STUDIES ON SARCOCYTOSIS AMONG SOME FARM ANIMALS II- EVALUATION OF BRADYZOITES ANTIGENS BY ELISA

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Abstract

The value of Enzyme Linked Immunosorbent Assay (ELISA) for diagnosing natural buffaloes and camels sarcocystosis, as well as experimental sheep sarcocystosis were studied. In ELISA, the sensitivity and specificity were 80.85% and 53.19% for diagnosing buffaloes naturally infected with *S.fusiformis*, while the sensitivity and specificity were 93.62% and 95.74%, respectively for diagnosing Sarcocystis in naturally infected camels.

The characterization of bradyzoites antigens (buffaloes and camels origin) using SDS-PAGE were studied. Concerning the experimentally infected sheep with sarcocystosis (buffaloes and camels origin), the antibody levels appeared at 2 weeks post-infection and gradually increased till 6 weeks post-infection and nearly at constant level till the end of experiment (8 weeks post-infection).

INTRODUCTION

Sarcocystosis is one of the most important parasitic infections among farm animals. The diagnosis of sarcocystosis is very difficult. Therefore, serological techniques are needed to increase sensitivity of detection and confirmation of the disease in its early stage. Shi and Zhao (1987) found that the sensitivity for diagnosis of sarcocystosis in cattle by ELIA was 79.25%. Haralampidis et al. (1987) found that the sensitivity for diagnosis of sarcocystosis in sheep and goats by ELISA was 83.2%

and 82.0%, respectively.

The present work was planned to evaluate the bradyzoites antigens by the Enzyme-Linked Immuno-Sorbent Assay (ELISA) for diagnosis of sarcocystosis among buffaloes and camels as well as the detection of antibody levels through experimentally infected lambs.

MATERIALS AND METHODS

Preparation of bradyzoites antigens :

Buffalo sarcocysts antigen was prepared from the macroscopic *Sarcocystis fusiformis* cysts found in the oesophagus of the slaughtered buffaloes. The camel and sheep sarcocysts antigens were prepared from microscopic sarcocysts obtained from the oesophagus of salughtered animals highly infected. The collection of sarocysts was done by the method of pepsin digestion technique according to Jacobs and Melton (1957). The preparation of bradyzoites antigens was done by the method of Smith and Herbert (1986).

Collection of serum samples :

Forty-seven blood samples were obtained from slaughtered buffaloes at Cairo abattoir naturally infected with *S.fusiformis*. The same number was obtained from salughtered camels naturally infected with *S.cameli* at Cairo abattoir. Each meat sample was examined by compressorium (EL-Afifi et al. 1963) and pepsin digetion technique (Jacobs and Melton 1957). Faecal examination and meat inspection of different organs, as well as blood examination were done to be sure that the slaughtered buffaloes and camels were nearly free from any parasitic infestation other than Sarcocystis. Fifteen blood samples from parasite-free slaughtered camels, as well as the same number from buffaloes were obtained from Cairo abattoir and considered as non-infected controls. The sera were collected from positive and negative blood samples separately.

Nine newly born lambs were chosen and isolated, divided and inoculated as described in the first part. The serum samples were obtained every week from each lamb of both infected and non-infected control groups till the 8 th week post-infection. Serum samples were stored at -20^{o}C till used for detection of specific antibodies against sarcocystosis by ELISA.

Enzyme-Linked Immuno - Sorbent Assay (ELISA) :

This test has been adopted as described by Smith and Herbert (1986)with some modifications. The optimal reaction conditions as regards sensitizing antigen concentration, antibody and conjugate dilution were chosen for use with micro-ELISA after preliminary checker board titration. In the present study, the optimum conditions were 25 µg/ml coating buffer antigen concentration, 1:50 serum dilution, 1:250 goat antibovine I gG, 1:250 rabbit anti-sheep IgG and 1:500 protein-A peroxidase, as the conjugate. All materials were used at 50 µl/well volumes and all incubation steps were carried out at 37°C in a moist chamber. The postivitity threshold value (Optical density, OD) was determined as equal to or greater than the mean OD values of negative sera + 2 standard deviations (mean + 2 SD).

Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) :

The sarcocysts antigens of buffalo and camel origin were analyzed by SDS-PAGE using Laemmli technique (1970) with some modifications. In the present study, we used 10% separating gel, and the two samples were treated separately with 20% by volume of sample buffer, 20% by volume of glycerol 50% and 5% by volume of bromo-phenol blue. The samples were then immersed separately in a boiling water bath for 5 minutes to ensure complete dissociation of the sample proteins. A sample of 10 µg concentration was applied to each well. The low range (18.5 - 106 KDa) molecular weight prestain standard mixture (Bio-Rad) was then applied to the first well. A current of 35 m Amp was applied per gel, and the run terminated when the bromophenol had reached the bottom of gel. The proteins of two samples were detected by silver stain method according to Berl *et al.* (1980)

RESULTS

The sensitivity and specificity of ELISA with Sarcocystis antigens for diagnosis of buffaloes and camels sarcocystosis were studied. Tables 1 and 2 represent the results of ELISA test on bradyzoites antigens with sera of buffaloes and camels naturally infected with sarcocystosis. The positivity threshold value is also shown in ELISA, and when using bradyzoites-antigen (buffalo origin), 38 buffalo sera had an optical density (OD) higher than the corresponding threshold value, indicating (38/47) 80.85% sensitivity, while 25 infeced camel sera gave negative result indicating

(25/47) 53.19% specificity.

