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SERODIAGNOSIS OF SARCOCYSTIS SPECIES IN CATTLE USING ELISA

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SUMMARY

This study was performed to compare the result of microscopical examination of oesophogeal muscles with that of Enzyme-linked immunosorbent assay (ELISA) for detecting *Sarcocystis* infection in cattle. For this purpose, one hundred and twenty samples of muscles and blood were collected and examined. *Sarcocystis* cysts were detected microscopically in 81.6 % of the examined cattle, while, by ELISA with two prepared antigens of bradyzoites from cattle *Sarcocystis spp.* and *S. fusiformis* of buffaloes detected specific antibodies in 83% and 85% of the tested serum samples, respectively. The sensitivity of both antigens was 100%, while the specificity was 91% and 82% for bradyzoites of cattle *Sarcocystis spp.* antigen and *S. fusiformis* antigen, respectively. Rabbit hyper-immune sera raised against bradyzoites of cattle *Sarcocystis spp.* antigen reacted against 2 bands with M.W. 26KDa and 28.5KDa in both antigens. One band with M.W. 31 KDa was recognized with bradyzoites of cattle *Sarcocystis spp.* antigen. ELISA using bradyzoites of cattle *Sarcocystis spp.* antigen was found to be suitable and can be utilized for diagnosis *Sarcocystis* infections in cattle.

INTRODUCTION

Sarcocystis spp. is heteroxenous cyst-forming coccidia commonly found throughout the world. The life cycle generally involves a herbivorous intermediate host, which is infected after ingesting sporocysts, shed in the feces of carnivorous definitive host. *Sarcocystis* infection is an important factor limiting animal production (*Dubey et al., 1989*). Acute sarcocystosis may cause death of the host and abortion (*Dubey and Bergeron,* 1982). However, most infections with *Sarcocystis spp.*

and poor milk production, thus affect significantly animal production. The presence of *Sarcocystis* infection in muscles produces a toxin sarcocystin, which affects on the heart and gastrointestinal tract (Herbert and Smith, 1987). Some *Sarcocystis spp.* lead to condemnation of the infected carcasses, as *Sarcocystis* infection renders meat unacceptable to consumer and causes health hazards.

There are three valid species of *sarcocystis* in cattle which act as intermediate host, *Sarcocystis cruzi* (*S. bovicanis*), *Sarcocystis hirusta* (*S. bovifelis*), *Sarcocystis hominis* (*S. bovihominis*) (Dubey et al., 1989). Bovine Sarcocystosis is known to cause considerable morbidity and mortality in cattle (*Dubey*, 1976).

Sarcocystis infections are conventionally diagnosed post-mortem by using compressorium to detect muscle cysts or during histopathological examination. As the clinical diagnosis of *Sarcocystis* infection is difficult due to the relatively non-specific clinical signs of the disease, various serological methods are used to detect the circulating antibodies produced due to *Sarcocystis* infection (O'Donoghue and Weyreter, 1983). *Fayer and Lunde* (1977) detected circulating antibodies in cattle infected with *Sarcocystis* by IHA test. *Shi* and Zhao (1987) used ELISA to detect antibodies in sera of cattle. Savini et al., (1994) examined serum samples for antibodies against crude antigens of cystozoites and merozoites of *S. cruzi, S. tenella*-using ELISA. In 1997, *Savini et al.*, compared the result obtained by digestion of oesophageal samples and ELISA using two antigens from merozoites and cystozoites of *S. cruzi*. Kalubowila et al., (2004) detected antibodies to *Sarcocystis* infection in cattle and buffaloes using ELISA with antigen derived from cystozoites of *S. cruzi*. *Singh et al.*, (2004) tested serum samples of cattle for antibodies against bradyzoites of *Sarcocystis spp.* antigen using double immuno diffusion, counter immunoelectrophoresis and dot-ELISA.

The present investigation was performed to compare the diagnosis of sarcocystosis using the parasitological examination with ELISA to detect antibodies against bradyzoites of *Sarcocystis spp.* antigen of cattle and *Sarcocystis fusiformis* antigen of buffaloes circulating during early infection. Immunoblot was developed to determine species specificity of the two antigens.

MATERIALS AND METHODS

I **Collection of samples:** Blood and oesophageal muscles were collected from 120 local cattle slaughtered at El-Bassatin abattoir. Parasitological examination of each muscle sample was carried out on the same day of meat collection 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 under the low power of light microscope. The infection was evaluated by counting the number of cysts per gram of meat. The infection was considered light, medium and heavy when counting 1-10, 11-25 and more than 25 sarcocystis cysts per gram respectively.

II **Collection of sera:** Serum samples were obtained from 120 cattle and stored in aliquots at –20 °C till use.

