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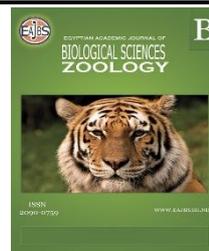


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## Serological Immunodetection of *Fasciola gigantica* Excretory/ Secretory Antigens in Naturally Infected Cattle and Human

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### ABSTRACT

The traditional diagnostic method of fascioliasis was based on the identification of the eggs in the stool, but this is not a reliable way as it has many restrictions. The present work was evaluated to immunodetect the Excretory/ Secretory antigens of *Fasciola gigantica* by sandwich Elisa in naturally infected cattle's sera and by dot blot in naturally infected human sera and compare these methods and the traditional methods. In this study, fresh adult *Fasciola gigantica* worms were collected to extract crude excretory/secretory (E/S) antigens. E/S was used to immunize rabbits and mice to raise polyclonal antibodies. The IgG fraction of rabbit and mouse anti-*Fasciola* antibodies was purified. The protein content of anti-*Fasciola* IgG antibody, was 8.8 and 5.2mg/ml, respectively. Sandwich ELISA was performed to detect *Fasciola* antigens in serum samples collected from 248 cattle. Also, a Dot blot was performed to detect *Fasciola* antigens in 38 human sera. Results showed that after parasitological stool examination, 26 human samples were positive and 12 human sera were healthy. The red-brown color appeared with all infected samples only. This technique is cheap, and saves time; multiple samples can make at the same time and not require expensive laboratory equipment. In conclusion, the sandwich ELISA and Dot blot assays were more reliable tools for early serodiagnosis of fasciolosis than traditional methods.

**Significance Statement:** Different antigenic fractions of *Fasciola* have been used for serological diagnosis of human fasciolosis.

### INTRODUCTION

*Fascioliasis* is a widespread foodborne neglected tropical parasitic infection (Caravedo and Cabada, 2020) and has been reported in 81 countries (Beesley *et al.*, 2017; WHO, 2017). It is a serious parasitic disease caused by infection with the trematodes of genus *Fasciola* sp. infecting both humans and animals (Ibrahim and Ahmed, 2019; Sugiyama *et al.*, 2021). Two species, *F. hepatica* and *F. gigantica*, have been recognized (Caravedo and Cabada, 2020; Sugiyama *et al.*, 2021). *Fasciola gigantica* is found in the tropical and subtropical regions in Africa and Asia (Ibrahim and Bakry, 2019). It caused great economic losses in the livestock industry, where this disease decreased milk yield, meat, carcass quality, and wool production and even caused sudden death (Caravedo and Cabada, 2020; Sugiyama *et al.*, 2021). About 250 million sheep and 350 million cattle are

at risk of *Fascioliasis* worldwide (Beesley *et al.*, 2017; Ibrahim and Bakry, 2019).

Human *Fascioliasis* zoonosis has an economic importance (Soliman, 2008), as it is affecting about 50 million people worldwide (Ibrahim and Ahmed, 2019; Rahman *et al.*, 2017; Villa-Mancera *et al.*, 2016). These flukes caused severe damage to the liver and bile duct of their definitive hosts (Amer *et al.*, 2016; Lee *et al.*, 2017). In Egypt, fasciolosis has a wide distribution in all the Egyptian Governorates and has a negative impact on the farming industry and human health (Ashour *et al.*, 2008; Elshraway and Mahmoud, 2017).

