



Diagnosis of Cryptosporidiosis Using Affinity Purified Antigen

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CRYPTOSPORIDIOSIS is a significant disease that causes diarrhea in humans and animals with relatively high morbidity and mortality. The present study aimed to adopt a purified and potent antigen for the accurate diagnosis of cryptosporidiosis. A total of 278 animal hosts (60 newborn calves and 218 buffaloes) were used in the current study. Sixty fecal samples were collected from new-born calves aged less than one month raised in the Beni-Suef and Qalyubia governorates. The samples were examined under a microscope after modified Ziehl-Neelsen staining, and *Cryptosporidium* oocysts were isolated from naturally infected calves. These oocysts were used in mice experimental infection. The oocyst antigen and coproantigen were prepared from the mice feces. The diagnostic efficacy of the two prepared antigens was evaluated using an Enzyme Linked Immunosorbent Assay (ELISA) with experimentally infected mice sera. The crude oocyst antigen proved to have higher diagnostic potential than coproantigen, so, it was chosen for purification using Cyanogen Bromide-activated Sepharose-4B affinity chromatography coupled with rabbit hyperimmune serum raised against oocyst antigen. The affinity purified fraction and its crude *Cryptosporidium* antigen were evaluated using the ELISA. The resulting purified fraction was 6733 fold increase in binding activity compared with its crude antigen. Characterization of the isolated fraction was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot, and amino acid analysis. SDS-PAGE clarified that the fraction contained three polypeptides of 94.7, 65, and 50 kDa, which were identified as immune-reactive components using a western blot analysis. The isolated fraction exhibited 17 amino acids and was rich in tyrosine, alanine, and phenylalanine. The affinity purified *Cryptosporidium* oocyst antigen effectively detected *Cryptosporidium* antibodies in experimentally infected mice sera and naturally infected buffalo sera with a sensitivity of 94.4% and 95.24 %, and a specificity of 100% and 93.33%, respectively. The purified fraction succeeded in diagnosis of cryptosporidiosis in 182 random serum samples collected from buffaloes with an incidence of 57.14 %. In conclusion, the affinity purified fraction of the *Cryptosporidium* oocyst antigen might be a good diagnostic candidate for cryptosporidiosis diagnosis and seroepidemiological surveillance.

Keywords: Cryptosporidiosis, ELISA, Affinity Chromatography, SDS, Western Blot, Amino Acid Analysis.

Introduction

Cryptosporidiosis is one of the most important zoonotic diseases causing intestinal infection in

humans and animals [1]. It is a major cause of mortality due to neonatal scour in pre-weaned calves and lambs and is considered a globally

endemic disease for cattle [2,3]. Its pathogenicity varies with the species of *Cryptosporidium* and the age, type, and immune status of the host, resulting in clinical or subclinical cryptosporidiosis [4,5]. The common symptoms are acute, moderate to severe, profuse, and watery diarrhea, weakness, depression, dehydration, loss of appetite [6]. Death can occur in severe cases [7]. The clinical signs in livestock range from asymptomatic infection to death, which causes severe economic losses [2].

Cryptosporidium oocysts are excreted into the environment with human and animal feces, and transmission occurs via food or drinking water contaminated with oocysts, creating a potential reservoir, and causing contamination of the environment [8-10]. Oocysts of *Cryptosporidium* spp. play an important role in the prevalence of disease, as they are resistant to numerous environmental exposures and tolerant to various chemicals and disinfecting agents including chlorine that is commonly used in water treatment [11]. In addition, clinical cryptosporidiosis is usually misdiagnosed in pre-weaned calves, lambs, and other young animals because the symptoms can mimic scour, which is similarly confused with many other entero-pathogen infections (bacterial and/or viral), as they are associated with the same clinical manifestations. Therefore, an appropriate diagnosis for cryptosporidiosis is required.

Choosing an appropriate method for *Cryptosporidium* diagnosis depends on the required sensitivity of the applied assay and specificity of the antigen, technical expertise, financial resources, and available time [12]. Direct detection of *Cryptosporidium* spp. oocysts in the feces of infected hosts is necessary for an accurate diagnosis of this parasite, as a clinical diagnosis is not sufficiently specific [13]; coprological diagnosis cannot determine the existence of a past infection because of the short and intermittent period of oocysts shedding [14]. Microscopic examination of stained fecal samples is traditionally used, and although it is simple and cost-efficient, it lacks sensitivity [15]; its accuracy depends mainly on the expertise of the microscopist [16], as misdiagnoses frequently occur because of insufficient knowledge about oocyst morphology and characteristics [11]. Also, the diagnostic accuracy is significantly reduced by lower numbers of oocysts, irregularly excreted oocysts and/or mechanically or enzymatically damaged oocysts [12,13].

