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Diagnosis of Eimeriosis in Cattle by ELISA Using Partially Purified Antigen

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Abstract: Chromatographic analysis of *Eimeria sp.* crude antigen was done by Sephacryl S-200 column. Four fractions were isolated by this approach. Comparative evaluation of the immunogenic binding activities of isolated fractions by ELISA proved the potency of fraction number one (F1) over the other three ones. Characterization of the isolated fraction (F1) by SDS polyacrylamide gel electrophoresis showed that the fraction consists of 3 bands of 225, 198, 114.5 KDa. Only one band of molecular weight 225 KDa was identified as immunogenic band by immunoblot assay in which cattle naturally infected sera were utilized. Diagnosis of *Eimeria* infection in cattle by ELISA in which F1 was utilized, recorded high infection percentage of 87.9% compared with coprological examination (24.2%). In conclusion: Fraction number one in the current research proved high diagnostic potential. It could be successfully utilized in the diagnosis of eimeriosis among cattle.

Key words: *Eimeria sp* · Chromatographic analysis · Diagnosis · SDS-PAGE · Immunoblot · ELISA

INTRODUCTOIN

Eimeria infections are one of the most common and important disease of cattle worldwide [1]. All age groups of cattle are susceptible to infection, but clinical eimeriosis is most common in young animals. Coccidiosis in cattle commonly occurs as subclinical disease and predisposing for great economical losses due to reduced appetite, reduced body weight, impaired feed conversion, unthriftness, diarrhea, dysentery, anemia and increased susceptibility to other diseases [2, 3]. The development of clinical coccidiosis in cattle mainly depends on factors like species of *Eimeria*, age of infected animal, number of oocysts ingested, presence of concurrent infections and type of production system and management practices [4]. Compared to clinical coccidiosis, subclinical coccidiosis is economically more important and may account for over 95% of all the losses associated with coccidiosis and can cost cattle ranchers more than US$400 million per annum. It can also delay growth of calves by as much as 2 months [5]. More than 20 species of *Eimeria* have been described in cattle, yet only a few cause significant diseases; for example, *Eimeria bovis* and *Eimeria zuernii* cause severe mortality rates, whereas *Eimeria auburnensis* and *Eimeria alabamensis* are considered mildly pathogenic [6].

Diagnosis of *Eimeria* infection is achieved by identifying oocysts in the faeces. However, faecal examination is time consuming and of low accuracy. So, immunodiagnosis which is based on detection of antibodies in the serum of infected animals [7] is a suitable diagnostic tool to apply early and accurate treatment to control the disease. ELISA and indirect immunofluorescent tests were developed for detection of antibodies to a protozoan parasite *Neospora sp*. in cattle by Pare *et al.* [8]. They proved that ELISA was more sensitive and specific test for serodiagnosis of *Neospora* infection. Moreover, the overall seroprevalence of *Neospora caninum* in dairy cattle was 13.3% by ELISA [9]. In addition, Hosseininejad *et al*. [10] used affinity purified 38 KDa *Neospora caninum* surface antigen (P38) for the sensitive and specific diagnosis of this infection in dog populations. In additon, a coproantigen ELISA test has been used successfully for immunodiagnosis of eimeriosis in cattle [11] and in calves by Ahmed and Hassan [12].

Sensitivity and specificity of serological tests were mainly affected by the antigen used. To increase the diagnostic potency of antigens, isolation of their immunodiagnostic fractions will be useful [13]. Consequently, the present study was designed to isolate partially purified antigen from *Eimeria sp.* oocysts and

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evaluate its potency in the diagnosis of cattle eimeriosis. Also, structural characterization of this antigen and detection of its immunogenic bands was another target.

MATERIALS AND METHODS

Animals: One hundred and fifty seven heads of cattle located at Lower Egypt during a period extended from 2008 to 2009, were used to perform this study.

Coprological Examination: Faecal samples were collected from the rectum into polyethylene sacs for parasitological examination. Samples were coprologically examined for the detection of *Eimeria sp*. oocysts using floatation technique as described by Soulsby [14].