Due to lacking the vaccine against *Fasciolosis*, the successful control of this disease depended on rapid and accurate diagnostic methods (Amiri *et al.*, 2021). The classical way of diagnosis is by detecting eggs in the stool or bile drainage or in the duodenal fluid (Sugiyama *et al.*, 2021). This method had many disadvantages, like eggs are not detected in the early period of infection and only appeared in the late period after the damage of the liver (Hannan Khan *et al.*, 2017; Saadh *et al.*, 2021). The most reliable methods were serological tests that used circulatory antigens. These techniques were preferred because they can detect the anti-*Fasciola* antibodies only two weeks after infection (Saadh *et al.*, 2021). The excretory-secretory products (ESP) of adult worms are excellent antigens that are shared between different helminthic parasites (Sugiyama *et al.*, 2021). The ES products of *F. gigantica* were collected and used for raising the polyclonal antibody in rabbits (Hannan Khan *et al.*, 2017). Different antigenic fractions of *Fasciola* have been used for serological diagnosis of human fasciolosis. Recently, many investigators used enzyme-linked immunosorbent assay (ELISA) and immunoblots for the immune diagnosis of fasciolosis because it is more rapid, sensitive and more specific than traditional methods (Abdolahi Khabisi and Sarkari, 2016; Acici *et al.*, 2017; Amiri *et al.*, 2021; Caravedo and Cabada, 2020). Also, ELISA and the dot blot assay could be used as standardized methods in mass screening programs to detect field infection (Santana *et al.*, 2013; Villa-Mancera *et al.*, 2016).

So, the present study was evaluated to immunodetect the Excretory/ Secretory antigens of *F. gigantica* by sandwich Elisa in naturally infected cattle's sera and by dot blot in naturally infected human sera in Egypt.

## MATERIALS AND METHODS

### Animals and Parasites:

Two New Zealand white rabbits (2 kg, 3 months old) and ten Albino BALB/c mice (20 gm, 6 weeks age) were used for immunization and harvesting of specific polyclonal antibodies. Only male animals were used in experiments. Adult worms of *F. gigantica* were obtained from an abattoir in Beni-suef governorate and were transferred in Hanks' buffer to the Zoology laboratory, Beni-Suef University.

### Blood samples

#### 1. Cattle:

Livers and gallbladders of cattle (248) in the local abattoir were investigated for the presence of *F. gigantica* adult worms. The outcome of the investigation was 168 infected, 50 co-infected with parasites other than *Fasciola* and 30 non-infected animals. Blood samples were collected during slaughtering and sera were prepared aliquoted and stored at -20°C until used.

#### 2. Human:

A total of 38 individuals (15 males and 23 females; age range, 6 to 12 years). A flotation test was carried out for 38 stool samples to investigate the presence of *F. gigantica* ova. The outcome was 26 positives (17 females and 9 males; age range, 7 to 12 years), and 12 negative samples. Blood samples (5 ml) were collected from positive and negative human individuals by vein puncture. Sera were prepared, aliquoted and stored at -70°C until used.

### 3. Preparation of E/S Antigens and Determination of Their Protein Content:

Mature *F. gigantica* adult worms were obtained from the bile ducts of infected cattle and washed six times in phosphate-buffered saline (PBS) to eliminate traces of bile and blood of host cells. The last time washing was in RPMI1640 medium containing 15 ml<sup>-1</sup>25 mM HEPES buffer, 7 ml 7.5% sodium bicarbonate, 100 U penicillin ml<sup>-1</sup> and 10 µg ml<sup>-1</sup>streptomycin. Excretory/ Secretory product (ESP) was prepared as described by (Khan *et al.*, 2009) with some modifications. The total protein concentration of the antigen was measured as described by (Bradford, 1976).

#### Immunization of animals for the Production of polyclonal Antibodies:

##### 1. Rabbits:

ESP antigen (250µg/ml) was emulsified with an equal quantity of Freund's complete adjuvant (Sigma Chemical Co., St. Louis, MO, USA) and administered subcutaneously under the skin of the neck (0.5ml) and intramuscularly at four different sites (0.5ml). The second immunization was given after two weeks after the first immunization using Freund's incomplete adjuvant (Sigma Chemical Co., St. Louis, MO, USA) instead. Two further immunizations were given with 100µg/ml, while the last immunization was given using 50µg/ml of ESP antigen. These last three immunizations were given at weekly intervals and without adjuvants. After one week of the last immunization, the rabbit was bled by cardiac venipuncture for the preparation of serum. Aliquots from the serum were prepared and stored at -20°C.