Enzyme Linked Immunosorbent Assay (ELISA) could offer higher sensitivity and specificity than conventional microscopy [12,19], with a recorded detection limit of about 10^3 – 10^4 oocysts/mL [20] and would reduce labor, cost, and time. The detection of circulating antibodies to *Cryptosporidium*-specific antigens in serum samples is an indirect diagnostic method for current infection or past exposure [21]. ELISA is a reliable technique that is mainly beneficial for the diagnosis and sero-epidemiological detection of cryptosporidiosis. Also, increasing its specificity by using specific antigens is needed to achieve better results. Purified immunodiagnostic fractions were better in diagnosis using ELISA as they gave higher sensitivity and specificity than crude extracts as previously proved in several studies with different parasites [22-24].

Since the diagnosis of cryptosporidiosis is essential for effective treatment, the present study aimed to develop a purified and effective antigen for the accurate diagnosis of cryptosporidiosis. The diagnostic efficacy of this purified antigen was evaluated by ELISA and its characterization was performed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), western blot, and amino acid analysis.

Materials and Methods

Ethical approval

All experimental procedures were conducted according to the guidelines laid down by the International Animal Ethics Committee and the institutional guidelines of the National Research Centre (NRC) Animal Research Committee under protocol number: 19-152.

Study design and animal sampling

Cattle and buffaloes

A total of 278 animal hosts (60 newborn cattle calves and 218 buffaloes) were organized into a cross-sectional study using a convenient sampling strategy from April 2021 to December 2021. Fecal samples were collected from 60 newborn cattle calves aged less than one month and reared by local farmers in Beni-Suef (40/60; 29° 03' 60.00" N, 31° 04' 60.00" E) and Qalyubia (20/60; 30° 7' 42.9996" N, 31° 14' 31.9992" E) governorates, Egypt. In addition, blood samples were collected from 218 buffaloes (3 mL of each buffalo) from the cut carotid artery or jugular vein immediately after slaughter at the main abattoir of El-Warak, Giza (29° 58' 27.00" N, 31° 08' 2.21" E), in plain Vacutainer tubes for serum

separation. The serum samples belonged to one of three groups—randomly collected serum (n = 182); negative control serum from apparently healthy buffaloes, (confirmed by examination of feces using microscopy, sedimentation, flotation [25], and modified Ziehl-Neelsen staining (MZN) [26]) (n = 15), and positive control serum from *Cryptosporidium*-infected buffaloes, confirmed by the same techniques (n = 21). All sera were labeled and stored at -20°C .

Lab animals (rabbits and mice)

Five healthy male New Zealand parasite-free rabbits weighing 1.5–2 kg each, and 60 parasite-free Swiss albino mice, aged two weeks old, were purchased. The animals were housed, fed a standard diet, and housed under sanitized conditions.

Parasite

Isolation of Cryptosporidium oocysts

A thin layer of each fecal sample was applied onto a glass slide, fixed with absolute methanol, stained with MZN [26], and examined under a light microscope (Olympus Corporation, Japan) with oil immersion.

Oocysts were concentrated from *Cryptosporidium*-positive feces by flotation using Sheather's sugar solution [27], collected, and stored in potassium dichromate solution (2.5%) at 4°C . Prior to the experimental infection of mice, oocysts were washed in a Phosphate Buffered Saline (PBS) solution.

Experimental infection

Fifty-five parasite-free Swiss albino mice were experimentally infected with a dose of 10^4 *Cryptosporidium* oocysts by gastric tubes in a single dose one hour before a meal [28]. After three days, animal fecal pellets were collected and examined using the MZN staining technique [26] to confirm infection. Five mice were sacrificed day by day, and blood samples were collected from the day zero through the 21st day. Five mice were used as a non-infected control group and sacrificed at the end of the experiment. Serum samples were separated and frozen at -20°C for further use.

Antigen preparation

Coproantigen

Fecal samples from mice experimentally infected with *Cryptosporidium* were shaken vigorously in an equal volume of 0.15 M PBS containing 0.3% Tween-20 until a slurry was formed. The samples were centrifuged at 15000 rpm for 45 minutes [26]. The supernatants were

frozen at -20°C until use.

Oocyst antigen

Cryptosporidium oocyst antigen was prepared according to Kaushik et al. [30]. Briefly, after the washing *Cryptosporidium* oocysts three times with PBS (15000 rpm for 15 min), they were resuspended in PBS and freeze-thawed for 20 cycles. The oocysts were then sonicated for 12 cycles of 30 seconds each and centrifuged (15000 rpm for 15 min at 4°C). The supernatants were frozen at -20°C until use.

Hyperimmune serum preparation

Hyperimmune serum against the oocyst antigen (40 μg antigen/kg body weight) was raised in five rabbits according to Fagbemi et al. [31]. Blood samples were collected from the ear vein four days after the last injection. The prepared hyperimmune serum was separated and frozen at -20°C until use.

Affinity purification of oocyst antigen

The prepared rabbit hyperimmune serum (raised against *Cryptosporidium* oocyst antigen) were dialyzed for three days in coupling buffer (0.1 M NaHCO_3 and 0.5 M NaCl, pH 8.4) and then coupled to cyanogen bromide (CNBr)-activated Sepharose-4B (Sigma-Aldrich) at the ratio of 2 mg/ml-swollen beads following the manufacturer instructions. The oocyst antigen was applied to the column (Flex-Column, Kimble, USA), and the bound fractions were eluted using 50 mM glycine containing 500 mM NaCl (pH = 2.3) and stored at -20°C until use.