Serum Samples: Blood samples corresponding to the faecal samples were collected (without anticoagulant) and centrifuged at 2000 rpm for 20 min. Sera were separated, labeled and stored at -20°C until be analyzed by ELISA.

Antigen Preparation: Sporulated oocyst antigen was prepared according to Kutkat *et al.* [15]. The oocysts were homogenized in 0.15 M phosphate buffer saline pH 7.2 using a Teflon glass homogenizer followed by sonication for 5 minutes to disrupt remaining intact oocysts. The homogenates were centrifuged at 15000 rpm for 45 min at 4°C. The protein content of the supernatant was determined according to Lowry *et al.* [16]. The supernatant was aliquoted and stored at -20°C until use.

Gel Filtration Chromatography: *Eimeria sp.* antigen was applied to Sephacryl S-200 column. Fractions were collected after the void volume had been passed. The absorbance of the eluate was monitored at 280 nm as described by McGonigle and Dalton [17].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): Eluted fractions of *Eimeria sp.* antigen were resolved by SDS-PAGE as described by Laemmli [18]. Molecular weight standards (Bio-Rad; USA) were applied in the same gel for calculating molecular weights of the examined components. The electrophoretically separated proteins were stained with Commassie brilliant blue dye.

Immunoblotting Assay: After electrophoresis, *Eimeria sp.* antigens were immunoblotted onto nitrocellulose membrane according to Towbin *et al.* [19] in a blotting system. Nitrocellulose membrane was incubated with *Eimeria* positive cattle sera from which infection with *Eimeria* was confirmed by detection of oocysts in their faeces.

Enzyme Linked Immunosorbent Assay (ELISA): Indirect-ELISA was adopted to evaluate the diagnostic potency of the four eluted fractions using pooled diluted naturally infected cattle sera (1:10, 1:100, 1:1000, 1:10000 and 1:100000). The assay was also utilized to assess the diagnostic potentials of the most potent purified fraction (F1) against sera samples collected from infected and non infected cattle. The optimum antigen concentration, serum and conjugate dilutions were determined by checkerboard titration and the test procedures were carried out according to Dumenigo *et al*. [20]. The cut-off values of optical densities were calculated as described by Hillyer *et al.* [21]. Serum samples from cattle in which infection with *Eimeria sp.* was confirmed by detection of oocysts in faeces were used to estimate sensitivity. Estimates of sensitivity were calculated according to Fleiss [22].

RESULTS

Fractionation of *Eimeria sp*. Oocysts Antigen by gel Filtration Chromatography: The elution profile of *Eimeria sp.* oocysts antigen from the Sephacryl S-200 column is depicted in a typical chromatogram with four peaks representing four fractions designated F1, F2, F3 and F4 (Fig.1).

Comparative Diagnostic Potency of Isolated Fractions: To assess the immunogenicity of chromatographically separated components of *Eimeria sp*. oocyst antigen to select the most potent one, the four fractions were examined by indirect-ELISA using different dilution of *Eimeria sp*. positive cattle sera. The comparative immunogenicity of these fractions is depicted in figure 2 with high potency associated with fraction 1.

Electrophoretic Profile of *Eimeria Sp*. Eluted Fractions: The electrophoretic profile of eluted fractions revealed 3 bands of 225, 198, 114.5 KDa for fraction 1 and 101, 87, 64 KDa for fraction 2 while, it revealed 6 bands of 53, 44, 37, 30, 22, 17 KDa for fraction 3 and 4 bands of 13, 10, 8, 6 KDa for fraction 4 as showed in figure 3.

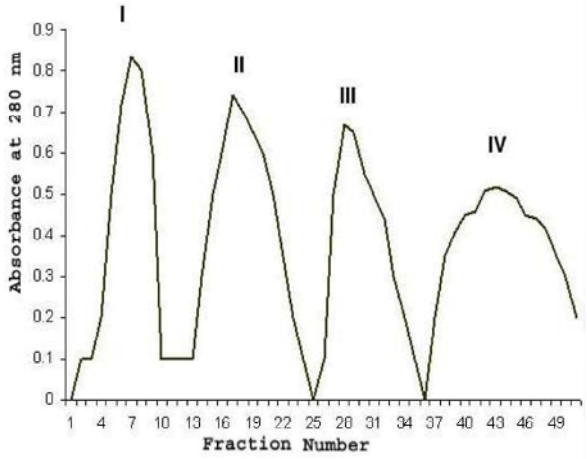

Fig. 1: Elution profile of *Eimeria sp*. antigen fractionated by gel filtration chromatography on Sephacryl S-200 column