III **Antigen preparation:** Two types of antigen, bradyzoites of *Sarcocystis spp.* of cattle and cystozoites of *S. fusiformis* from buffaloes (*Bubalus bubalis*) were used to examine serum samples and the results of both antigens were compared.

a- Bradyzoites antigen: Bradyzoites were obtained from heavily *Sarcocystis* infected oesophageal muscles of naturally infected cattle by digestion technique (Singh et al., 2004). Forty grams of tissue were chopped into small pieces and incubated in 1% pepsin –HCl digestion solution for 1 hour at 37 °C with agitation. The digestible muscle fluid was sieved and the filtrate was centrifuged at 500G for 5 min. The supernatant was removed and the sediment was resuspended in PBS pH 7.2 and washed 5 times by centrifugation till white bradyzoites appeared. Then, repeated freezing and thawing three times followed by sonication ruptured bradyzoites. The suspension was centrifuged at 14,000 rpm for 45 minutes and the supernatant was collected and aliquoted. The protein concentration of the antigen was determined (Lowry et al. 1951).

***b- Sarcocystis fusiformis* antigen:** Large visible cysts (40/ml PBS pH 7.2) were harvested from the oesophageal muscles of naturally infected buffaloes slaughtered at El-Bassatin abattoir. The antigen was prepared from the collected cysts as the same described for bradyzoites antigen.

IV Preparation of hyper-immune sera:

Hyper-immune sera against bradyzoites of *Sarcocystis spp.* in cattle was produced in New

Zealand white rabbits at the age of 2 months. Two rabbits were primed subcutaneously with 200-ug protein of the prepared bradyzoites antigen emulsified in Freund's complete adjuvant. They were boosted 14 and 28 days after priming with 150-ug 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 Serodiagnostic techniques:

a- Enzyme- linked immunosorbent assay (ELISA): Specific antibody against *Sarcocystis* antigens (bradyzoites of *S. spp.* of cattle and *S. fusiformis* antigens) were detected in the cattle tested sera by ELISA (*Iacona et al.*, 1980). Wells in ELISA plates were coated with 100 ul of different *Sarcocystis* antigens at the rate of 40 ug /ml of coated buffer overnight at 4°C. After washes with PBS, wells were saturated with 100ul/well of PBS containing 5% BSA for 1hour. One hundred ul serum diluted 1:100 in PBS-T was added to each well after washing and incubated for 1h. Plates were washed and then incubated with Alk. Phosphates (1:5000) (Sigma com.). After washing P-nitrophyenyl phosph. Substrate (1tab/5ml buffer) was added. 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.

b- Immunoblots: Protein bands of both antigens 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 2h on rocker platform. Rabbit sera diluted at 1:100 in 5% BSA/PBS-T were reacted with both fractionated *Sarcocystis* antigens 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 1h on rocker platform. The chromogen AEC substrate (Sigma com.) was added to NC strips and allowed to develop for 10 min. The reaction was visualized by the naked eye.

RESULTS

Ninety-eight of 120 fresh oesophogeal muscle samples of slaughtered cattle were found to be positive for *Sarcocystis spp.* cysts using compression technique with a prevalence of infection 81.66%. Out of the 98 *Sarcocystis* infected cattle, 17 (18.75%) had light infection with 1-10 cysts/g, 62 (60.9%) had moderate infection with 11-25 cysts/g and 19 (20.3%)

was considered heavily infection which contained more than 25 cysts/g.

The ELISAs using the antigen from bradyzoites of *Sarcocystis spp.* from cattle and *S. fusiformis* from buffaloe detected levels of antibodies in 100 (83%) and 102 (85%) of sera of the examined cattle, respectively. Two and four samples out of 22 negative samples for *Sarcocystis* by compression technique tested positive by ELISA using bradyzoites of cattle *Sarcocystis spp* and *S. fusiformis* antigens, respectively. Positive serum samples had higher OD readings with bradyzoites of cattle *Sarcocystis spp.* antigen (0.62±0.035) than *S. fusiformis* antigen (0.45±0.021). A very high correlation was observed between the parasitological results and the results obtained from ELISA which used antigens from either bradyzoites of cattle *Sarcocystis spp.* or *S. fusiformis* as the sensitivity was 100% for both antigens, while the specificity was 91% and 82% for the two antigens, respectively (Table1).

The transferred protein bands to nitrocellulose membrane of both bradyzoites of cattle *Sarcocystis spp.* and *S. fusiformis* antigens were reacted with rabbit anti-sera raised against bradyzoites of cattle *Sarcocystis spp.* antigen. Both antigens exhibited similar reactions against 2 bands but 31 KDa was recognized with bradyzoites of cattle *Sarcocystis* antigen. The hyperimmune sera were reacted strongly against 26 KDa of both antigens and slightly weaker against 28.5 KDa. (Plate 1).

