2022, Scienceline Publication
World's Veterinary Journal
World Vet J, 12(3): 237-249, September 25, 2022
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis

Dina M. H. El Akkad[1], Reem M. Ramadan[2], Hend M. Auda[3], Yousra N. Abd El-Hafez[4], M.M El-Bahy[2], and Shimaa Abdel-Radi[2*]

[1]*Department of Parasitology, Faculty of Medicine, Cairo University, Giza, Egypt*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*
[3]*Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*
[4]*Department of Parasitology, Faculty of Medicine, Fayoum University, Egypt*

*Corresponding author's Email: Shimaa.abdelradi@cu.edu.eg

ORIGINAL ARTICLE
pii: S232245682200030-12
Received: 28 June 2022
Accepted: 17 August 2022

ABSTRACT

Clinicians face significant problems in the diagnosis of zoonotic coenurosis. The current study aimed to develop an improved dot-Enzyme-linked-immunosorbent assay (dot-ELISA) for the diagnosis of zoonotic coenurosis using sheep *Coenurus cerebralis* scolices purified antigen (CcS-Ag) and to compare the obtained results with those of indirect ELISA and Enzyme-linked immunoelectrotransfer blot technique (EITB). Sera were collected from humans and sheep infected or suspected of infection with *Coenurus cerebralis*, control cases, and cases infected with other parasites. The CcS-Ag was proved to be the most specific antigen. This antigen was fractionated, and its specific polypeptides against anti-*C. cerebralis* antibodies (ACc-Ab) were identified using EITB. Fractions at the molecular weight (MW) of 48 and 58 kDa were proved as the only specific ones, eluted from the gel and concentrated, then dotted on the NC sheet as pooled antigen before its evaluation in the diagnosis of infection using dot-ELISA. Dot-ELISA demonstrated absolute 100% sensitivity and 100% specificity as recorded by EITB, compared to both fractions on a nitrocellulose (NC) sheet using surgically proved infected human or sheep sera as a gold standard. Diagnosis by ELISA using crude CcS-Ag revealed similar sensitivity but lower specificity (75%). The diagnostic accuracy of dot-ELISA was proved by comparing its results with postmortem data obtained post slaughtering of 20 suspected sheep and patients investigated by computed tomography (CT) and magnetic resonance imaging (MRI). In conclusion, the selection of specific fractions after EITB to be used in dot-ELISA improved the diagnostic value of the test as a diagnostic tool gathering the benefits of ELISA and EITB.

Keywords: Antigen,*Coenurus cerebralis*, Dot-ELISA, Human, Sheep, Scolices

INTRODUCTION

Clinicians face significant issues when dealing with neurological cases for several reasons, including parasites, which can infect the central nervous system (CNS). Unspecific clinical features associated with the absence of specific diagnostic methods have exaggerated the problem (Varcasia et al., 2022; Yamazawa, 2020). One of these issues is coenurosis, a worldwide zoonotic parasitic infection with the larval stage (*Coenurus*) of *Taenia multiceps*, *T. serialis*, and *T. brauni* that occurs mainly in sheep, goats, horses, cattle, buffaloes, yaks, rodents, rabbits, and humans that act as intermediate hosts (IMH) for the adult worms which develop in the small intestine of some canines, causing taeniasis (Lotfy, 2020). *Taenia multiceps* larvae *(Coenurus cerebralis)* have been thought to be the only ones capable of infecting the CNS, with only one incidence of *C. serialis* infection in the brain found in Iran (Rostami et al., 2013; Yamazawa, 2020). The definitive hosts are infected by eating raw or undercooked tissues infected with *C. cerebralis* cysts, which then develop into adult worms in the small intestine. Afterward, the eggs or gravid proglottids pass into the final host's feces (Ajaj et al., 2021). However, IMH becomes infected after ingesting food or water contaminated with *T. multiceps* eggs or gravid segments of the parasite. In the small intestine, the parasite oncospheres hatch from the eggs and penetrate the intestinal wall. Then, most of the parasites migrate through the blood to the CNS subcutaneously, forming cysts commonly detected in the brain, cerebellum, and subarachnoid spaces (Lescano and Zunt, 2013). Acute or chronic nervous manifestations (headaches, vomiting, ataxia, blindness, and papilloedema) due to intracranial pressure led to death in some cases (Varcasia et al., 2022). These symptoms have been detected in some cases in Egypt described by Antonios and Mina (2000), who found patients infected with *C. cerebralis*. Moreover, *C. cerebralis* causes sheep coenurosis, which is common in Egypt, in which the metacestodes develop in the brain and spinal cord mainly, causing nervous signs, depression, circling, convulsions, head deviation, blindness, and ataxia leading to animals' death and severe economic

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.,* 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

losses (Zhang, 2019). Dogs are a definitive host for *Taenia* species; a high incidence of infection by this metacestode in sheep should be reflected in humans in the same localities (Rostami et al., 2013).

Infection in sheep is diagnosed mainly at Post-Mortem (P.M.) inspection of the suspected cases (Desouky et al., 2011). In contrast, there is no accurate clinical way to differentiate cases of cerebral coenurosis from those of cysticercosis, echinococcosis, or other CNS infection. Moreover, some patients have sterile cysts, making diagnosis challenging with some imaging techniques (Lotfy, 2020). Therefore, the availability of accurate and applicable serological field tests associated with sonography and other clinical-pathological investigations can facilitate the diagnosis.

The diagnostic assay ELISA is simple to use and can evaluate numerous samples simultaneously. However, due to a high percentage of false-negative results and occasional cross-reactions with other parasite disorders, its sensitivity and specificity are influenced by the degree of purity and specificity of the used antigens (Sun et al., 2015). While more sensitive diagnostic methods, such as Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) antigen fractionation and Western blot or enzyme-linked immunoelectrotransfer blot (EITB) fractionation can be applied, they are not considered to be field tests (Sabry, 2007; Mahdy et al., 2017).

Dot-ELISA is a special modification for ELISA assay. It has more advantages than the indirect ELISA as it can be performed using a minute volume of reagents and is easily read visually. For this reason, dot-ELISA is considered an accurate test, gathering the benefits of both ELISA and EITB (Taher et al., 2017).

Due to the previous explanations, this study aimed to create an improved dot-ELISA assay for diagnosing human and animal coenurosis, which was performed by using specific protein fractions extracted from sheep *C. cerbralis*. Regarding positive and negative sheep and patient sera, the sensitivity and specificity of the assay were evaluated and compared to those of indirect ELISA and EITB techniques. Comparing the test results with the P.M. data of suspected sheep was also considered.

MATERIALS AND METHODS

Ethical approval

This research was carried out per the principles of the Helsinki Declaration. The procedure was accepted by the Faculty of Medicine, Cairo University's Scientific Research Ethics Committee (Vet CU20022020132). All Patients enrolled in the study were informed verbally about the aim of the study and the future examination that would be applied to their collected stool and blood samples. The institutional review board approved the procedures for handling and sample collections from animals of the Institutional Animal Care and Use Committee (IACUC) of Cairo University, Egypt. The research took place at Cairo University's Department of Parasitology in the Faculty of Veterinary Medicine from September 2021 to March 2022.

Collection and identification of cysts

Symptomatic Coenurusosis suspected 1-year-old Baladi sheep males of 60± 5.5 kg weight, showing nervous manifestations (depression, circling, head deviation, blindness, and ataxia) were gathered from private sheep farms at Fayoum Governorate, Egypt. Then, they were sent for Halal slaughtering at Fayoum slaughterhouse, Fayoum Governorate, Egypt. After that, transverse sectioning of their heads was carried out during the P.M. examination. Then, bladder-like cysts present in the cerebrum and cerebellum of their brains were collected (Figure 1). The collected cysts were transferred in an icebox to the Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, for further examinations and antigen preparation. The collected cysts were examined parasitologically and identified according to Desouky et al. (2011). Identified blood samples of the inspected infected sheep were also collected from the jugular vein into a 10-ml centrifuge tube without anticoagulant (Abdel-Rahman and Abdel-Radi, 2022).

