies on *Cysticercus bovis* in Egyptian cattle and water buffaloes." M.V.Sc., Faculty of Veterinary Medicine, Beni Sueif University, Egypt.


El-Saieh, A.F. (1982): "Incidence of bovine cysticercosis in slaughtered animals in upper Egypt." M.V.Sc., Faculty of Veterinary Medicine, Assiut University, Egypt.

El-Saieh, A.F. (1998): "Incidence of toxoplasma and sarco-
sporidia in slaughtered animals in Qena Governorate." Ph.D. Thesis, Faculty of Veterinary Medicine, Assiut University, Egypt.


Maha, El.B. (1994): "Serological studies on cysticercosis and hydatidosis in cattle and buffaloes." M.V.Sc., Faculty of Veterinary Medicine, Cairo
University.


Mohamed, M.S. (1996): "Muscular parasites in slaughtered animals in Assiut Governorate." Ph.D., Faculty of Veterinary Medicine, Assiut University.

Mousa, W.M.A. (1992): "Studies on the cross reactivity among some helminthes of veterinary and medical importance." Ph.D., Faculty of Veterinary Medicine, Cairo University, Egypt.


تقييم مستضد السيستيركس بوفيز لتشخيص السيستيركس بوفيز في الإبكار
إبراهيم جودة حافظ رضوان
قسم الطفيلييات - معهد بحوث صحة الجيوبان

الملخص العربي

لقد أجريت هذه الدراسة لتقييم معامل الضد للسيستيركس بوفيز في تشخيص حويصلات السيستيركس في الإبكار وكذلك تحديد مدى التفاعل التداخللي بين السيستيركس والساركوسست في الإبكار بواسطة اختبار الألبزا. لهذا الغرض تم تجميع 80 عينة من دم وعضلات القلبي واللسان والفك لتشخيص السيستيركس بوفيز كذلك تم تجميع دم وعضلات من المرئ لتشخيص الساركوسست من نفس الإبكار. وقد وجد أن نسبة الإصابات بالفحص المخبري هي 1,20٪ بينما كانت نسبة الإصابة بالساركوسست في الإبكار بالفحص الميكروسكوبى هي 85٪.

وقد كانت نسبة الإصابة بالسيستيركس بوفيز في الحالات المصابة بحليبة باستخدام أختبار الألبزا هي 6 حالات بينما كانت هناك 58 حالة سلبية و57 حالة بها أصابة كاذبة. وقد تبين أن نسبة الإحساسية والتخصصية لمستضد السيستيركس بوفيز لتشخيص حويصلات السيستيركس في الإبكار هي 100٪ و77,1٪ على التوالي.

وباستخدام الطريقة المناعية لمستضد السيستيركس بوفيز باستخدام المصل العالي المناعي من الأراب المصنعة حيث أظهرت 5 حلقات بيتيدية أورانها الزيتية تتراوح بين 210 و 12 كيلودالتون. وفي المقابل ظهرت حلقات بيتيدية عند استخدام المصل المناعي من آبيير محضية طبيعية بدلاً من السيستيركس بوفيز والساركوسست مع مستضد السيستيركس بوفيز وقد وجد أن هناك حلقة بيتيدية ممتدة 115 كيلودالتون ظهرت بين كل منهما وقد تبين من هذا السبب في التفاعل التداخللي بينهما عند استخدام أختبار الألبزا.

المكونين:
أ.د. حسين عمر
أ.د. أبوالعجم محمد