Protein determination

The protein concentrations of coproantigen, oocyst antigen, affinity purified antigen and the hyperimmune serum were estimated as described by Lowry et al. [32].-

SDS-PAGE and western blot

Crude *Cryptosporidium* oocyst antigen and its isolated fraction were mixed separately with a reducing buffer containing 5% 2-mercaptoethanol and electrophoresed using SDS-PAGE with a 10% slab gel according to Laemmli [33]. The gel was stained using a silver stain [34]. The molecular weight standard (Genedirex, USA) was electrophoresed on the same gel to measure the relative molecular weights of examined antigens.

Two gels of SDS-PAGE-separated oocyst antigen and its specific fraction were electroblotted onto two nitrocellulose membranes (Himedia Labs., India), as described by Towbin et al. [35],

in a blotting system. One of them was incubated with serum from buffaloes naturally infected with *Cryptosporidium*, diluted 1:200 in dilution buffer (0.01 M Tris buffer saline PH 7.4 containing 0.5% bovine serum albumin and 0.05% Tween-20), and the other was incubated with serum from mice experimentally infected with *Cryptosporidium* (1:200 in dilution buffer) overnight at 4°C. The two membranes were washed and incubated with anti-bovine IgG horseradish peroxidase conjugate (Sigma-Aldrich) and anti-mice IgG horseradish peroxidase conjugate (Santa Cruz Biotechnology, Inc.), separately, at 1:2000 dilutions in dilution buffer. Immuno-reactive bands were visualized with 4-chloro-1-naphthol (Sigma-Aldrich) as a substrate. The two nitrocellulose membranes were analyzed using a Molecular Imager Gel Doc™ XR with Image Lab Software.

Amino acid analysis

One hundred microliters of purified fraction were hydrolyzed with 10 mL HCl-phenol solution (10 ml 6 M HCl containing 100 mg phenol) for 24 hours at 110°C. The resultant mixture was evaporated at 40°C and 100 rpm. Ten microliters of citrate buffer (0.1 M, pH: 2.2) were added to the dried residue, and 1 mL was filtered and injected into an HPLC system (Agilent 1260 series, USA) [36].

ELISA

Indirect ELISA was carried out three times according to Lind *et al.* [37] with some modifications.

Firstly: to evaluate the diagnostic potential of the prepared antigens (oocyst antigen and coproantigen) against two-fold serially diluted experimentally infected mice sera. The conjugate anti-mice IgG horseradish peroxidase (Santa Cruz Biotechnology, Inc.) was used.

Secondly: to evaluate the success of the purification process by determining the antigenic activities of the eluted specific bound fractions compared to unbound ones and its crude extract, against two-fold serially diluted sera of *Cryptosporidium* naturally infected buffaloes and experimentally infected mice, independently. Anti-bovine IgG horseradish peroxidase (Sigma-Aldrich) and anti-mice IgG horseradish peroxidase (Santa Cruz Biotechnology, Inc.) conjugates were used based on the host serum species.

Thirdly: to validate and evaluate the diagnostic potency of the affinity purified fraction in cryptosporidiosis diagnosis using the experimentally infected and non-infected mice sera and the collected random buffalo sera. Anti-bovine IgG horseradish peroxidase conjugate (Sigma-Aldrich) and anti-mice IgG horseradish peroxidase conjugate (Santa Cruz Biotechnology, Inc.) were used for buffalo and mice sera, respectively. Ortho-phenylenediamine (Sigma-Aldrich) substrate buffer was used, and the plates were read spectrophotometrically at 450 nm using ELISA reader (BIO-TEK, ELx800UV, USA). ELISA OD cut off values were calculated according to Allan *et al.* [29]. Specificity, sensitivity, positive and negative predictive values, and diagnostic efficacy percentages were calculated according to Parikh *et al.* [38].

Results

Coprological examination of the collected fecal samples:

Forty-eight out of the collected 60 calves' fecal samples (80%) were infected with cryptosporidiosis. Collected oocysts were used in the experimental infection of mice (Fig. 1).

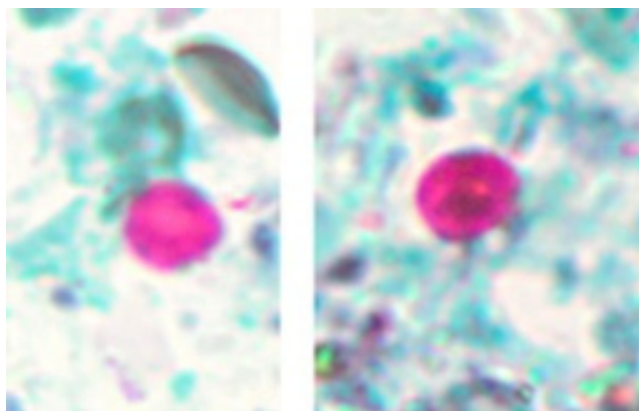


Fig. 1. *Cryptosporidium* oocysts in calf fecal smears stained with modified Ziehl-Neelsen (X1000).