Preparation of *Coenurus cerebralis* antigens

Cysts were collected from all positively infected sheep which proved after P.M. inspection, but only fresh extracted non-calcified cysts with numerous macroscopic scolices, intact transparent wall, and clear cystic fluid were used for antigens preparation as follows:

Coenurus cerebralis crude fluid antigens

Coenurus cerebralis crude fluid antigens (CcF-Ag) were prepared as described by Jeyathilakan et al. (2021) with slight modification. Briefly, after washing the cyst using 0.01 M sterile phosphate buffer saline (PBS), with a pH of 7.4, its fluid was aspirated using a needle. The fluid was centrifuged at 8,000 g for 30 minutes by using IEC Centra CL2 General-purpose benchtop centrifuge (United States). Then the supernatant was collected, and 0.02% sodium azide was added. It was dialyzed for 24 h in the refrigerator using a dialysis membrane (6000-8000 MW cut-off) (Sigma, USA) against 5mM Tris–HCl (pH 7.4). The fluid was then concentrated versus poly-ethylene glycol 6000 (SRL, China). Using

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.*, 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

the Bradford method (Bradford 1976), its content was measured, allocated into a 1.0 ml Eppendorf tube, and kept at -20 °C.

Coenurus cerebralis scolices crude antigens

Coenurus cerebralis scolices crude antigens (CcS-Ag) were prepared as previously described by Hassanain et al. (2016) with few modifications. By dissecting the scolices neck away from the cyst wall with sterile fine forceps and scissors, the scolices were removed from the cyst wall. They were collected in a sterile tube and washed with PBS (pH 7.4) three times. The tissue was suspended in ten volumes of sterile PBS. The mixture was exposed to three cycles of freezing and thawing. Then, the contents were homogenized using a homogenizer (ULTRA- TURRAX Janke and Kunkel KG) for 15 minutes in an ice bath. The content was sonicated using Cole Parmer ultrasonic homogenizer for 5 minutes (under 150-watt interrupted pulse output with 10-second bursts and five seconds intervals). The mixture was centrifuged at 10,000 g at 4 °C for 30 min. After adding 0.02% sodium azide to the collected supernatant, it was dialyzed, and its protein contents were measured, allocated, and stored at -20°C.

Coenurus cerebralis wall crude antigens

As previously described, for the preparation of the scolices antigens, a clean, sterile cyst wall was washed with PBS (pH 7.4) and then dissected into small pieces using sharp forceps and scissors. The pieces were suspended in a ten-time volume of PBS and then exposed to freezing and thawing three times. The mixture was homogenized, then sonicated and processed as in the case of scolices antigen preparation. The supernatant was collected, and its protein contents were measured, allocated, and stored at -20°C.

Selected sera

Sheep blood samples were collected from the jugular vein into 10 ml tube without anticoagulant. Using venipuncture, human blood samples were collected from a superficial vein in the upper limb In addition, serum samples were prepared and then stored at -20°C to be used in the study.

Human sera

A total of 85 human serum samples were included in the study. Five serum samples from surgically proved *C. cerebralis* infected patients in the first group (G-1, 30-45 years-old males), 20 serum samples from suspected *C. cerebralis* infected people in the second group (G-2, 32-50 years-old males) at high risk of coenurosis infection, who worked in shelters to collect stray dogs, and went to private neurology clinics, tropical medicine outpatient clinics, Cairo University Hospitals, and Fayoum University Hospital. Those people complained of some neurological symptoms, including chronic headaches, nausea, weakness, weight loss, disturbances of personality, visual disturbance, drowsiness, facial palsy, paraesthesia, ataxia, hemiparesis, and sensory impairment with or without shadows in Computed tomography (CT) of the brain.

Forty serum samples in group 3 (G-3) were used to assess cross-reactivity in the study which were collected from patients with known infection with other parasites previously confirmed by stool, blood smears, and other specific tests such as serological tests (ELISA and IHA). These serum samples included 20 infected by *Schistosoma mansoni* (*S. mansoni*), ten harbor *Taenia* eggs in the stool, and ten surgically proved that they were infected with *Hydatid* cysts (Hc) in their lungs. A total of 20 serum samples from healthy individuals were collected in fourth group (G-4).

Sheep sera

To analyze the sheep, 10 serum samples were collected from *C. cerebralis* infected sheep proved during the P.M. examination of their heads and were selected as a positive control (G-1). The second group (G-2) included 20 samples from suspected *C. cerebralis* infected sheep suffering from clinical signs of coenurosis (depression, circling, head deviation, blindness, and ataxia), and the authors could inspect these animals post slaughtering, were selected to evaluate the sensitivity of the diagnostic technique. For the third group (G-3), 50 serum samples were used to assess cross-reactivity of *C. cerebralis* tested antigens. The samples were collected from sheep with known infection by other parasites previously confirmed by stool, blood smears, and other specific tests. These serum samples included 20 infected by *Cysticercus ovis* (*C. ovis*), ten harbored *Hydatid* cysts, and 20 infected by *C. tenuicollis*. Finally, the fourth group (G-4) entailed 20 samples from healthy non-infected sheep, which were used as a negative control.

Indirect ELISA

Briefly, the assay was performed as described by Liu et al. (2015) with few modifications. Following checkerboard titration, 200 µl/well of each antigen (4 mg/ml) were separately added to the 96-well Poly-ethylene ELISA plates in carbonate buffer (pH 9.6), and the plates were then incubated for 1 hour at 37 °C. The wells were washed three times in washing buffer (0.5% Tween 20 in PBS pH 7.3 0.2), and then blocked with 200 ml of PBS-0.05% Tween 20 (PBST) containing 0.5% bovine serum albumin (BSA) and incubated as before. Following washing, the following reagents were subsequently added (100 ml/well), and each step was incubated at 37°C for 1 hour, firstly tested sera at a 1:100 dilution in PBST, and then horseradish peroxidize HRP-conjugated anti-sheep IgG (Sigma, USA) diluted 1:2500.

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.*, 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

After adding O-phenylene diamine dihydrochloride (OPD, Sigma, USA) as a substrate and 100 ml/well H2O2, the reaction was observed, then it was stopped by adding 1 N H2SO4 (50 ml/well). Optical density (OD) values were measured at 450 nm using a microplate reader (Titerteck multiskan ELISA reader). Serum samples were tested in duplicate reference; known infected and non-infected sera were associated with each plate. The cut-off values were calculated as double the mean negative controls (Lardeux et al., 2016). Mean standard deviation, sensitivity and specificity were calculated statically.

This assay was used for two purposes; the first was a selection of the best crude larval antigens (CcF-Ag, CcS-Ag, and *C. cerebralis* wall crude antigens [CcW-Ag]) in capturing their specific anti-Cc antibodies (ACc- Ab) and exclusion of non-specific antibodies present in sera infected with other parasites. The second purpose was to evaluate this assay in diagnosing coenurus infection using these selected antigens compared with the other improved dot-ELISA assays.

Fractionation of *Coenurus cerebralis* crude antigen and transferring of protein

Under reduced circumstances, SDS-PAGE analysis of CcS-Ag was performed using a 12 % non-gradient slab gel and a 5 percent stacking gel (Laemmli, 1970). A 20 mA current was used to fractionate the antigen. The gel was calibrated using standard markers and the molecular weight (MW) (Sigma SDS-100B). According to a previous report by Towbin et al. (1979), CcS-Ag fractionated proteins were transferred from the gel onto nitrocellulose paper (NC) overnight at 10 V, 100 mA, and 4 C. Drying and storing the NC sheet at -20 C.