Table (1): Effectiveness of ELISA using two antigens for detection of antibodies *to Sarcocystis spp.* in naturally infected cattle.

Antigen type	Serological results (ELISA)	Infection status examination of compression technique		
		Positive	negative	total
		98	22	120
Bradyzoites of cattle *Sarcocystis spp.*	Positive	98	2	100
	Negative	0	20	20
S. fusiformis	Positive	98	4	102
	negative	0	18	18

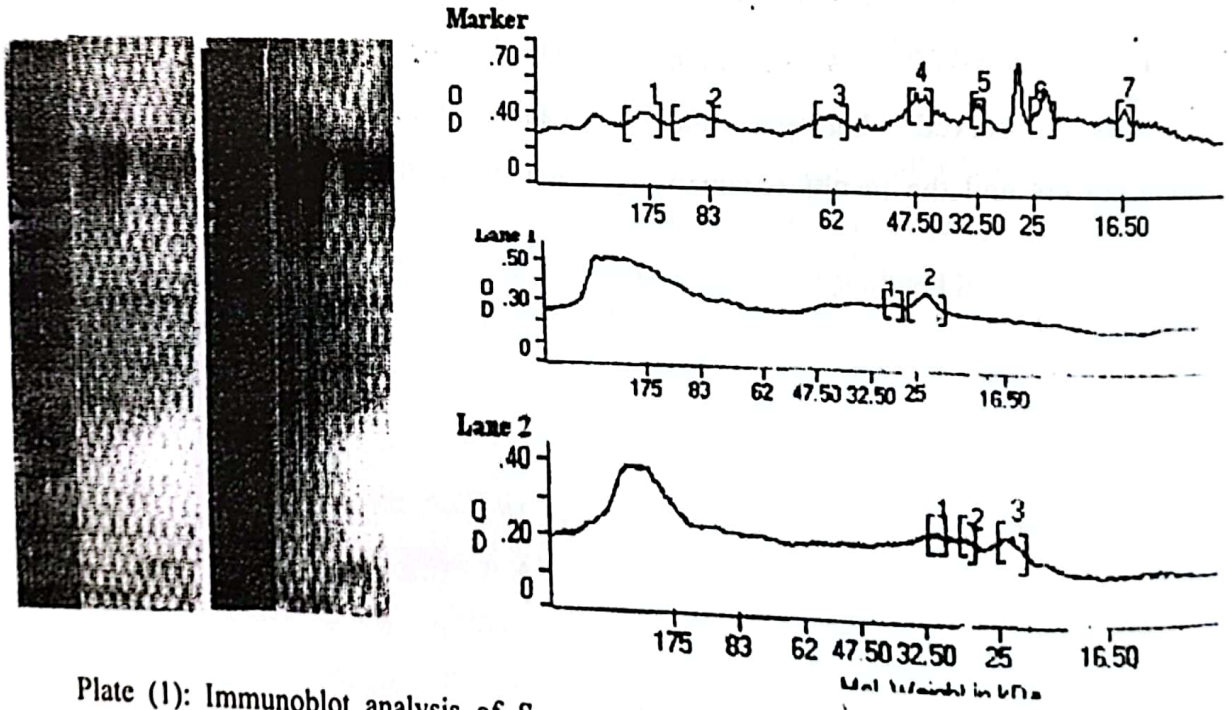

Plate (1): Immunoblot analysis of *Sarcocystis* antigens using hyper-immune sera against cattle Sarcocystis spp. Antigen.
M (marker): Prestained broad range molecular weight markers (Bio-lab com.).
Lane (1): Immunoblot analysis of *S. fusiformis* antigen.
Lane (2): Immunoblot analysis of cattle *Sarcocystis spp.*antigen.

DISCUSSION

Sarcocystis spp. is considered one of the causes of severe and even fatal disease in cattle (*Dubey*, 1976). In the present study out of 120 oesophageal muscle samples of cattle, 98(81.66%) were found to be positive for *Sarcocystis spp.* cysts using compression technique. This incidence was related to that of *El-Saieh* (1998) who stated that the incidence of *Sarcocystis* infection in adult cattle was 75.12% in Qena governorate. Also *Hassanien* (1992) recorded that 78.85% of examined cattle were infected with *Sarcocystis spp.* in Kalubia governorate.

On the other hand, *Mohamed* (1996) detected low incidence (30%) of *Sarcocystis* infection among the examined cattle in Assiut governorate. The difference of the incidence of *Sarcocystis* infections may be attributed to the difference in localities and the methods used for diagnosis. Oesophogeal muscles were selected to examine in this study, which were considered the most heavily infected organ with *Sarcocystis* in cattle (*El-Saieh*, 1998).