Table 1. Percentage of sensitivity by ELISA with Sarcocystis antigens (buffalo and camel origin) against IgG antibodies for diagnosis of sarcocystosis.

	Buffalo antigen against buffalo sera	Camel antigen against camel sera
Number of tested sera	47	47
Number of positive sera	38	44
Number of false negative sera	9	3
Sensitivity %	80.85	93.62

Table 2. Percentage of sepcificity by ELISA with Sarcocystis antigens (buffalo and camel origin) against IgG antibodies for diagnosis of sarcocystosis.

	Buffalo antigen against camel sera	Camel antigen against buffalo sera
Number of tested sera	47	47
Number of positive sera	22	2
Number of false negative sera	25	45
Sensitivity %	53.19	95.74

On the other hand, when using bradyzoites antigen (camel origin), 44 camel sera had an optical density higher than cut-off value indicating (44/47) 93.62% sensitivity. On contrast, 45 infected buffalo sera had an optical density lower than the cut-off value indicating (45/47) 95.74 % specificity.

In the present study, the bradyzoites antigens (buffalo and camel origin) were characterized with SDS-PAGE, and then, stained with silver stain. They showed 15 bands with molecular weights ranging between 175-14 KDa with bradyzoites antigen buffalo origin, but 11 bands with molecular weights ranging between 175-14 KDa with bradyzoites antigen camel origin (fig. 1).

Also, the reaction of sera from sheep experimentally infected with sarcocystosis (buffalo and camel origin) with bradyzoites antigen by ELISA were studied. Analysis of the obtained ELISA data showed a significant antibody levels at 2 weeks post-infection and gradually increased till 6 weeks post-infection. They nearly remained constant till the termination of the experiment after 8 weeks post-infection (Fig.2).

DISCUSSION

In the present study, the diagnosis of sarcocystosis by ELISA was studied. It was found that, the sensitivity was 80.85% in naturally infected buffaloes with *S.fusiformis* which was in agreement with that obtained by Shi and Zhao (1987) in diagnosis in cattle, while the sensitivity of sarcocystosis among camels was 93.62%. These data disagreed with Haralampidis et al. (1987) who found that the sensitivity for diagnosis of sarcocystosis among sheep and goats was 83.2% and 82.0%, respectively. These variations might be related to the difference of the host and the species of sarcocystis.

The specificity of bradyzoites antigens (buffalo and camel origin) was 53.19% and 95.74%, respectively. This cross-reaction between two antigens may be related to the similarity of some antigenic components (by SDS-PAGE) between the two bradyzoites antigens in the present study.

Concerning the detection of antibody levels by ELISA in the experimentally infected lambs with Sarcocystis from buffaloes and camels, they appeared 2 weeks post-infection, gradually increased till 6 weeks post-infection and became nearly