##### 2. Mouse:

ESP antigen (50µg/ml) was emulsified with an equal quantity of BCG as adjuvant (obtain from Vaccera, Cairo, Egypt) and administered subcutaneously under the skin of the neck (0.1ml) and intramuscularly at four different sites (0.1ml). The second immunization was given after two weeks after the first immunization using BCG as an adjuvant also (obtain from Vaccera, Cairo, Egypt) instead. Two further immunizations were given with 20µg/ml, while the last immunization was given using 5µg/ml of ESP antigen. These last three immunizations were given at weekly intervals and without adjuvant. After one week of the last immunization, the mouse was bled by cardiac venipuncture for the preparation of serum. Aliquots from the serum were prepared and stored at -20°C.

#### Characterization of anti-*Fasciola gigantica* Polyclonal Antibodies by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE):

This characterization is according to Laemmli, (Laemmli, U.K., 1970).

#### Detection of Circulating *Fasciola* Antigens in Cattle Animal's Sera by Sandwich Enzyme-Linked Immunosorbent Assay (ELISA):

ELISA was based on the original method of (Engvall and Perlmann, 1971) and was used with the microplate modification of (Nilsson *et al.*, 1990) and (Venkatesan and Wakelin, 1993). Also, Sandwich ELISA was made to detect circulating *Fasciola* antigen in sera of naturally infected animals with other parasites such as *Schistosoma*, *Hookworm*, *Hydatid* and *Trichostrongyloid*.

#### Detection of Circulating *Fasciola* Antigens In Human-Infected Sera By Dot Blot According to (Yamaura *et al.*, 2003):

##### Statistical Analysis:

All statistical analyses were done with the SAS/STAT (2013). The cut-off values for ELISA experiments were calculated by ANOVA test. Sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) were assessed according to the formula of (Lukambagire *et al.*, 2021). A P value of <0.05 was considered statistically significant.

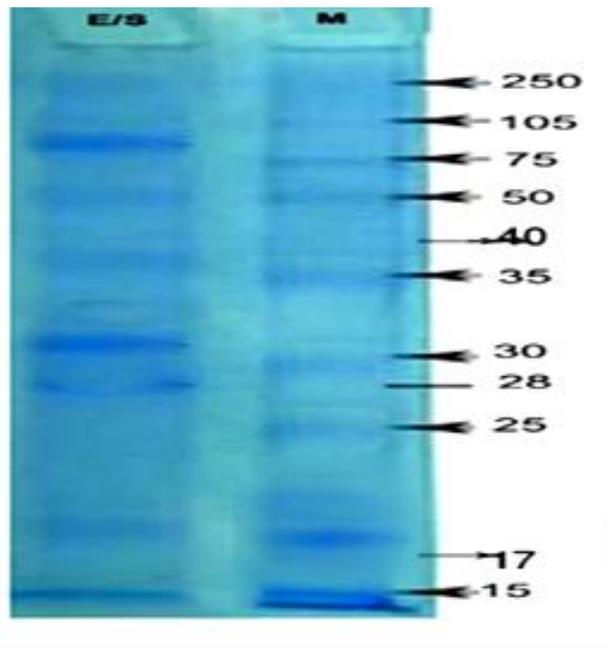
## RESULTS

### 1. Estimation of the Total Protein Content of *Fasciola gigantica* Antigens :

Protein content assays of ESP (Excretory and Secretory products) obtained from adult *Fasciola* worms indicated that 2-, 4- 6-, and 8-h ESP preparations contained an average of 2, 5.1, 6.8 and 4.7 mg/ml protein, respectively measured as a total protein by Bio-Rad Protein assay. So, only 6-h ESP preparation was used in the present study.

### 2. Gel electrophoresis for ESP:

The SDS-PAGE analysis of ESP antigen was resolved by SDS-PAGE (12.5%) under reducing conditions (+2-mercaptoethanol) and stained with Coomassie Blue. Protein bands that appeared at 15, 17, 28, 32, 40, 60 and 84 kDa were observed (Fig. 1).