The compression technique for diagnosis of *Sarcocystis spp.* and other methods as digestion and histopathological methods in cattle tissues are not suitable for large scale screening programs or for diagnosing infections in live animals. Also, no practicable procedures can detect microscopical *Sarcocystis* cysts, and the carcass may pass for human consumption. So, serological diagnosis appears to provide suitable alternative methods for diagnosing *Sarcocystis* infection in cattle. ELISA results showed that, anti-*Sarcocystis* antibodies were detected using bradyzoites of cattle *Sarcocystis spp.* and *S. fusiformis* cyst antigens. The sensitivity was 100% for both antigens, whereas specificity was 91% and 82% respectively. This result indicated that, *S.fusiformis* antigen of buffaloes generally crossly reacted with bradyzoites of cattle *Sarcocystis spp.*. So, both antigens were able to differentiate between naturally infected and uninfected cattle sera. The present study confirmed other studies using serological assay for diagnosing *Sarcocystis spp.* in naturally infected cattle. *Shi and Zhao* (1987) evaluated ELISA for detection the antibodies against *Sarcocystis spp.* in naturally infected cattle. They detected *Sarcocystis* specific antibodies in 79.25% cattle. *Savini et al.* (1997) found that, sensitivity and specificity of the ELISA using antigen derived from merozoites were 98% and 97%, respectively, whereas, when antigen from cystozoites was used, the values were 95% and 84%, respectively. Also *Singh et al.* (2004) recorded that, the prevalence of *Sarcocystis*

spp. in cattle by DID, CIE and dot-ELISA as26%, 30% and 46%, respectively. In the present studies, from 22 samples with microscopy negative results using compression technique, 2 and 4 were positive serologically using bradyzoites of cattle *Sarcocystis spp* and *S. fusiformis* antigens, respectively. Therefore, animals may be in early stages of infection, and the cysts had not been composed yet, so can not be detected microscopically.

Concerning immunoblot, the anti-bradyzoites of cattle *Sarcocystis spp.* antigen reacted with both antigens at two bands with molecular weight 26 and 28.5 KDa. This result suggested that, the major components of two antigens were similar and the two species of *Sarcocystis* shared with many common epitopes. The obtained result agreed with *O'Donoghue et al.* (1990) who stated that immune sera collected from sheep experimentally infected with *S.gigentea* or *S.tenella* found to be highly cross-reactive for a variety of cystozoite antigens. However, single band with M.W. 31 KDa was detected in bradyzoites antigen. This indicated that 31 KDa may be responsible to specificity of bradyzoites of cattle *Sarcocystis spp.* and can characterize it. The strong recognized band with molecular weight 26 KDa can be used for diagnosis infection with *Sarcocystis spp.* in cattle.

This study showed that ELISA, using bradyzoites of *Sarcocystis spp.* antigen, can be effectively used for diagnosis *Sarcocystis* infections in cattle, and sero-epidemiological surveys on live stock can be carried out without the need to examine the animals after slaughtering.

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التشخيص السيرولوجي للساركوسيست في الأبقار باستخدام اختبار الامتصاص الإنزيمي المناعي

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أجريت هذه الدراسة لمقارنة نتائج الفحص الميكروسكوبي لعضلات المرئ مع اختبار الامتصاص الإنزيمي المناعي للكشف عن الإصابة بطفيل الساركوسيست في الأبقار. ولهذا الغرض تم تجميع وفحص 120 عينة من عضلات المرئ والدم من الأبقار وقد تم الكشف عن حويصلات الساركوسيست بالفحص الميكروسكوبي في 81.6% من الأبقار المفحوصة بينما تم الكشف عن الأجسام المناعية المتخصصة في أمصال الأبقار بواسطة اختبار الامتصاص الإنزيمي المناعي باستخدام مستضدين محضرين من براديزويت الساركوسيست بالأبقار والساركوسيست فيوزيفورم بالجاموس بنسبة 83% و85% على التوالي. وقد تبين ان نسبة الحساسية للمستضدين هي 100% بينما نسبة التخصص لمستضد براديزويت الساركوسيست بالأبقار والساركوسيست بالجاموس هي 91% و82% على التوالي. أما المصل العالي المناعة المحضر بالأرانب ضد مستضد براديزويت الساركوسيست بالأبقار قد تفاعل مع حزمتين اوزانهما الجزيئية 26كيلودالتون و28و5كيلودالتون في المستضدين بينما حزمة واحدةوزنها الجزيئ 31كيلودالتون تم الكشف عنها في مستضد براديزويت الساركوسيست بالأبقار ولذلك فإن اختبار الامتصاص الإنزيمي المناعي باستخدام مستضد براديزويت الساركوسيست بالأبقار هو الأنسب للاستخدام في تشخيص عدوى الساركوسيست بالأبقار