Comparative evaluation of the diagnostic potency of the prepared crude oocyst and copro-antigens

The result of the comparative diagnostic potential of coproantigen and oocyst antigen against experimentally infected mice sera demonstrated that the oocyst antigen had a higher capacity in detecting anti-*Cryptosporidium* antibodies as proved by ELISA (Fig. 2).

Purified specific fraction of the oocyst antigen

As shown in Table 1, about 72.64% of the initial antigenic activities in crude *Cryptosporidium* oocyst antigen were recovered in the bound and eluted fraction which represents only 1.25% of the total protein in crude *Cryptosporidium* oocyst

antigen applied to the column, giving 6733-fold increases in the specific activities compared to the crude antigen.

Immunogenic activities of the purified specific fraction of the oocyst antigen

The affinity purified *Cryptosporidium* oocyst fraction showed higher potency than its crude antigen in cryptosporidiosis diagnosis in two-fold serially diluted naturally infected buffalo sera (Fig. 3) and two-fold serially diluted experimentally infected mice sera (Fig. 4) by ELISA. The immunogenic diagnostic potency of the fraction was still present when the serum samples were diluted up to 1:4096 (Fig. 3,4).

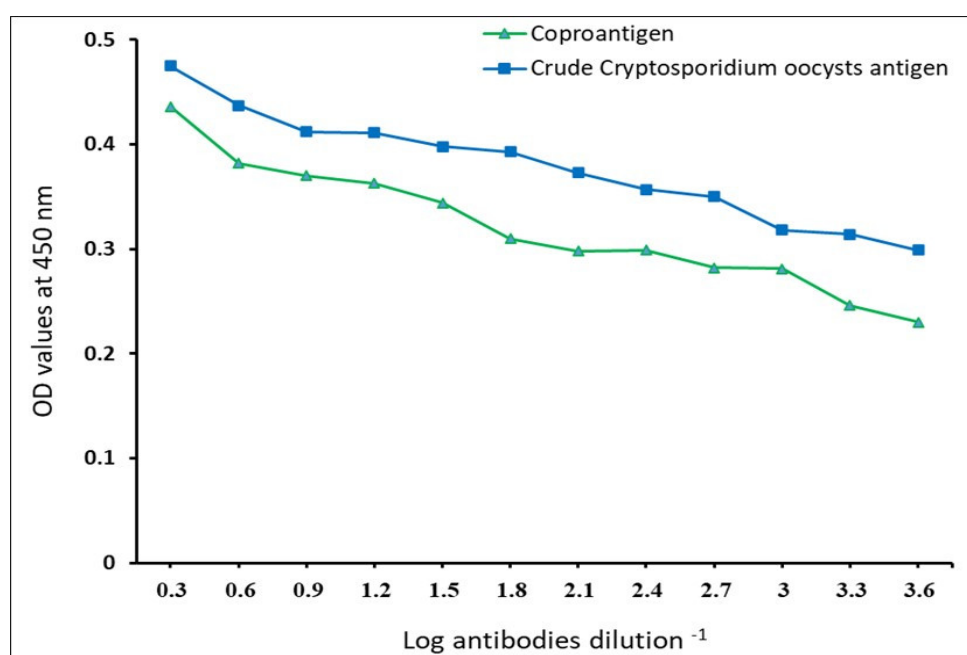


Fig. 2. Comparative diagnostic potency of the *Cryptosporidium* coproantigen and crude oocyst antigens against two-fold serially diluted experimentally infected mice sera.

TABLE 1. Quantitative summary of *Cryptosporidium* oocyst purification.

Fraction	Total protein ^a ($\mu\text{g} \times 10^{-4}$)	Activity unit ^b (Au $\times 10^{-4}$)	Specific activity ^c (Au/ $\mu\text{g} \times 10^{-2}$)	Purification Fold	Yield (%)
Crude <i>Cryptosporidium</i> oocyst	24.0	4.1	0.17	1	100
Unbound to column	6.46	0.4	0.061	0.133	9.8
Bound and eluted fraction	0.3	3.0	9.3	6733	72.64

^a Protein was measured as described by Lowry et al. (1951).

^b Activity unit (Au): defined as the amount of required protein to give one well of agglutination.

^c Specific activity: defined as the number of activities per microgram of protein and is related to the starting crude *Cryptosporidium* oocyst.