Determination of *Coenurus cerebralis* specific protein fractions using Enzyme-linked immunoelectrotransfer blot technique

Longitudinal NC strips (15X 0.5 cm) representing the fractionated antigen were cut out. The strips were incubated with 3% BSA at 37 °C for 1 hour to block the non-specific antibody binding sites. According to Towbin et al. (1979), the strips were washed in PBS and then incubated with 2.0 ml of serum/strip and a 1:100 dilution of known positive and negative control serum samples for 2 hours using EITB. After being washed, each strip was treated in a blocking buffer containing HRP-conjugated anti-human or anti-sheep IgG (Sigma, USA) at a dilution of 1:1.000 for 1 hour at 37°C. While being continuously observed, the addition of peroxidase substrate (4-chloro-1-naphthol, Sigma) revealed the interaction between IgG and the antigen.

Nitrocellulose paper strips were reacted against positive and negative sera from cases with known infection history to detect *C. cerebralis*-specific diagnostic protein fractions. The specific fractions are those reacting positively with *C. cerebralis* infected cases and did not react with negative control or those infected with other parasites. To identify the specific protein bands throughout the entire NC sheet in relation to the MW standard, these strips were retained in their original positions on the NC sheet. Moreover, the identified specific fractions were used to identify infection in all tested serum samples by EITB as a diagnostic technique to be compared with the accuracy of dot-ELISA.

Elution and concentration of the specific protein bands from the gel

Elution and concentration of the specific protein fractions from the gel were performed as described by Taher et al. (2017). In brief, longitudinal strips containing the MW standards and the outermost portion of the fractionated antigen were cut out following the SDS-PAGE analysis of Cc-Ag. Tsai and Frasch (1982) reported that coomassie blue was used to dye the strips. The proteins with MWs of 48 kDa and 58 kDa were identified and transverse gel strips corresponding to those proteins were cut out horizontally across the whole gel. This gel strip was moved to an elution tube with a membrane connecting it (6-8 MW cut-off) from Spectrum Medical Inc. in Los Angeles, California, and filled with PBS (pH 7.4). The process was carried out overnight at 10 V, 100 mA under 4 °C with the tubes stored in a Bio-Rad elution unit. According to Bien et al. (2013), 0.02% sodium azide was added to the collected solution after the gel material was removed, resulting in a smaller amount of solution, compared to poly-ethylene-glycol in the molecular porous membrane tubing (6-8 MW cut-off). Five elution runs were performed after cutting the gel slice containing the kDa from thick (1 ml thick) gel to obtain enough amount of this specific fraction to perform the study. The protein content of the concentrated eluted material was calculated and placed in a 250 MM tube, which was then stored at -70°C.

Dot-ELISA technique

The technique was carried out as described by Taher et al. (2017) with slight modification using a piece of NC described by Boctor et al. (1987). The eluted concentrated pooled protein fractions with MWs of 48 and 58 kDa were dot-plotted onto a 10X 10 cm NC sheet that had been separated into 10X 10 mm squares using a pencil. The sheet was then

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.*, 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

maintained in an appropriate Petri dish. Tests on Sera were conducted twice. The sheet was immersed in 3.0% BSA to block the non-specific binding sites. After being washed three times by PBS-T, they were allowed to dry once more before 3 µL of the tested and control sera (1: 100 dilution in duplicate) were spotted on each dot. They were then allowed to dry for 15 minutes before being washed once more by PBS-T. Then, each square received 3 µL from 1:1000 HRP-conjugated anti-Human or anti-sheep IgG (Sigma, USA) and was dotted with the solution before being allowed to dry. After being washed three times, the sheet in the dish was immersed in 5 ml of the substrate solution (4-chloro-1-naphthol, 340 mg/ml substrate buffer, and 0.03 percent hydrogen peroxide solution). The discs were observed; typically, the color changed after 10 to 15 minutes. A well-defined blue-purple spot was considered positive and evaluated in comparison to the reference control and tested sera after the dot's color changed, which was visible with the naked eye (Figure 2). Additionally, the sensitivity and specificity were computed.

Statistical analysis

The statistical program SPSS, version 28, was used to code and enter the data for this investigation (IBM Corp., Armonk, NY, USA). Mean, standard deviation, median, minimum, and maximum were used to describe quantitative data, while frequency (count) and relative frequency were used to do so for categorical data (percentage). To compare numerical variables, the non-parametric Mann-Whitney test was applied (Chan, 2003a). Using the Chi-square (c^2) test, categorical data were compared. When the anticipated frequency was less than 5, the exact test was utilized (Chan, 2003b). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic efficacy of common diagnostic indices were calculated as indicated by (Galen, 1980). Area under curve (AUC) analysis was used to construct the Receiver operating characteristic **(**ROC) curve and find the optimal ELISA cut-off value for infection detection. A P-value of less than 0.05 was regarded as statistical significance

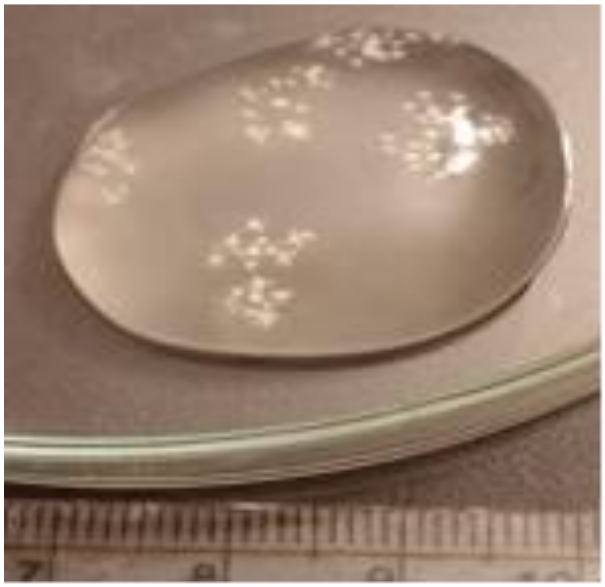

Figure 1. *C. cerebralis* bladder-like cyst freshly extracted from Baladi sheep brain (about 30 mm in diameter)

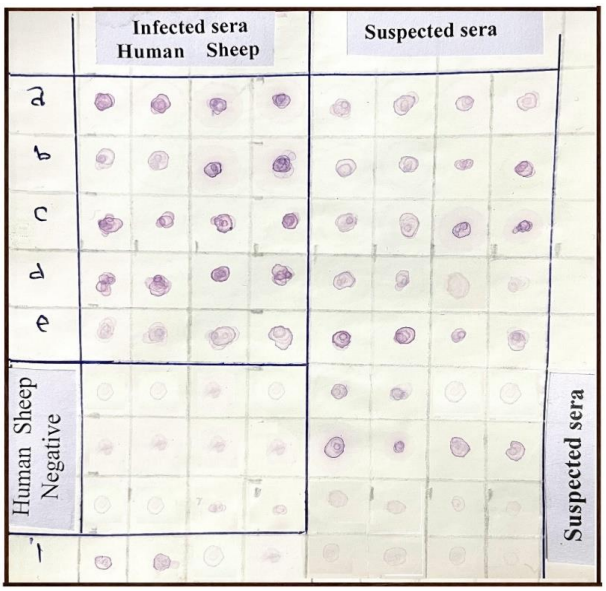

Figure 2. Improved Dot-ELISA on a nitrocellulose sheet using specific CcS-Ag protein fractions versus surgical proved infected patients, infected Baladi sheep sera, negative control, and some suspected sera.

RESULTS

Selection of the most diagnostic *Coenurus cerebralis* crude antigen

The data in Table 1 revealed that CcF-Ag and CcS-Ag showed absolute (100%) sensitivity in the diagnosis of anti-Cc antibodies (ACc-Ab) in sera of infected sheep in comparison with CcW-Ag that failed in capturing specific Ab in two positive serum samples of infected sheep that were proved to be infected after P.M. inspection of their brains. CcS-Ag had high mean specificity than the other two Ag reaching 88.57%, while it was 80% and 74.28% for CcF-Ag and CcW-Ag respectively. Both CcF-Ag and CcS-Ag demonstrated the same specificity in the diagnosis of anti-*C. ovis* Ab in infected sheep while CcW-Ag showed a high level of cross-reaction versus anti-*Hc* -Ab and anti-*C. tenuicollis* Ab in infected sheep sera. These data proved that CcS-Ag could be used as a diagnostic Ag more accurately than the other *C. cerebralis* tested Ag.