**Fig. 1.** Molecular weight bands of ESP antigen in SDS-PAGE (12.5%) stained by Coomassie blue.

ESP: Excretory Secretory product; M: standard molecular weight.

### 3. Reactivity of Anti-ESP Polyclonal Antibodies with *Fasciola gigantica* Antigens and Different Parasite Antigens by Indirect ELISA:

Reactivity of anti-ESP polyclonal antibodies with different parasite antigens such as *S. mansoni*, Hydatid and Hook Worm were strongly reactive with sera of naturally infected cattle with *F. gigantica* worms, while no cross-reactions were recorded with sera of other parasites (Table 1).

**Table 1:** Shows reactivity of anti-ESP polyclonal antibodies with different parasite antigens by indirect ELISA.

Serum samples	OD readings at 492 nm $\pm$ SD
<i>Fasciola</i>	1.12 $\pm$ 0.311
<i>Schistosoma mansoni</i>	0.488 $\pm$ 0.261
Hydatid	0.122 $\pm$ 0.098
Hook worm	0.147 $\pm$ 0.071

OD= optical density; SD= standard deviation.

#### 4. Purification of Anti-*Fasciola* Antibodies

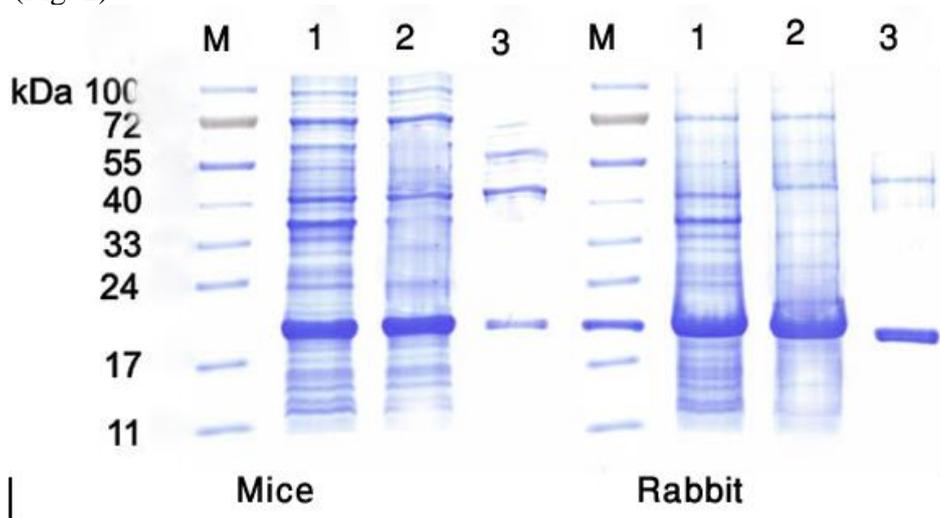
##### 4.1. Purification of Rabbit Anti-*Fasciola* Antibodies:

The total protein content of crude rabbit serum containing anti-*Fasciola* antibody was 13.7 mg/ml. The yield of purified anti-*Fasciola* IgG antibody following each purification step was determined by the assessment of protein content. Using the 50% ammonium sulfate precipitation method, the protein content was 8.8 mg/ml, while following the 7% caprylic acid precipitation method the content dropped to 5.2 mg/ml.

##### 4.2 Purification of Mouse Anti-*Fasciola* Antibodies:

The total protein content of crude mouse serum containing anti-*Fasciola* antibody was 7.5 mg/ml. The yield of purified anti-*Fasciola* IgG antibody following each purification step was determined by the assessment of protein content. Using the 50% ammonium sulfate precipitation method, the protein content was 4.1 mg/ml, while following the 7% caprylic acid precipitation method the content dropped to 2.7 mg/ml. Analysis of 50% ammonium sulfate-precipitated proteins by 12.5% SDS-PAGE under reducing conditions showed that precipitated proteins appeared as several bands.