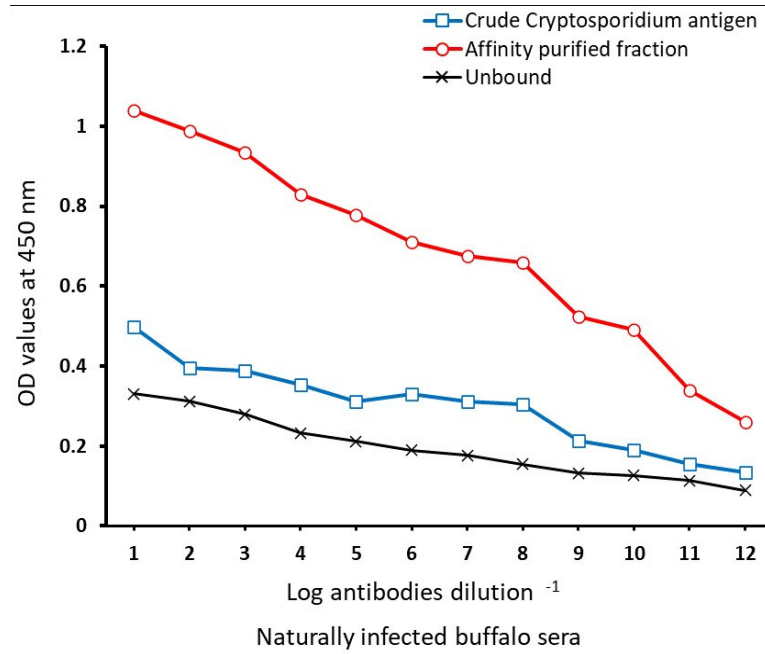


Fig. 3. Reactive binding activities in sera from buffalo naturally infected with *Cryptosporidium* toward the affinity purified fraction, its crude antigen and unbound antigen.

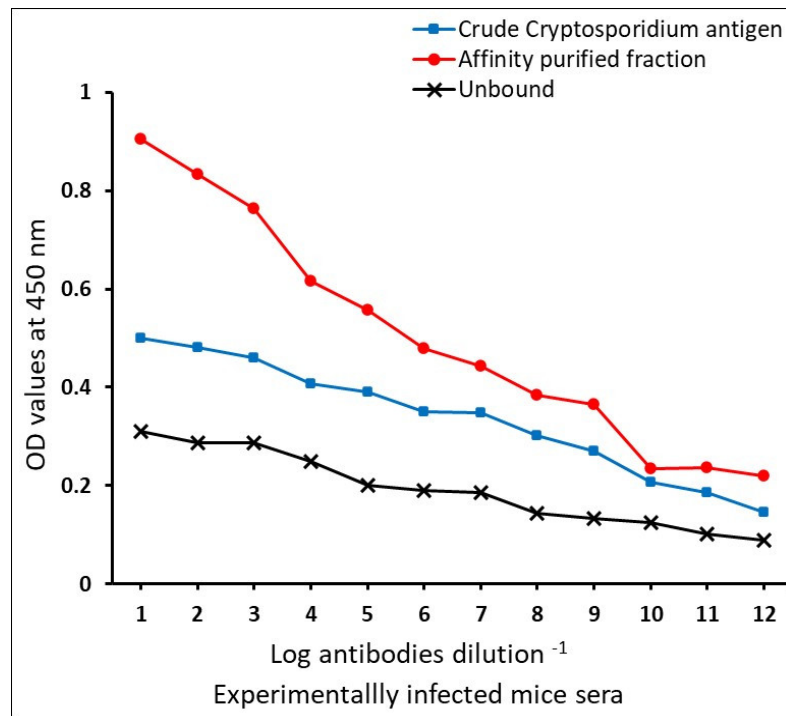


Fig. 4. Reactive binding activities in sera from mice experimentally infected with *Cryptosporidium* toward the affinity purified fraction, its crude antigen and unbound antigen.

Electrophoretic profile of the affinity purified fraction and crude antigen

Under reducing conditions in an SDS-PAGE 10% slab gel, the affinity purified fraction was resolved into three bands with molecular weights of 94.7, 65 and 50 kDa (Lane B), compared to the crude extract, which migrated into twelve bands at molecular weights 203.9, 130, 94.7, 86.8, 65, 50, 45, 33, 29, 19.4, 17 and 11kDa (Lane A), as shown in Fig. 5.

Immunogenic reactive components of the affinity purified fraction by western blot assay

The immunogenic reactive components of the specific purified fraction of the *Cryptosporidium* oocyst antigen recognized by sera from experimentally infected mice (Fig. 6, A, Lane 2) and sera from naturally infected buffaloes (Fig. 6, B, Lane 2) were 94.7, 65 and 50 kDa. Whereas the immunogenic reactive bands of its crude extract with sera from experimentally infected mice were 203.9, 94.7, 65, 50, 47, 32, and 11 kDa (Fig. 6, A, Lane 1), and 203.9, 94.7, 65, 50, 29, and 11 kDa were identified against sera from naturally infected buffaloes (Fig. 6, B, Lane 1).

Amino acid analysis of the affinity purified fraction

The purified fraction exhibited 17 amino acids (Table 2) with high proportions of tyrosine (90.595 µg/mL), alanine (87.1 µg/mL) and phenylalanine (82.595 µg/mL), while glycine had the lowest concentration (37.535 µg/mL).