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.,* 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

Table 1. Sensitivity and specificity of *Coenurus cerebralis* crude antigen in the diagnosis of infection in Baladi sheep using indirect ELISA

	Tested sera collected from		Reaction of tested sera versus C. *cerebralis*					
			Fluid Ag		Scolices Ag		Wall Ag	
	sheep infected by	No. exam	No. +Ve	Percentage	No. +Ve	Percentage	No. + Ve	Percentage
Sensitivity	*C. cerebralis*	10	10	100	10	100	8	80
Specificity	*C. ovis*	20	2	90%	2	90%	3	85
	Hydatid cyst	10	5	50	3	70	6	40
	C. tenuicollis	20	5	75	3	85	5	75
	Non infected sheep	20	2	90	0	100	4	80
	Total Mean specificity	70	14	80%	8	88.57	18	74.28

No. exam: Number of examined cases, *C. cerebralis*: *Coenurus cerebralis*, Ag: antigen

Determination of *Coenurus cerebralis* reacted polypeptides in scolex crude antigen using Enzyme-linked immunoelectrotransfer blot technique

The treatment of NC strips carrying the fractionated CcS-Ag of sheep origin against infected and controlled sheep and human sera using EITB revealed several specific and cross-reacted fractions (Figure 3). Seven reactive bands, corresponding to MW standard at 18,22, 26-34, 48, 58, 75, and 112 kDa were recognized compared to control infected sheep sera (Figure 3, Lane 1).

Nine bands reacted positively after the treatment by *C. cerebralis* of similar strips from surgically approved infected patients at MW of 18, 22, 26-34, 48, 58, 63, 75, and 98 kDa (Figure 3, Lane 2).

The fractions at MW of 18, 22, 29, 38, 63, 75, 98, and 112 kDa were cross-reacted against Hydatid cyst (H. cyst) infected patient sera (Figure 3, Lane 3). The fractions at kDa 22, 24, 29, 63, and 75 kDa were also reacted against *S. mansoni* infected patient sera (Figure 3, Lane 4), while the fractions at MW 18, 22-24, 35 and 63 kDa (Figure 3, Lane 5) were cross-reacted against sera of *Taenia* spp. infected patients. At the same time, fractions with MW of 18, 24, and 63 kDa were falsely reacted against intact sera (Figure 3, Lane 6), and fractions with MW of 11-15, 18-22, and 34-36 kDa were falsely reacted against healthy sheep sera (Figure 3, Lane 7).

Among all the previous fractions, the two fractions at MW 48 and 58 kDa in strips 1 and 2 were considered *C. cerebralis-specific* fractions. These fractions were recognized only in sheep and humans surgically approved *as C. cerebralis* (gold standard) infected sera and not recognized in sera of patients infected with other parasites, as well as in human and sheep negative control sera (Figure 3).

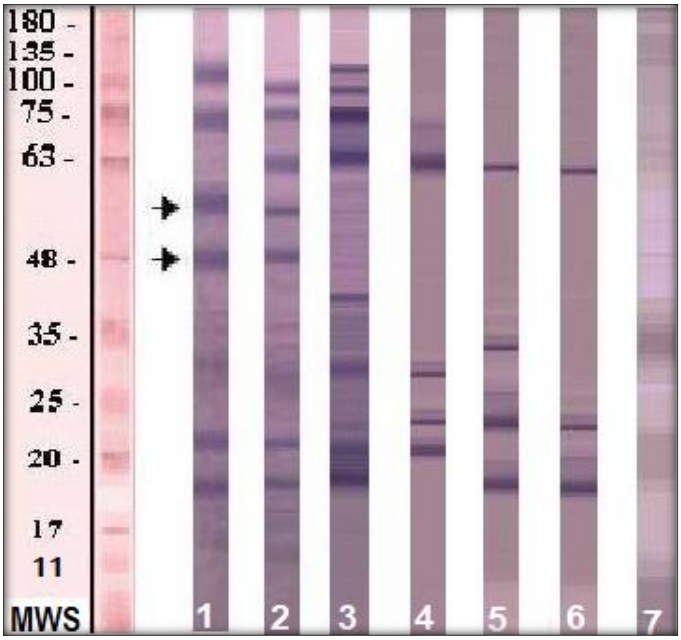

Figure 3. Reacted polypeptides in fractionated CcS-Ag of Baladi sheep on NC strips treated with infected and control sera using EITB. Treated versus *C. cerebralis* infected sheep sera (Lane 1), treated by *C. cerebralis* infected patients' sera (Lane 2), treated by *Hydatid* cyst infected patients sera (Lane 3), treated versus *S. mansoni* infected patients sera (Lane 4), treated by *Taenia* spp. infected patients sera (Lane 5). Treated by healthy control in human sera (Lane 6), control non-infected sheep sera (Lane 7). MWst.: Molecular weight protein slandered (Sigma).

Efficacy of indirect, Dot-ELISA, and EITB for the diagnosis of infection

Comparing the efficacy of indirect ELISA using crude CcS-Ag and dot-ELISA that was performed using pooled eluted concentration fractions (MW 48 and 58 KDa) in the diagnosis of infection, the data revealed that both assays showed absolute (100%) sensitivity in the diagnosis of ACc-Ab in sera of surgically proven infected patients and sheep (Table 2). Concerning the test specificity, indirect ELISA showed low specificity in the diagnosis of antibodies of other parasites present in sera from patients infected with H. cyst, *S. mansoni*, and *Taenia* spp. eggs like 40%, 25%, and 30% from the sera infected by the previous parasites, respectively. They reacted as false positive against the used CcS-Ag. This cross-reaction decreased the test's specificity to 60%, 75%, and 70% in these patients' sera. These false-positive cases' mean OD values were 0.463 ± 0.020, 0.420 ± 0.019, and 0.375± 0.041, respectively. At the same time, 15% of the healthy people demonstrated false reactions against this crude antigen with a low mean OD value (0.403 ± 0.034) as well

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.*, 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

as four sheep from the control non-infected group also reacted as false positive with a mean OD value of 0.460 ± 0.053 (Table 2).

On the contrary, screening of the same previous sera using EITB or dot-ELISA did not show any false-positive reactions against the control negative or other sera as they revealed absolute (100%) sensitivity and specificity using the previous two specific fractions (MW 48 and 58 KDa). Using EITB, these two fractions demonstrated significant reactions during the treatment of NC strips against control infected samples. In contrast, it did not react against healthy control. Moreover, they did not cross-react with antibodies in patients' sera infected with H. cysts, *S. mansoni*, or *Taenia* spp. Using dot-ELISA to diagnose anti-Cc-Ab in the previous sera revealed consistent results with the previously recorded EITB, as both showed 100% specificity corresponding to 75% only for screening the same sera against CcS-Ag by indirect ELISA (Table 2).

Table 2. Difference in diagnostic sensitivity and specificity between indirect and Dot-ELISA assay

Tested human and sheep infected and control sera		No. exam.		Diagnosis by ELISA versus Crude CcS-Ag			Using EITB or dot-ELISA	
				No. + Ve	SP (%)	ELISA OD (Mean ± SD.)	No. + Ve	SP (%)
Human	*C. cerebralis*	5	TP	5	100	0.660 ± 0.093	5	100
			FN	0			0	
	H. cyst	10	TN	6	60%	0.143 ± 0.023	0	100
			FP	4		0.463 ± 0.020	0	
	S. mansoni	20	TN	15	75%	0.159 ± 0.015	0	100
			FP	5		0.420 ± 0.019	0	
	Taenia spp.	10	TN	7	70%	0.165 ± 0.022	0	100
			FP	3		0.375 ± 0.041	0	
	Healthy control	20	TN	17	85%	0.148 ± 0.023	0	100
			FP	3		0.403 ± 0.034	0	
	Mean specificity	60	TN	45	75%		0	100
			FP	15			0	
Sheep	*C. cerebralis*	10	TP	10	100	0.708 ± 0.076	10	100
			FP	0			0	
	Healthy control	20	TN	16	80%	0.123 ± 0.006	0	100
			FP	4		0.460 ± 0.053	0	

No. exam: Number of examined cases. TP: True Positive, FP: false Positive, TN: True negative, FN: false negative, SP: Specificity, OD: Optical density, SD: Standard deviation.