The purity of IgG after each step of purification was assayed by 12.5% SDS-PAGE under reducing conditions. The purified pAb (polyclonal antibody) IgG was represented by H- and L-chain bands at (53 and 18 KDa) respectively. The pAb appears free from other proteins (Fig. 2).



**Fig. 2:** SDS-PAGE (12.5%) for anti-ESP IgG pAb before and after purification with 7% caprylic acid and stained with Coomassie blue. Lane 1, anti-ESP serum; Lane 2: Precipitated proteins of anti-ESP serum with 50% ammonium sulfate; Lane 3: Purified anti-ESP serum IgG pAb by caprylic acid; M, Molecular weight of standard protein.

#### 5. Conjugation and Purified Rabbit & Mouse Anti-*Fasciola* IgG:

The results of titration showed that the 1/20  $\mu\text{g/ml}$  concentration of the conjugate gave the highest OD reading against *Fasciola* ESP after subtraction of the background and was chosen as the working dilution for subsequent assays.

#### 6. Detection of Circulating *F. gigantica* Antigen in Sera of Naturally Infected Cattle:

In order to measure the incidence of positivity for *Fasciola* E/S in the studied sera, it was necessary to determine the cut-off point for positivity or the line of demarcation between positive and negative. The cut-off point for positivity was measured as the mean OD reading of negative controls + 2 standard deviation (SD) of the mean. Tested samples showing OD values > cut-off value were considered positive for *Fasciola*.

- Developing a sandwich ELISA to detect E/S antigens of *Fasciola* through the production of Anti-*F.gigantica* IgG polyclonal sera in two different animals (mouse and rabbit) by using different coating and conjugate Anti- *F. gigantica* IgG. There are four cases that were used in Sandwich Elisa:

**Case (1):** Using rabbit anti- *F.gigantica* IgG for Coating (primary Ab) and rabbit anti-*F.gigantica* IgG Conjugated with peroxidase as conjugate (secondary Ab) ( Figs. 3; A& B).

**Case (2):** Using mouse anti- *F.gigantica* IgG for Coating (primary Ab) and mouse anti-*F.gigantica* IgG Conjugated with peroxidase as conjugate (secondary Ab). The cut-off values for positivity were calculated as mean + 2 SD (Figs.3; C& D).