Assessment of the antigenic activities and the diagnostic immunological values of the isolated fraction of the oocyst antigen

The diagnostic potentiality of the affinity purified fraction of the oocyst antigen was validated using the positive and negative *Cryptosporidium*-infected and non-infected mice sera controls. The indirect ELISA test scored a sensitivity of 94.4 % and the specificity of this purified fraction in the detection of the anti-*Cryptosporidium* IgG was 100%. The positive predictive value (PPV) and negative predictive value (NPV) were 100% and 91.7%, respectively, while the recorded diagnostic efficacy was 96.6%. Additionally, the success of the *Cryptosporidium* diagnostic fraction in the detection of specific buffalo anti-*Cryptosporidium* IgG was assessed using positive and negative naturally infected and non-infected buffalo sera controls. The affinity purified *Cryptosporidium* oocyst antigen fraction had a 93.33% specificity, 95.24% sensitivity, 95.2% PPV, 93.3 % NPV, and 94.4% diagnostic efficacy.

Detection of Cryptosporidium infection in random buffalo sera

Anti-*Cryptosporidium* antibody detection in 182 collected random buffalo sera was performed using indirect ELISA based on the affinity purified fraction. The isolated fraction detected anti-*Cryptosporidium* antibodies in 57.14% (104 positive out of 182) of the collected random buffalo sera samples at a cutoff value of 0.4659. The optical density (OD values) ranged from 0.049 to 1.111 (Fig. 7).

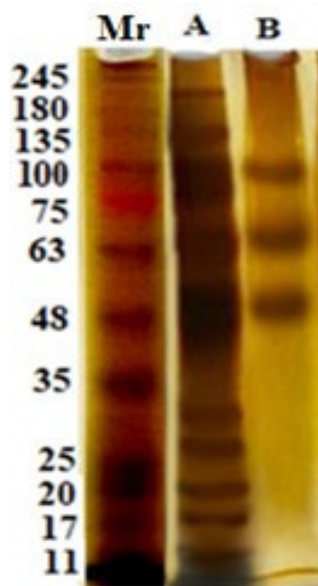


Fig. 5. 10% SDS-PAGE of the crude *Cryptosporidium* oocyst antigen (Lane A), affinity purified *Cryptosporidium* oocyst antigen fraction (Lane B) and molecular weight standard in kDa (Lane Mr).



Fig. 6. Immunogenic reactive bands profile of crude *Cryptosporidium* oocyst antigen (Lane 1) and affinity purified *Cryptosporidium* oocyst antigen fraction (Lane 2) reacted against experimentally infected mice sera (A), and naturally infected buffalo sera (B). Molecular weights standards in kDa (Lane Mr).

TABLE 2. Analysis of amino acids in *Cryptosporidium* oocyst antigen purified fraction.

Compound	Concentration ($\mu\text{g/mL}$)
Aspartic Acid	66.555
Glutamic Acid	73.565
Serine	52.545
Histidine	77.577
Glycine	37.535
Threonine	59.56
Alanine	87.1
Arginine	44.545
Tyrosine	90.595
Cystine	60.075
Valine	58.575
Methionine	74.605
Phenylalanine	82.595
IsoLeucine	65.585
Leucine	65.585
Lysine	73.095
Proline	57.565

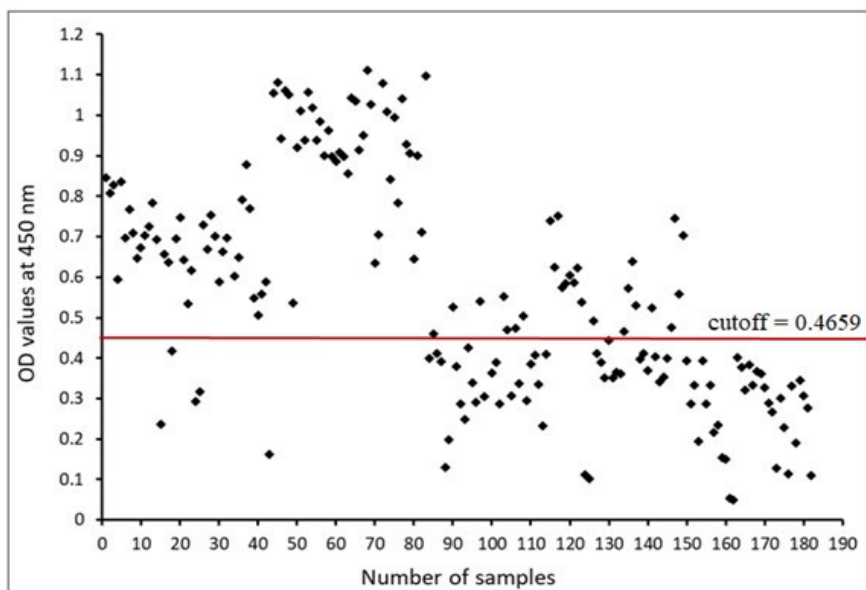


Fig.7. Diagnostic potential of the affinity purified fraction in the detection of *Cryptosporidium* antibodies in the randomly collected buffalo sera.