Efficacy of indirect, Dot-ELISA, and EITB for the diagnosis of infection in suspected cases

Investigating the efficacy of the used assays in the diagnoses of infection in 20 symptomatic *C. cerebralis* suspected patients and sheep, the results obtained after EITB was compatible with that of dot-ELISA. At the same time, both tests showed high sensitivity than indirect ELISA (Table 3). Indirect ELISA showed a false diagnosis of infection in 11 patients from 20 suspected (55%). Screening the same patients' sera using EITB or Dot-ELISA revealed infection in 4 cases (20%). Further investigation of these 4 cases using CT and MRI inspection proved that they had cysts in their brain.

For the sensitivity of the used assays in the diagnoses of infection in 20 suspected sheep isolated from private farms with the coenurosis characteristic symptoms, indirect ELISA was used to diagnose the parasite-specific antibodies in 12 animals (60%). A re-examination of this group of sheep using EITB or by the improved dot-ELISA identified another three more positive sheep as infected by the parasite which confirmed by detection of *C. cerebralis* cysts in their brain of them at their inspection post slaughtering (Table 3).

The performance of both types of ELISA in the diagnosis of coenurosis in suspected patients and sheep was statistically evaluated. Receiver operating characteristic **(**ROC) curve was developed for the results of tested serum samples of positive cases, with confirmed coenurosis-infected cases proved after surgical removal or P.M. inspected sheep (Figure 4 and Table 4). The statistics for ELISA results are described in Table 5.

For all positive cases diagnosed by EITB or dot-ELISA were proved to have parasite cysts, the sensitivity and specificity of both tests reached 100%, while as described in Table 5, the sensitivity and specificity of ELISA in the suspected patients were 100% and 72.22%, respectively. In addition, it was 80% and 100% in the suspected sheep, respectively. At the same time, the PPV of the test was very low in patients than in sheep, while the NPV was considered high in patients than in symptomatic sheep. The test reported 75.00% and 85.00% accuracy in patients and sheep, respectively (Table 6).

The data in Table 7 revealed direct relation between the mean value of ELISA OD and the strong positive color determined using dot-ELISA visual reading. The mean of the OD duplicates was used to calculate the Area under the Curve (AUC), which represents the test's accuracy. In the case of suspected patients, the AUC was 0.976, whereas, in the case of suspected sheep, it was 0.987. The best cut-off points, determined after ROC analysis, were 0.403 and 0.157 for

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.,* 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

the OD values in both cases. These values showed ELISA sensitivity and specificity of 100% and 94.4%, respectively. In the case of suspected patients, they were 93% and 100% in cases of suspected sheep. Inspection of the relation between mean ELISA OD and visuals values of Dot-ELISA revealed a significant relation between the degree of darkness of the fraction in EITB or the dot on the NC sheet and the mean OD value of indirect ELISA. This was observed in *C. cerebralis* surgically proved infected cases in humans and sheep. After screening by dot-ELISA, false-positive cases in non-infected controls with low ELISA OD values appeared negative. Also, in the patients infected with other parasites (four by H. cyst, five by *S. mansoni* and three by *Taenia* spp. eggs), they previously demonstrated moderate to low positive ELISA OD values but were negative when screened with dot-ELISA.

Table 3. Diagnostic efficacy of improved dot- ELISA in comparison with indirect ELISA for detection of the infection in suspected cases

	No. exam.		Diagnosis by indirect ELISA versus Crude CcS-Ag			Using EITB or dot- ELISA	
			No.	Percentage	ELISA OD (Mean ± SD.)	No.	Percentage
Suspected people	20	+ Ve	11	55	0.434 ± 0.018	4	20%
		- Ve	9	45	0.148 ± 0.020	16	80%
Suspected sheep	20	+ Ve	12	60	0.469 ± 0.175	15	75%
		- Ve	8	40	0.101 ± 0.040	5	25%

No. exam: Number of examined cases, SD: Standard deviation, OD: Optical density, CcS-Ag: *Coenurus cerebralis* scolex antigen.

Table 4. ROC curve values for prediction of infection using indirect ELISA

	AUC	P value	95% Confidence Interval		Cut off value	Sensitivity (%)	Specificity (%)
			Lower Bound	Upper Bound			
Patients	0.976	< 0.001	0.931	1.021	0.403	100	94.4%
Sheep	0.987	< 0.001	0.946	1.028	0.157	93.3	100

Table 5. Value of ELISA in differentiation between positive and negative suspected samples

	Dot-ELISA						
	Positive			Negative			P value
	Median	Minimum	Maximum	Median	Minimum	Maximum	
Patients	0.439	0.408	0.451	0.163	0.116	0.142	0.001
Sheep	0.515	0.141	0.666	0.088	0.058	0.152	< 0.001

Table 6. Accuracy of indirect ELISA in the prediction of infection

Indices	Suspected patients		Suspected sheep	
	Value (%)	95% CI	Value (%)	95% CI
Sensitivity	100.00	39.76 to 100.00	80.00	51.91 to 95.67
Specificity	72.22	54.81 to 85.80	100.00	47.82 to 100.00
PPV	28.57	19.11 to 40.38	100.00	
NPV	100.00		62.50	37.72 to 82.10
Accuracy	75.00	58.80 to 87.31	85.00	62.11 to 96.79

PPV: Positive predictive value, NPV**:** Negative predictive value, CI: Confidence interval.

Table 7. Relation between positive ELISA value and positive dot- ELISA reading

	Value of mean indirect ELISA OD of positive serum samples							Level of positive dot-ELISA				
	No. of positive samples in infected cases	High OD. (~ 0.6)		Moderate OD (~ 0.45)		Low OD. (~ 0.3)		No.+Ve	Strong positive		Weak positive	
		No.	%	No.	%	No.	%		No.	%	No.	%
Human	*C. cerebralis* (5/5)	5	100					5	5	100	-	-
	Suspected (11/20)	2	18.18	4	36.36	5	45.45	4	2	50	2	50
	H. cyst (4/10)	2	50	1	25	1	25	0	-	-	-	-
	S. mansoni (5/20)	-	-	3	60	2	40	0	-	-	-	-
	Taenia spp. (3/10)	-	-	3	100	-	-	0	-	-	-	-
	Control (3/20)	-	-	-	-	3	100	0	-	-	-	-
Sheep	*C. cerebralis* infected (10/10)	6	60	4	40	-	-	10	10	100	-	-
	Suspected (12/20)	8	66.6	4	33.3	-	-	15	8	53.33	7	46.66
	Control (4/20)	-	-	-	-	4	100	0	-	-	-	-

No: Number; OD: Optical density; *C. cerebralis: Coenurus cerebralis*; *S. mansoni*: *Schistosoma mansoni.*

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.,* 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