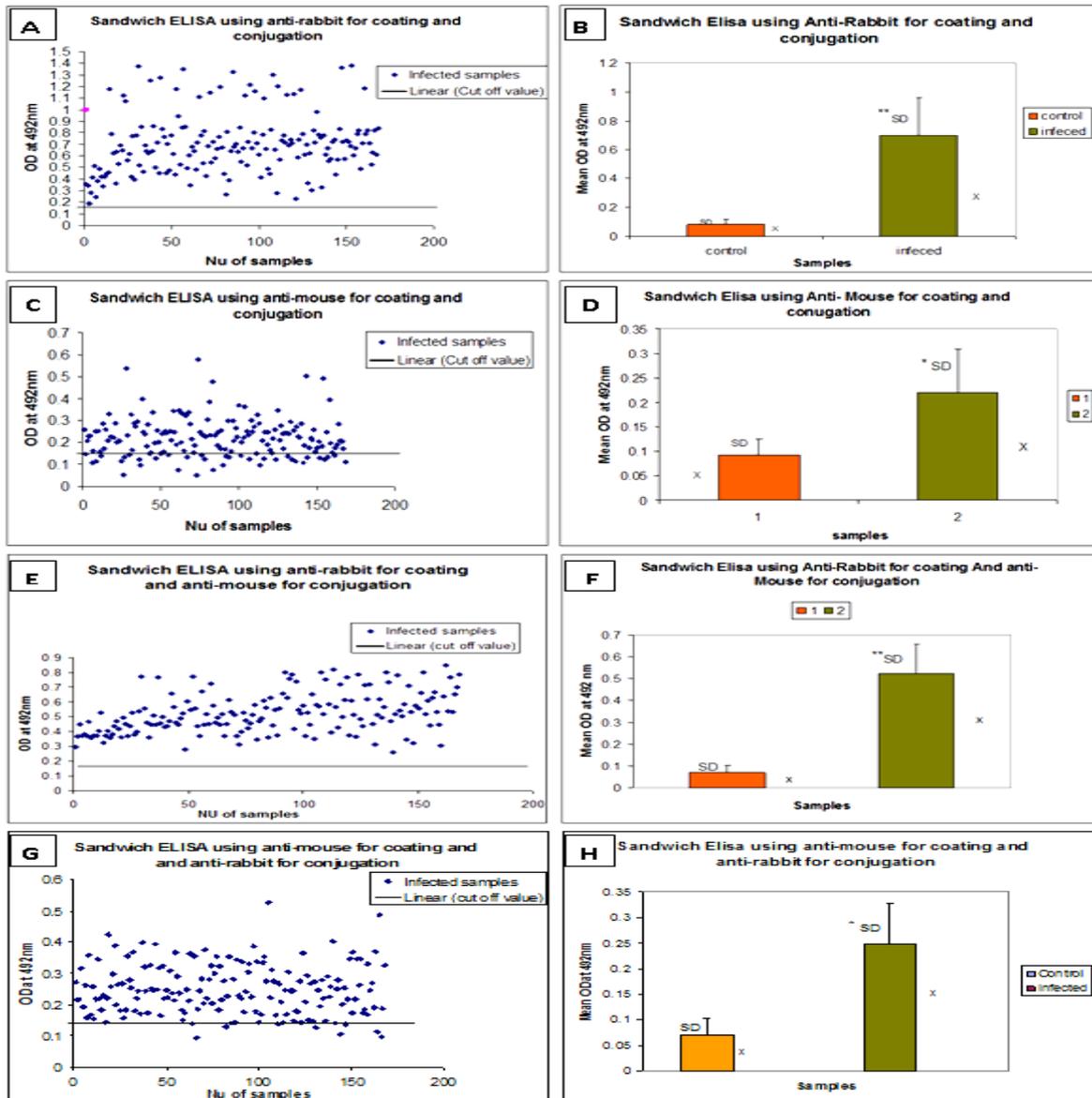
**Case (3):** Using rabbit anti- *F.gigantica* IgG for Coating (primary Ab) and mouse anti-*F.gigantica* IgG Conjugated with peroxidase as conjugate (secondary Ab). The cut-off values for positivity were calculated as mean + 2 SD (Figs.3; E& F).

**Case (4):** Using mouse anti- *F.gigantica* IgG for Coating (primary Ab) and rabbit anti-*F.gigantica* IgG Conjugated with peroxidase as conjugate (secondary Ab). The cut-off values for positivity were calculated as mean + 2 SD (Figs. 3; G& H).

By comparing the results of these four cases with each other conclude that, the highest sensitivity was found in the first three cases 100%, 100% and 94% respectively, but the lowest one was in the last case 73.23%. While the highest specificity was in the last case (100%) and the other three cases also give high specificity 96.67%, 93.34% and 96.67%, respectively. Positive predictive values were high in the four cases 99.4%, 98.83%, 99.38% and 100%, respectively. While the highest negative predictive values in the first two cases were 100%, the lowest one was in the last case 66.67% and the third case was 74.36% (Table 2).

**Table 2:** The summarized table shows the Sensitivity, specificity, positive predictive value and negative predictive value of sandwich ELISA used for the detection of *Fasciola* E/S antigens in serum samples of *Fasciola* naturally infected cattle. Using the four cases mentioned above.