Discussion

In the present study, weakness, mild fever, diarrhea, lack of appetite, and dehydration were the major observed symptoms, which coincides with previous studies in which diarrhea was a typical clinical sign of human and animal cryptosporidiosis; *Cryptosporidium* has been increasingly recognized as one of the major causes of moderate to severe diarrhea [39]. Also, dehydration, fever, weakness, lack of appetite and anorexia have been reported as the symptoms of the infected hosts [40]. In addition, it has been found that clinical and subclinical cryptosporidiosis are associated with malnutrition and low weight gain in young animals [41].

In recent years, many researchers have encountered *Cryptosporidium* and have tested different commercial diagnostics that depend on the coproantigen [42,43]; however, some of these immunological tests are not enough qualified to detect the anti-*Cryptosporidium* antibodies in sera of different cattle and buffalo breeds [44]. Therefore, an ELISA assay was adopted in the present study to compare the diagnostic potential of two prepared antigens: coproantigen and crude oocyst antigen. Additionally, the diagnostic potential of the specific fraction purified from the more potent antigen in detecting anti-*Cryptosporidium* antibodies was evaluated and validated.

The comparative diagnostic potential study of the copro- and oocyst antigens using sera from experimentally infected mice showed that the oocyst antigen was preferable and more effective in detecting anti-*Cryptosporidium* antibodies as demonstrated by ELISA (Fig. 2). This variation might be due to different antigen origins or the preparation method [45], or different quantities or qualities of the chemical reagents used [46]. Furthermore, the antibody level against the antigen might differ with the immune response condition of the host, circulating immune complexes, and the mechanisms of immune evasion [44,45,47].

Consequently, in this study, the crude oocyst antigen was subjected to affinity chromatography in order to specifically purify and get the immunogenic fraction recognized by the coupled hyperimmune serum. Moreover, affinity chromatography is a beneficial and highly selective method to keep the protein in a folded conformation [48]. In the current study, the purified fraction represented only 1.25% of total protein in the crude oocyst antigen and had increased specific activities by 6733-fold. The purification process of the crude oocyst antigen using CNBr-activated Sepharose-4B produced one specific fraction. The fraction was composed of three bands at molecular weights of 94.7, 65, and 50 kDa using a reducing condition SDS-PAGE 10% slab gel (Fig. 5). These bands are immunogenically recognized by both naturally

and experimentally *Cryptosporidium*-infected sera of buffaloes and mice, respectively, and this could be responsible for the specificity of crude antigen and purified fraction.

This purified specific fraction revealed a higher specificity of 100% when tested using ELISA against positive and negative control mice sera with nearly the same sensitivity as 94%–95% of ELISA when using buffalo sera. The resultant high sensitivity proved the reliability of ELISA as a gold standard immunological method to be used in cryptosporidiosis diagnosis. This finding is in accordance with data mentioned by other authors who investigated the diagnosis of *Cryptosporidium* infection using ELISA in both humans [20,49-51] and animals [45, 52-55]. In the current study, indirect ELISA was used for serodiagnosis of *Cryptosporidium*-infection by affinity purified fraction recording a sensitivity of 94.4% and a specificity of 100% in mice sera, and a sensitivity of 95.24% and a specificity of 93.33% in buffalo sera. These results are better than those of a previous study by Danisová *et al.* [44], who used three commercially available immunological tests (enzyme immunoassay, ELISA, and immuno-chromatographic tests) in the diagnosis of *Cryptosporidium* species in animals and revealed a lower sensitivity, 63.6%, 40.9%, and 22.7%, respectively, whereas specificity was 75.9%, 78.9% and 100%, respectively. These differences might be due to different antigens as Danisová *et al.* [44] used coproantigen. Moreover, the specificity of the purified fraction could be returned to the purification process that maximizes the ability of the obtained fraction in the detection of true negative cases of negative control mice sera [24,56]. Also, as previously proved in several studies on other apicomplexan parasites [23, 57-61], it was found that using purified fractions was better in diagnosis, having higher sensitivity and specificity of ELISA, than crude extracts.

In the present study, the purified fraction exhibited 17 amino acids with high proportions of tyrosine, alanine, and phenylalanine. The high specificity of the purified fraction could be attributed to the amino acid composition of the obtained fraction which is composed of a high proportion of tyrosine and aromatic residues like phenylalanine. This composition comes in agreement with the fact suggested that tyrosine and aromatic residues have a positive contribution and, indeed, are favored in the antigen-antibody

interaction sites between the cognate protein and their antibodies [62-64]. On the other hand, the false-positive cases of reactivity cases that occurred with the buffalo sera and reduced the overall specificity of this antigen to 93.33% might be attributed to the existence of anti-*Cryptosporidium* antibodies from past infections or infections eliminated by anti-protozoal medications [44,45]. Interestingly, the diagnostic efficacies achieved by both the crude (94.4%) and the affinity purified fraction (96.6%) of the oocyst antigens were nearly similar. These results might be attributed to the shared immunogenic reactive bands that were detected by buffalo and mice sera with both compared crude and purified antigens, which might have led to these diagnostic efficacies. Therefore, the affinity purified fraction of the *Cryptosporidium* oocyst antigen succeeded in the detection of anti-*Cryptosporidium* antibodies in the randomly collected buffalo sera. Taken together, the specific purified fraction of the *Cryptosporidium* oocyst antigen could be a useful diagnostic candidate that could be used in the diagnosis and seroepidemiological surveillance of *Cryptosporidium* infections.