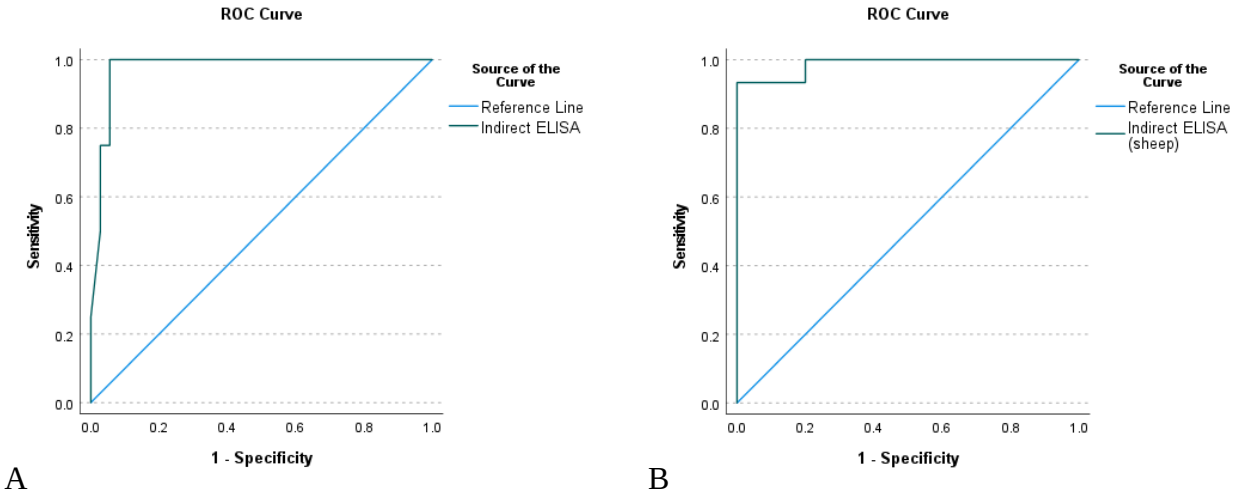

Figure 4. Receiver operating characteristic (Roc) curve for prediction of infection using indirect ELISA. A: Suspected patients, B: Suspected Baladi sheep

DISCUSSION

Coenurosis infection in people or animals is symptomatically and clinically misdiagnosed with several other CNS infections, such as metastatic brain tumor, glioblastoma, brain abscess, primary CNS lymphoma, and neurocysticercosis caused by *T. solium*. The CT, MRI, and sonography with the symptoms and clinical data are beneficial in reaching an accurate diagnosis. Tests, such as MRI, CT, and sonography, are expensive, time-consuming, and considered less sensitive, especially for diagnosing early infection. Therefore, an accurate diagnosis was performed by postmortem (P.M.) examination in animals and surgical removal in humans (Lotfym, 2020; Yamazawa et al., 2020).

The availability of sensitive serological tests that can diagnose the specific Anti-Cc-Ab in sera could be an extra tool supporting the other diagnostic methods. However, these techniques (ELISA, EITB) face other problems, such as selecting a filed applicable test and a source for the specific diagnostic antigen that can capture the characteristic Cc-Ab in infected cases. The present study investigated the importance of *C. cerebralis* antigens extracted from cysts of sheep origin to be used to diagnose infection in patients.

ELISA is one of the most common field serological tests that are easily applied and valuable for simultaneously examining a large number of samples. ELISA specificity is usually affected by the degree of purity and specificity of the antigens (Sabry, 2007; Taher et al., 2017). EITB technique is considered a highly specific diagnostic technique, but it is unsuitable for field applications (Mahdy et al., 2017; Ramadan et al., 2021). The identification of specific diagnostic protein fractions then elution and concentration of this fraction to use in modified ELISA technique after dotting a minute amount of this purified antigen on NC sheet or even in micro-ELISA technique introduce more specific diagnostic assay described as dot-ELISA. This technique was first described by Boctor et al. (1987) for diagnosing schistosomiasis in Egypt. Then, it was modified by Taher et al. (2017) and successfully applied in diagnosing trichinosis infection. Later, Mahdy et al. (2020) used it in hydatidosis diagnosis. Dot-ELISA still has the advantage of the original assay regarding its simplicity, non-time consuming, low cost, and easy investigation of large samples in a short time. Moreover, the test is superior to the original ELISA assay as it can be performed using a minute volume of reagents, serum, and antigens can be evaluated visually without special readers. So, Dot-ELISA is considered a specific test, gathering the benefits of both ELISA and EITB (Taher et al., 2017).

Three types of antigens can be extracted from fresh *C. cerebralis* cysts, including fluid antigen (CcF-Ag), scolices antigens (CcS-Ag), and cyst wall (CcW-Ag). Scolices and fluid antigens are the most commonly used diagnostic antigens in similar metacestodes (Mahdy et al., 2017). The first part of this study used indirect ELISA to test the diagnostic specificity and sensitivity of these crude antigens in capturing anti-Cc-Ab in sera of surgically proven *C. cerebralis* infected sheep, control non-infected sheep, and sera collected from sheep infected by other parasites in order to identify the most diagnostic *C. cerebralis* antigen. CcF-Ag and CcS-Ag proved to have higher diagnostic sensitivity and specificity than CcW-Ag (Huang et al., 2016; Mahdy et al., 207; Ramadan et al., 2021). In contrast, CcS-Ag proved to have higher diagnostic specificity than the other two antigens. The superiority of scolices antigens over fluid antigens in this subject disagreed with Carmena et al. (2006). They described fluid antigens of the bladder worms as specific and high protein content than other antigens. The selection of scolices antigen in this field was supported by Zhang et al. (2012) and Ramadan et al. (2021). As they mentioned, fluid antigens of metacestodes usually contain some proteins from the

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.*, 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

hosts, such as IgG, that might interfere with the accuracy of diagnosis because it cross-reacted with the specific immunoglobulin present in infected host sera.

The identification of the polypeptide profile of this scolices antigen versus known infected and control sera using EITB revealed seven and nine reacted bends versus infected sheep and patients' sera. These fractions in the MW range of 18, 22, 26-34, 48, 58, 75, and 112 kDa were versus infected sheep sera and corresponded to MW of 18, 22, 26-34, 48, 58, 63, 75, and 98 kDa versus control infected patient sera. Most of these fractions cross-reacted with one or more of the tested serum groups from non-infected patients or those infected by H. cyst, *S. mansoni*, or *Taenia* spp. infected patients. The only two fractions at MW 48 and 58 kDa were *C. cerebralis* specific fractions, as they were recognized only by sheep and human sera infected by *C. cerebralis*.

Most of the previously recorded cross-reacted protein fractions with Ab of other bladder worms except those of 48-58 kDa were previously mentioned by Jeyathilakan et al. (2021). They identified the fractions at MW of 12, 28, 42, 52, 98, and 112 kDa in fractionated CcS-Ag of sheep origin that were cross-reacted versus Ab present in experimentally produced anti-hydatid cysts and Ab present in experimentally produced hyper-immune sera (HIS) after immunization of rabbits by Hc scolices antigens. This high number of cross-reacted fractions might be related to the nature of the tested experimentally produced HIS, not versus naturally infected sera as in the present study. The first part of this work succeeded in identifying CcS-Ag and the two fractions at MW of 48 and 58 KDa as specific crude and purified antigens, respectively. The study then used EITB and dot-ELISA to examine the efficacy of indirect ELISA utilizing crude Ag or the value of specific fractions in diagnosing infection in various known infected and control sera.

Using indirect ELISA to diagnose anti-Cc-Ab in tested sera revealed absolute sensitivity for this test with high ELISA OD values. Still, cross-reaction with antibodies of other parasites was recorded, decreasing the mean specificity of the test to 75% with low OD values in all the cross-reacted cases. The recorded absolute sensitivity could be attributed to the source of this control sera as they were from patients or animals that were surgically proved to be infected. This IgG Ab was developed during prolonged migration of the parasite and its survival as an active infection. However, the test revealed several false-positive reactions in control healthy samples. This result agreed with Sun et al. (2015) and Taher et al. (2017) because using crude antigens in this technique adversely affected its specificity for excluding other cross-reacted parasitic Ab as that of hydatidosis, schistosomiasis, and taeniasis in the present study. The authors agreed with Jeyathilakan *et al.* (2021), who mentioned that selecting specific antigens was important for developing specific diagnostic assays and overcoming the indirect ELISA disadvantages concerning the specification of the used antigens.

In comparison to the previously detected specific KDa at MW of 48 and 58 KDa that were previously diagnosed by reference control sera, the screening of the same previous sera utilizing EITB revealed that all infected sera succeeded in producing clear, sharp reactions on the NC sheet. This finding revealed absolute 100% sensitivity and 100% specificity. The specificity and sensitivity of EITB in diagnosis were previously mentioned by several authors against different parasites. However, this technique is considered a time-consuming and non-applicable field test (Mahdy et al., 2017; Taher et al., 2017).