<i>Fasciola</i> antigen detected in serum of naturally infected cattle	Sensitivity	Specificity	+ve predictive value	-ve predictive value
A) Using rabbit anti- <i>F.gigantica</i> IgG for Coating and rabbit anti- <i>F.gigantica</i> IgG Conjugated with peroxidase as conjugate	100%	96.67 %	99.4 %	100 %
B) Using rabbit anti- <i>F.gigantica</i> IgG for Coating and mouse anti- <i>F.gigantica</i> IgG Conjugated with peroxidase as conjugate	100%	93.34 %	98.83 %	100 %
C) Using mouse anti- <i>F.gigantica</i> IgG for Coating and rabbit anti- <i>F.gigantica</i> IgG Conjugated with peroxidase as conjugate	94%	96.67 %	99.38 %	74.36 %
D) Using mouse anti- <i>F.gigantica</i> IgG for Coating and mouse anti- <i>F.gigantica</i> IgG Conjugated with peroxidase as conjugate	73.23%	100 %	100 %	66.67%



**Fig. 3:** Shows distribution of OD at 492 of A) 168 naturally infected cattle and the cut-off value. B) Control samples (n=30) and naturally infected samples (n=168), where x=mean, SD= standard deviation and P<0.01 for case (1). C) 168 naturally infected cattle and the cut-off value for the second case; D) Control samples (n=30) and naturally infected samples (n=168) for case (2). E) 168 naturally infected cattle and the cut-off value for case (3); F) Control samples (n=30) and naturally infected samples (n=168) for case (3). G) 168 naturally infected cattle and the cut-off value for case (4); H) Control samples (n=30) and naturally infected samples (n=168) for case (4).

**9. Detection of Heterologous Parasite Antigens:**

In order to verify if antigens from other parasites could be detected by the assay, using rabbit anti- *F.gigantica* IgG for Coating and rabbit anti-*F.gigantica* IgG Conjugated with peroxidase as conjugate (Table 3).

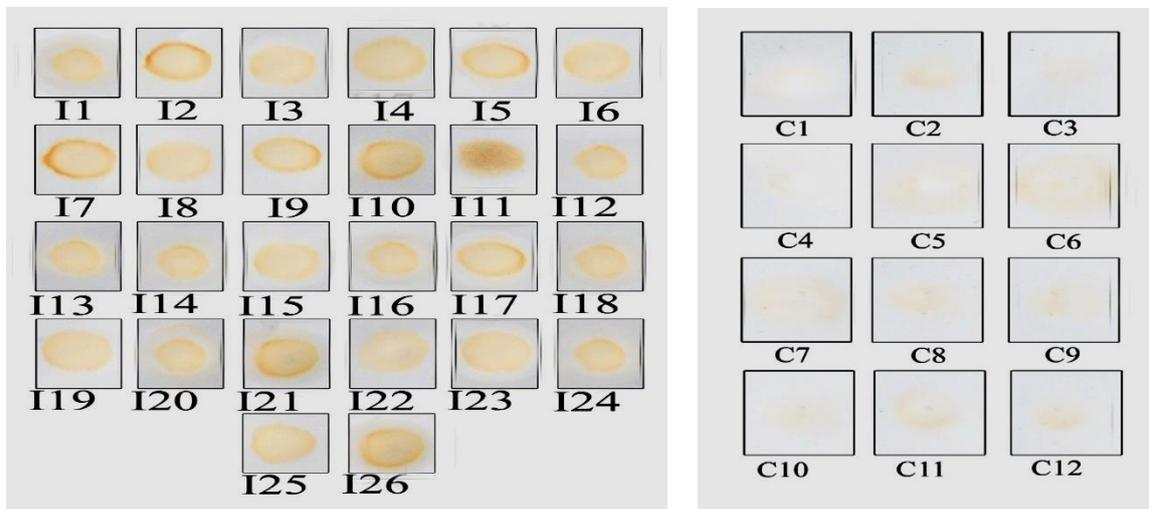
**Table 3:** Detection of circulating *Fasciola* antigen in serum of naturally infected animals with other parasites.