Conclusion

This study was designed to isolate and identify an effective antigen for the diagnosis of cryptosporidiosis. This local affinity purified antigen isolated by an easy, and low-cost purification method proved to exhibit high diagnostic potency for the diagnosis of *Cryptosporidium* infection in animals. This affinity purified fraction might be a useful diagnostic candidate and, in the future, could be used in vaccine manufacture.

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Competing Interests

The authors declare that they have no competing interests.

Authors' contribution

All authors contributed to the study conception and design. All authors shared in the laboratory work, data analysis and interpretation. The first draft of the manuscript was written by Dina Aboelsoued, Nagwa I. Toelab and Seham H.M. Hendawy then all authors discussed the results and provided critical feedback. All authors read and approved the final manuscript.

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تشخيص مرض الكريبتوسبورidium باستخدام مولد ضد منقى بواسطة عمود الصلة الكروماتوجرافي

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مرض الكريبتوسبورidium هو مرض هام يسبب الإسهال لدى البشر والحيوانات وينتج عنه معدلات إصابة ووفيات عالية نسبيًا. وتهدف هذه الدراسة لاجاد مولد ضد منقى وفعال من أجل التشخيص الدقيق لمرض الكريبتوسبورidium. في الدراسة الحالية تم استخدام ٢٧٨ حيوان (٦٠ عجول ابقار حديثي الولادة و٢١٨ جاموسًا). وقد تم جمع ٦٠ عينة براز من عجول ابقار حديثة الولادة نقل أعمارها عن شهر تمت تربيتها بمحافظتي بني سويف والقليوبية وتم فحص العينات تحت الميكروسكوب باستخدام صبغة الزيل نيلسين المعدلة (Modified Ziehl-Neelsen) وعزل حويصلات الكريبتوسبورidium من العجول المصابة تم استخدمت هذه الحويصلات في العدوى التجريبية للفئران. ثم تم تحضير مولد ضد من الحويصلات المعزولة (oocyst antigen) ومولد ضد من براز الفئران المصابة (coproantigen). وبعد ذلك تم تقييم مولدي الضد في مصلى الفئران المصابة تجريبيا باستخدام اختبار الاليزا. وأظهرت النتائج أن مولد الضد المحضر من الحويصلات لديه قدرة تشخيصية أعلى من مولد الضد المحضر من البراز. ثم تمت تنقية مولد الضد المحضر من الحويصلات (الأفضل تشخيصيا) باستخدام عمود الصلة الكروماتوجرافي المكون من مادة Cyanogen Bromide Sepharose 4B المتحددة مع الأجسام المضادة لمرض الكريبتوسبورidium المحضرة في الأرانب (Hyperimmune serum) وتم بعد ذلك تقييم مولد الضد المنقى مقارنة بمولد ضد الحويصلات الخام باستخدام اختبار الاليزا وتوصيفهما باستخدام الفصل الكهربائي واختبار الطبع المناعي. وتم أيضا تحليل الاحماض الامينية لمولد الضد المنقى. وأظهرت نتائج الفصل الكهربائي ان المولد الضد المنقى يتكون من ثلاث بوليبيبتيدات الوزن الجزيئي لها ٩٤,٧ و ٦٥ و ٥٠ كيلو دالتون والتي وجد ان جميعها متفاعلة مناعيا باستخدام اختبار الطبع المناعي. كما أوضح تحليل الاحماض الامينية لمولد الضد المنقى وجود ١٧ حمض اميني منهم كميات كبيرة من التيروسين والألانين والفينيل ألانين. وأوضح اختبار الاليزا ان رد الفعل المناعي لمولد الضد المنقى للكشف عن مستويات الاجسام المضادة لطفيل الكريبتوسبورidium في مصلى الفئران المصابة تجريبيا وأمصال الجاموس الإيجابية لمرض الكريبتوسبورidium والأمصال السالبة للمرض ولأي طفيلية أخرى نسبة حساسية ٩٤,٤٪ للفئران و ٩٥,٢٤٪ للجاموس وخصوصية ١٠٠٪ للفئران و ٩٣,٣٣٪ للجاموس. وأظهرت النتائج ان نسبة الإصابة ٥٧,١٤٪ باستخدام مولد الضد المنقى في تشخيص مرض الكريبتوسبورidium في ١٨٢ عينة مصلى عشوائية تم جمعها من الجاموس. ويتضح مما سبق ان مولد الضد المنقى من مولد الضد المحضر من الحويصلات مرشحًا جيدًا لتشخيص مرض الكريبتوسبورidium.

الكلمات الدالة: مرض الكريبتوسبورidium- الاليزا- عمود الصلة الكروماتوجرافي- الفصل الكهربائي- الطبع المناعي- تحليل الاحماض الامينية.