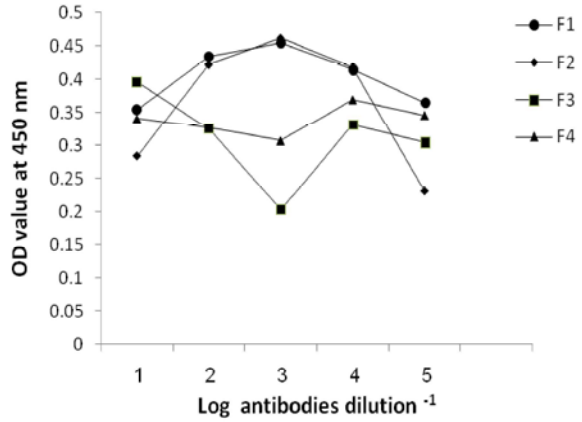

Fig. 2: Comparative diagnostic potency of *Eimeria sp.* fractions collected from Sephacryl S-200 column against different diluted positive cattle sera by ELISA

Western Blot Analysis of *Eimeria Sp*. Eluted Fractions: The immunoreactive bands of eluted fractions were identified with *Eimeria sp*. positive cattle sera by western immunoblot assay. One immunogenic band (225 KDa) was identified in fraction 1 (Fig. 4 lane 1). While, three reactive components of molecular weights 101, 87 and 64 KDa were detected in fraction 2 (Fig.4 lane 2). Also, fraction 3 have three reactive components of molecular weights 37, 30, 22 KDa (Fig. 4 lane 3) ,while fraction 4 was inactive (Fig. 4 lane 4).

Diagnostic Potentials

Coprological Examination: Coprological examination revealed that 38 faecal samples from 157 (24.2%) were positive for *Eimeria* infection (Table 1).

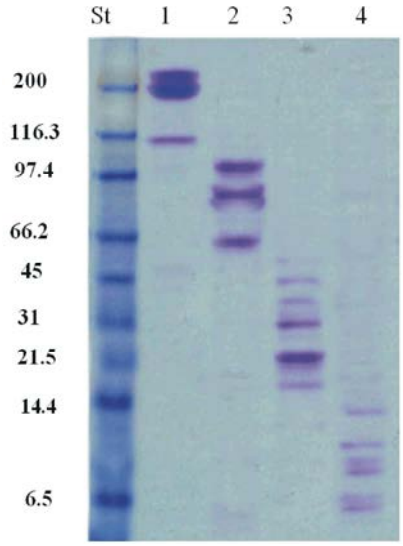

Fig. 3: Electrophoretic profile of *Eimeria sp.* eluted fractions: Lane 1 (F1), Lane 2 (F2), Lane 3 (F3), lane 4 (F4) and Molecular weight standards in KDa (St)

Fig. 4: Immunoreactive bands identified by positive cattle sera: Lane 1(F1), Lane 2 (F2), Lane 3 (F3) and Lane 4 (F4)

Table. 1: Comparative diagnosis of *Eimeria sp*. infection in cattle using coprological examination and ELISA.

		Coprological examination		
		Positive	Negative	Total
ELISA	Positive	38 (24.2%)	100 (63.7%)	138(87.9%)
	Negative	0.0 (0%)	19 (12.1%)	19(12.1%)
Total		38 (24.2%)	119 (75.8%)	157