The ability to utilize a modified ELISA assay depends on using specific purified antigens that can partially overcome the drawbacks of indirect ELISA. Thus, during the present study, the assay was performed by directedly dotting the purified eluted concentrated pooled antigens from the two specific fractions at MW of 48 and 58 kDa that were previously identified by EITB on a piece of NC sheets divided by a pencil into 10 X 10 mm squares. Then, testing its diagnostic efficacy after being absorbed in the NC sheet. The assay revealed the same results as previously reported after EITB, with 100% sensitivity, specificity, and accuracy that were better than indirect ELISA. Moreover, the assay did not demonstrate false-positive reactions versus control negative human and sheep sera.

Mixing both fractions (48 and 58 kDa) as pooled antigens after elution initiated a synergistic action increasing the concentration of specific antigen per dot, causing a darker color per dot in comparison with the relatively faint reaction per band obtained per each separate specific band after EITB. After using dot-ELISA, the high protein concentration per dot enhanced the number of strong positive results while decreasing the number of weak positive reactions. With a fixed amount of protein/dot, the obtained color of the dot was directly related to the level of ACc-Ab in the tested sera, which was directly related to the mean OD values of ELISA. This result was in agreement with Taher et al. (2017).

The described dot-ELISA had all indirect ELISA benefits and overcame its drawback related to the degree of antigen purification, which minimized the level of cross-reaction with other parasites. It was more economic because it used a small number of reagents. In addition, this assay could be easily performed and could be evaluated by the naked eye or a densitometer. The test gathered the benefits of ELISA because it could be performed on a large number of samples simultaneously in a short time and had the accuracy of EITB as it used a specific purified protein fraction.

The thickness of the gel slide and the quantity of initially added antigen had an impact on the protein content of the gel strip that was produced. It just required dotting a tiny amount of protein/square of 2-3 µl/dot to run several samples using the concentrated eluted fractions that were produced (about 0.2 mg protein). Additionally, it was able to prepare any amount of pooled antigen by repeatedly fractionating and eluting the sample. The three diagnostic assays were evaluated to accurately diagnose the infection of 20 suspected patients and sheep. Statistical analysis for their diagnostic efficacy

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.,* 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

revealed very low PPV for diagnosis by indirect ELISA in suspected patients (28.75%), while it was 100% in suspected sheep. In the author's opinion, this finding was related to the experiences of veterinarians in diagnosing the disease from its apparent characteristic symptoms. The finding was in agreement with Desouky et al. (2011), who reported that the infection was 100% in suspected sheep. In contrast, Amer et al. (2017) revealed that the infection was 26.4% in clinically suspected sheep. Patients usually attend the clinic for several causes of headaches with numerous incriminated diseases. For this reason, the PPV in sheep was considered to be high in comparison with patients.

Both EITB and Dot-ELISA techniques excluded seven false-positive cases from the suspected patients that were previously determined as positive by ELISA. On the contrary, both methods determined three sheep as being infected more than the 12 cases previously diagnosed by ELISA. Interestingly, the four patients proved truly infected after CT and MRI sonography, and the 15 positive sheep proved that they harbored the parasite cyst in their brains at P.M. inspection post slaughtering. CT was done using a state-of-the-art 64-channel multislice machine (Toshiba Aquillion). While MRI was performed using 1.5 Tesla MR imaging unit (Intera, Philips Medical Systems, Netherlands).

In the study, the sheep were primarily used to increase the number of known positive controls and test the accuracy of the results produced by the improved dot-ELISA on symptomatic harmed living sheep and the exact results after slaughtering and examining these animals.

The small sample size was the study's principal limitation in determining the cut-off point. There was difficulty in obtaining a large number of surgically proven coenurosis-infected patients and serum samples from a large number of sheep infected by this parasite only. At the same time, the availability of reference sera from known infected patients or sheep was considered more valuable than using hyper-immune sera prepared in an experimental animal after injection by pure or crude antigens as described by Jeyathilakan et al. (2021).

CONCLUSION

Investigation of *C. cerbralies* infected or suspected human or sheep sera by EITB or dot-ELISA depending on reaction versus pooled purified 48 and 58 kDa fractions of CcS-Ag revealed absolute (100%) sensitivity and specificity. Indirect ELISA using CcS-crude antigen revealed absolute sensitivity, while its specificity reached 75% versus non-infected and other parasitic infected sera. EITB and dot-ELISA showed high accuracy in the diagnosis of infection in suspected cases proved by sonography in patients and PM inspection in sheep. The described dot-ELISA using this pooled purified antigen proved an easily applicable diagnostic tool gathering the benefits of both ELISA and EITB. The assay can be applied on the field level after producing enough of these specific fractions. Further study is continued to identify the relation between genotypes of *C. cerbralis* species extracted from humans and sheep in the near future.

DECLARATIONS

Authors' contributions

All authors consented to submit the manuscript to the current journal, gave final approval of the version to be published, and agreed to be responsible for all aspects of the work. They also significantly contributed to the conception, design, data collection, analysis, and interpretation.

Competing interests

There are no competing interests with regard to this work, according to the authors.

Acknowledgments

The Faculty of Veterinary Medicine, Cairo University, Egypt, for its cooperation in providing research facilities, and the veterinary technical team for their assistance during sample collection are acknowledged by the authors. In addition, the authors introduce special thanks for the enormous help performed by Dr. M. Auda; specialist and previous head of surgical Dept. in Shubra hospital, Cairo, Governorate, for supporting the work by controlling infected cases and reviewing some investigation.

Ethical consideration

The authors have checked for ethical issues, such as plagiarism, approval of public misconduct, data fabrication or falsification, duplicate publishing or submission, and redundancy.

REFERENCES

Abdel-Rahman GH and Abdel-Radi S (2022). Synergistic action of Viteselen with anti-Fasciola drug as a tool for improving fertility and hemato-biochemical biomarkers in Fasciola infected sheep. Journal of Parasitic Diseases, 46: 103-112. DOI: https://www.doi.org/10.1007/s12639-021-01423

Ajaj EA, Mohammad HA, and Gharban HAJ (2021). First molecular confirmation of *Coenurus cerebralis* in sheep and goats with neurological behaviors in Iraq. Veterinary World, 14(6): 1420-1425. DOI: https://www.doi.org/10.14202/vetworld.2021.1420-1425

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.*, 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

Amer S, ElKhatam A, Fukuda Y, Bakr LI, Zidan S, Elsify A, Mohamed MA, Tada C, and Nakai Y (2017). Prevalence and identity of *Taenia multiceps* cysts "*Coenurus cerebralis*" in sheep in Egypt. Acta Tropica, 176: 270-276. DOI: https://www.doi.org/10.1016/j.actatropica.2017.08.012

Antonios SN and Mina SN (2000). A case report of human *Coenurus cerebralis* in Tanta, Egypt. Journal of the Egyptian Society of Parasitology, 30(3): 959-960. Available at: https://pubmed.ncbi.nlm.nih.gov/11198393/

Bien J, Cabaj W, and Moskwa B (2013). Recognition of antigens of three different stages of the Trichinella spiralis by antibodies from pigs infected with *T. spiralis.* Experimental Parasitology, 134(2): 129-137. DOI: https://www.doi.org/10.1016/j.exppara.2013.02.007

Boctor FN, Stek MJ, James J, Peter B, and Kamal R (1987). Simplification and standardization of dot-ELISA ELISA for human *schistosomiasis mansoni*. The Journal of Parasitology, 73(3): 589-592. DOI: https://www.doi.org/10.2307/3282141

Bradford MMA (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2): 248-254. DOI: https://www.doi.org/10.1016/0003-2697(76)90527-3