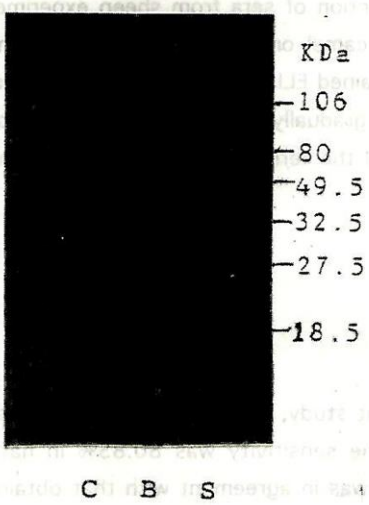

Fig.1. Characterization of bradyzoites antigens by SDS-PAGE

C. Bradyzoites antigen of Sarcocystis (camel orignin)

B. Bradyzoites antigen of Sarcocystis (buffalo orignin)

S. Pre-stained standard (Bio-Rad)

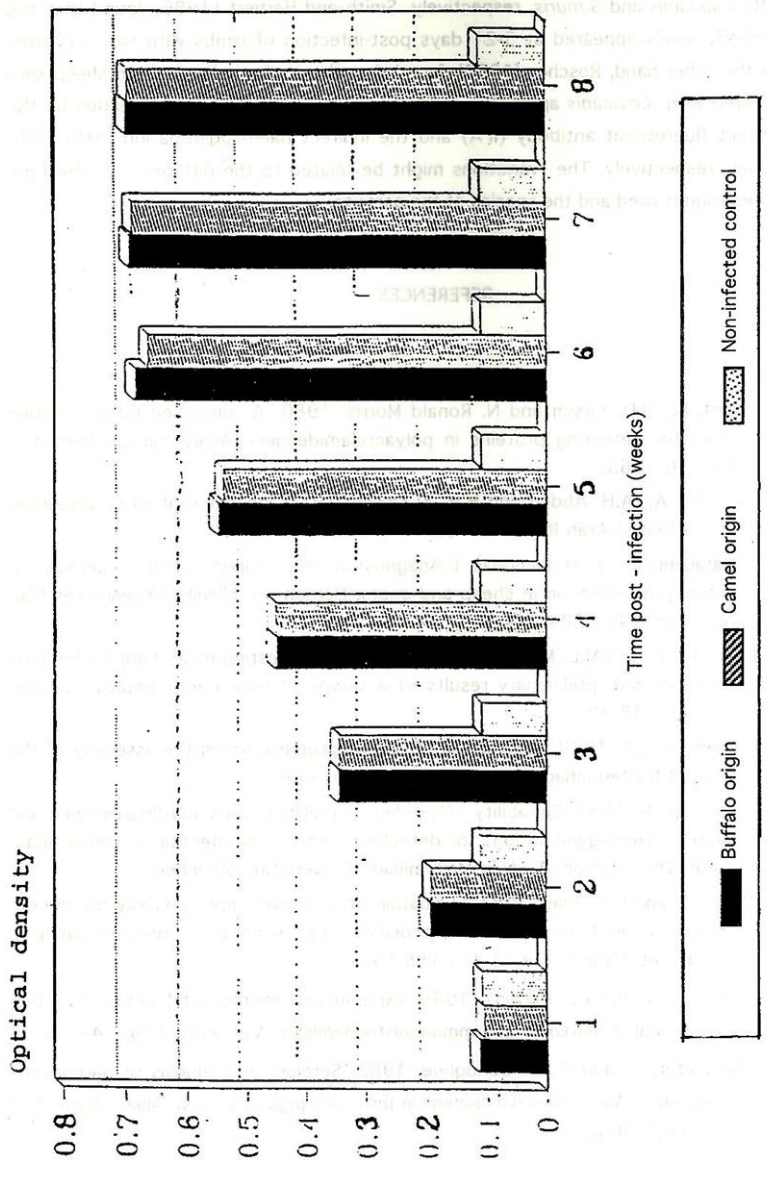

Fig. 2. Reactivity of bradyzoites antigen with sera from experimentally sarcocystosis lambs against IgG antibodies by ELISA.

tioned that the antibody levels appeared at 17 days post-infection of pigs and mice with *S.suicanis* and *S.muris*, respectively. Smith and Herbert (1986) found that the antibody levels appeared at 5-21 days post-infection of lambs with sarcocystosis. On the other hand, Roscher (1980) found that the antibody levels from sheep sera infected with *S.ovicanis* appeared at 4-12 days and 7-14 days post-infection by the indirect fluorescent antibody (IFA) and the indirect haemagglutination (IHA) techniques, respectively. The variations might be related to the difference of the type of techniques used and the species of the parasite.

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دراسات على الساركوسيستوزيس فى بعض حيوانات المزرعه
٢- تقييم المولد المضاد (الأنتيجين) باستخدام إختبار التحليل المناعى الإنزيمى المترابط (الإليزا)

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تم استخدام إختبار التحليل المناعى الإنزيمى المترابط (الإليزا) فى تشخيص الساركوسيستوزيس فى الجاموس والجمال المصابة طبيعياً وكذلك الحملان المصابة معملياً بالساركوسيست من أصل جاموسى وأصل جملى.

وجدت نسبة الحساسية ٨٠,٨٥ ٪ ونسبة التخصصية ٥٣,١٩ ٪ فى الجاموس، بينما كانت النسبة ٩٣,٦٢٪ ، ٩٥,٧٤٪ على التوالى فى الجمال. وقد ظهر حدوث نسبة من التفاعل التداخلى وتم توضيحه عن طريق فصل المولدين المضادين بإستخدام التحليل الكهربى فى البولى أكريلاميد إلى أجزائه المختلفة. وقد بينت الدراسة أن هناك أجزاء متشابهة بين المولدين المضادين يعزى اليهما حدوث التفاعل التداخلى بينهما.

كذلك أوضحت الدراسة ظهور الأجسام المضاده فى مصل الحملان المصابة معملياً بالساركوسيست من أصل جاموسى وجملى ، بعد أسبوعين من بدء العدوى ، وزادت تدريجياً حتى الأسبوع السادس ثم أصبحت ثابتة تقريباً حتى نهاية التجربة فى الأسبوع الثامن.