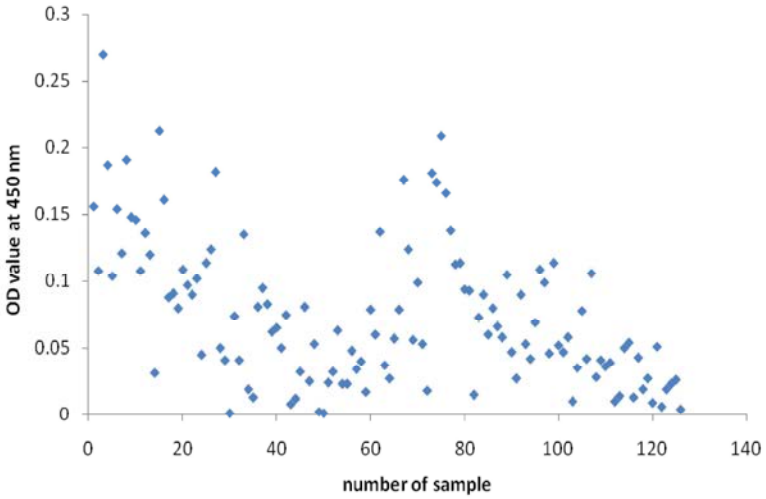

Fig. 5: Diagnostic potential of *Eimeria sp*. F1 in the diagnosis of eimeriosis among cattle

ELISA: The most potent fraction (F1) showed high diagnostic potency (87.9%) When tested against 157 cattle sera. Out of 138 samples positive by ELISA, 100 (63.7%) were negative by coprological examination (Table 1). All *Eimeria* infected cattle sera (confirmed by detection of oocysts in faeces) were positive reactors using ELISA recording 100% sensitivity (Fig. 5).

DISCUSSION

In grazing ruminants, diarrhea may be caused by protozoan pathogens belonging to the genus *Eimeria* of the subclass *Coccidia*. In cattle, several *Eimeria* species have been shown to be capable of establishing clinical disease associated with watery to bloody diarrhea, high morbidity and, depending on which *Eimeria* species is dominant, high mortality rates as well [23].

Concerning coprological examination, prevalence of *Eimeria sp.* infection (24.2%) in the present study was in line with the reported of 24.9% in Ethiopia [24]. However, our incidence was lower than the report of 48.2% in Germany [23], 67.4% in Kenya [25], 68% in Turkey [26], 70% in South Africa [27] and 70.7% - 75.8% in cattle calves in Kashmir [28]. This variation could be likely attributed to the differences in agro-ecology, management and husbandry practices of the study animals in different countries.

Regarding serodiagnosis, ELISA using the isolated fraction proved higher potency in the diagnosis of *Eimeria* infection among cattle compared with parasitological examination. ELISA succeeded to detect infection (positive cases) in 100 (63.7%) of the negative cases examined by parasitological technique. ELISA as a sensitive technique was previously proved [12, 30, 31]. But, the advantage in the current research is the fraction which was isolated from crude extract by gel filtration chromatography using Sephacryl S -200.

In the current research, purification by gel filtration chromatography revealed four fractions. Fraction number one (F1) was the most potent as judged by ELISA using naturally infected cattle sera. The fraction was composed of three bands of molecular weights 225, 198, 114.5 KDa as obtained from SDS-PAGE. Previous characterization of *Eimeria sp.* antigen was performed by Ahmed and Hassan [32] who used ion exchange chromatography assay in the purification of antigen. Their study revealed 8 bands of molecular weights ranged from 15-130 KDa are associated with the most potent fraction. The difference in the molecular weights of isolated fraction may be due to different used techniques.

In the present study, immunoblot assay was used to detect the immunoreactive bands of isolated fractions using cattle naturally infected sera. The most immunogenic fraction (F1) has one immunogenic band of molecular weight 225 KDa. Using the same technique in which buffaloes naturally infected sera were utilized [32], 4 immunogenic bands with lower molecular weights were detected in the most potent fraction.This difference in the number and molecular weights of immunogenic bands may be due to different utilized sera.

In conclusion, the present research introduced a model of partial purification of *Eimeria sp*. antigen by gel filtration using Sephacryl S-200. The most potent fraction exhibited 100% sensitivity in the diagnosis of infection among cattle. This trial is one of the very few studies concerned with fractionation

of *Eimeria sp*. antigen and the use of isolated fraction in diagnosis of *Eimeria* infection in cattle. In further investigation, fraction number 1 could be utilized on large scale in seroprevalence of *Eimeria* infection among bovine.

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