Carmena D, Benito A, and Eraso E (2006). Antigens for the immuno diagnosis of *Echinococcus granulosus*. An update. Acta Tropica, 98(1): 74-86. DOI: https://www.doi.org/10.1016/j.actatropica.2006.02.002

Chan YH (2003a). Biostatistics102: Quantitative data – parametric & non-parametric tests. Singapore Medicine Journal, 44(8): 391-396. Available at: https://medicine.nus.edu.sg/rsu/wp-content/uploads/sites/15/2020/02/biostat102_resources4.pdf

Chan YH (2003b). Biostatistics 103: Qualitative data –tests of independence. Singapore Medicine Journal, 44(10): 498-503. Available at: https://medicine.nus.edu.sg/rsu/wp-content/uploads/sites/15/2020/02/biostat103_resources5.pdf

Desouky EA, Badawy AI, and Refaat RA (2011). Survey on coenurosis in sheep and goats in Egypt. Veterinaria Italiana, 47(3): 333-340. Available at: https://www.izs.it/vet_italiana/2011/47_3/333.pdf

Galen RS (1980). Predictive values and efficiency of laboratory testing. Pediatric Clinics of North America, 27(4): 861-869. DOI: https://www.doi.org/10.1016/S0031-3955(16)33930-X

Hassanain MA, Shaapan RM, and Khalil FA (2016). Sero-epidemiological value of some hydatid cyst antigen in diagnosis of human cystic echinococcosis. Journal of Parasitic Diseases, 40: 52-56. DOI: https://www.doi.org/10.1007/s12639-014-0443-5

Huang X, Xu J, Wang Y, Guo C, Chen L, Gu X, Lai W, Peng X, Yang G (2016). GP50 as a promising early diagnostic antigen for *Taenia multiceps* infection in goats by indirect ELISA. Parasites & Vectors, 9(1): 618. DOI: https://www.doi.org/10.1186/s13071-016-1915-5

Jeyathilakan N, Abdul Basith S, John L, Chandran ND, and Dhinakarraj G (2021). Immunochemical localization of cross-reactive proteins of bladder worms with special reference to detection of specific antigen in cystic echinococcosis of sheep. Indian Journal of Veterinary and Animal Sciences, 50(2): 37-47.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature, 227: 680-685. DOI: https://www.doi.org/10.1038/227680a0

Lardeux F, Torrico G, and Aliaga C (2016). Calculation of the ELISA's cut-off based on the change-point analysis method for detection of *Trypanosoma cruzi* infection in Bolivian dogs in the absence of controls. Memorias do Instituto Oswaldo Cruz, 111(8): 501-504. DOI: https://www.doi.org/10.1590/0074-02760160119

Lescano AG and Zunt J (2013). Other cestodes: Sparganosis, coenurosis and *Taenia crassiceps* cysticercosis. Handbook of Clinical Neurology, Chapter 27, 114: 335-345. DOI: https://www.doi.org/10.1016/B978-0-444-53490-3.00027-3

Liu P, Cui J, Liu RD, Wang M, Jiang P, Liu LN, Long SR, Li LG, Zhang SB, Zhang XZ et al. (2015). Protective immunity against *Trichinella spiralis* infection induced by TsNd vaccine in mice. Parasites & Vectors, 8: 185. DOI: https://www.doi.org/10.1186/s13071-015-0791-8

Lotfy W (2020). Neglected rare human parasitic infections: Part II: Coenuriasis. Parasitologists United Journal, 13(2): 72-75. DOI: https://www.doi.org/10.21608/PUJ.2020.30935.1074

Mahdy OA, Mousa WM, Abdel-Maogood SZ, Abdel-Radi S, and El-Bahy MM (2017). Characterization of immunogenic protein fractions of sheep cysticercosis in Cairo, Egypt. Journal of the Hellenic Veterinary Medical Society, 68(3): 291-298. DOI: https://www.doi.org/10.12681/jhvms.15471

Mahdy OA, Mousa WM, Abdel-Maogood SZ, Nader S, and Abdel-Radi S (2020). Molecular characterization and phylogenetic analysis of Toxocara species in dogs, cattle and buffalo in Egypt. Helminthologia, 57(2): 83-90. DOI: https://www.doi.org/10.2478/helm-2020-0013

Ramadan RM, Khalifa MM, El-Akkad DM, Abdel-Wahab AM, and El-Bahy MM (2021). Animal hydatid cyst genotypes as a potential substitute for human hydatid cyst as a source of antigen for diagnosis of zoonotic hydatidosis. Journal of Parasitic Diseases, 45(2): 424-434. DOI: https://www.doi.org/10.1007/s12639-020-01309-2

Rostami S, Salavati RR, Beech N, Sharbatkhori M, Babaei Z, Saedi S, and FasihiHarandi M (2013). Cytochrome c oxidase subunit 1 and 12S ribosomal RNA characterization of *Coenurus cerebralis* from sheep in Iran. Veterinary Parasitology, 197(1–2): 141-151. DOI: https://www.doi.org/10.1016/j.vetpar.2013.07.001

Sabry MA (2007). Advanced concepts in diagnosis of hydatidosis in human and living animals. Journal of Biological Sciences, 7(5): 720-728. DOI: https://www.doi.org/10.3923/jbs.2007.720.728

Sun GG, Wang ZQ, Liu CY, Jiang P, Liu RD, Wen H, Qi X, Wang L, and Cui J (2015). Early serodiagnosis of trichinellosis by ELISA using excretory-secretory antigens of *Trichinella spiralis* adult worms. Parasites and Vectors, 8: 484. DOI: https://www.doi.org/10.1186/s13071-015-1094-9

Taher EE, Meabed EM, El Akkad DM, Kamel NO, and Sabry MA (2017). Modified dot-ELISA for diagnosis of human trichinellosis. Experimental Parasitology, 177: 40-46. DOI: https://www.doi.org/10.1016/j.exppara.2017.04.002

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.,* 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30

Towbin H, Stachelin T, and Gordon J (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedures and some applications. Proceedings of the National Academy of Sciences, 76(9): 4350-4354. DOI: https://www.doi.org/10.1073/pnas.76.9.4350

Tsai CM and Frasch CE (1982). A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Analytical Biochemistry, 119(1): 115-119. DOI: https://www.doi.org/10.1016/0003-2697(82)90673-X

Varcasia A, Tamponi C, Ahmed F, Cappai MG, Porcu F, Mehmood N, Dessì G, and Scala A (2022). *Taenia multiceps coenurosis*: A review. Parasites & Vectors, 15: 84. DOI: https://www.doi.org/10.1186/s13071-022-05210-0

Yamazawa E, Ohno M, Satomi K, Yoshida A, Miyakita Y, Takahashi M, Satomi N, Asanome T, Maeshima A, Shiotsuka M et al. (2020). First case of human neurocoenurosis caused by *Taenia serialis:* A case report. International Journal of Infectious Diseases, 92: 171-174. DOI: https://www.doi.org/10.1016/j.ijid.2020.01.004

Zhang W, Wen H, Li J, Lin R, and Mc Manus DP (2012). Immunology and immunodiagnosis of cystic echinococcosis: An update. Journal of Immunology Research, 2012: 101895. DOI: https://www.doi.org/10.1155/2012/101895

Zhang XY, Jian YN, Duo H, Shen XY, Ma YJ, Fu Y, and Guo ZH (2019). Coenurosis of yak, Bos grunniens, caused by *Taenia multiceps*: A case report with molecular identification in Qinghai Tibetan Plateau area, China. Korean Journal of Parasitology, 57(4): 423-427. DOI: https://www.doi.org/10.3347/kjp.2019.57.4.423

To cite this paper: El Akkad DMH, Ramadan RM, Auda HM, Abd El-Hafez YN, El-Bahy MM, and Abdel-Radi S (2022). Improved Dot-ELISA Assay Using Purified Sheep *Coenurus cerebralis* Antigenic Fractions for the Diagnosis of Zoonotic Coenurosis. *World Vet. J.,* 12 (3): 237-249. DOI: https://dx.doi.org/10.54203/scil.2022.wvj30