Group (no of animals)	Positive cases		Negative cases	
	No.	X± SD	No.	X± SD
Healthy control (n= 20)	--	--	20	0.167 ± 0.098
<i>Schistosoma</i> (n= 16)	2	0.313 ± 0.216	14	0.156 ± 0.179
Hookworm (n= 12)	--	--	12	0.117 ± 0.091
<i>Hydatid</i> (n= 20)	--	--	20	0.148 ± 0.109
<i>Trichostrongyloid</i> (n= 15)	--	--	15	0.121 ± 0.189

X= mean; SD= standard deviation.

#### 10. Immunoblotting (Dot-BLOT):

Two groups consisting of Serum samples obtained from 26 suspected cases of patients infected with fasciolosis (n= 26) and healthy individuals (n= 12), were implicated in the test (Fig. 4).



**Fig 4.** Dot blot showing the appearance of the dot with human naturally infected with *Fasciola* (n=26); I= Infected sample. B) Dot blot showing disappearance of dots with healthy human individuals, (control samples) (n= 12); C= Control sample.

## DISCUSSION

The urgent need for sensitive and specific diagnostic tools for *Fasciola* has been developed (Caravedo and Cabada, 2020; Sugiyama *et al.*, 2021; Villa-Mancera *et al.*, 2016). These tools could detect the presence of flukes within the parasitized animal throughout the prepatent infections and the migration process (Amiri *et al.*, 2021; Caravedo and Cabada, 2020). (El Ridi *et al.*, 2007) suggested that *F. gigantica* total (Excretory/Secretory) (ESP) might be used as easily available, safe and inexpensive antigens for immunodiagnosis. The ESP antigens of *Fasciola spp* or their partially purified component are the commonest source of antigens used in protection trials and serodiagnosis (Dar *et al.*, 2016).

The SDS-PAGE analysis of Fg-E/S antigens in the present study showed that many bands have appeared, and the most prominent of which were of 15, 17, 28, 40, 60 and 84 kDa. Some bands (40, 60, and 84) were shown to be derived from the worm tegument (Viyanant *et al.*, 1997), and they are the major recognition targets in ESP-based immunodiagnosis, while the 15, 17 and 28 kDa species appeared to be released from the cells lining the gut (Anuracpreeda *et al.*, 2006; Arjmand Yamchi *et al.*, 2016). (Awad *et al.*,

2009) showed that the *Fg*-ES antigen revealed seven protein bands with molecular weights of 15, 28, 31.6, 32.9, 39.4, 83.3 and 101.7 kDa. (Fagbemi and Guobadia, 1995) have identified four polypeptide antigens that were specific to *F. gigantica* and reported that the 17 kDa antigen was recognized early in *F. gigantica* infection in sheep.

Reactivity of anti-ESP polyclonal antibodies with different parasite antigens such as *S. mansoni*, Hydatid and Hookworms were strongly reactive with sera of naturally infected cattle with *F. gigantica* worms, while no cross-reactions were recorded with sera of other parasites and this proved that the ability of the produced polyclonal antibodies to bind with, is the target antigens (ESP of *Fasciola*) not with the other antigens.

ELISA is the most reliable and widely used method in fasciolosis diagnosis due to its simplicity and easy steps (Amiri *et al.*, 2021; Awad *et al.*, 2009; Saadh *et al.*, 2021). (Molloy *et al.*, 2005) conclude that the ELISA will be a valuable tool for diagnosing *F. hepatica* infections in cattle and sheep. The ability to diagnose and treat infections early is a big advantage of ELISA because it minimized the tissue damage in the infected animals that is caused by the immature flukes (Kahl *et al.*, 2021). Also, sandwich ELISA was evaluated for its ability to detect E/S antigens in naturally infected cattle by using anti-*F. gigantica* antibodies (Hannan Khan *et al.*, 2017).

The present study developed a sandwich ELISA to detect ESP antigens of *F. gigantica* in sera of naturally infected cattle through the production of Anti-*F. gigantica* IgG polyclonal sera in two different animals (albino mouse and new-Zeeland rabbit), Polyclonal antibodies against ESP antigens were employed, one for coating and the other one as a peroxidase-conjugated antibody, therefore using two different coating and conjugate Anti-*F. gigantica* IgG in Sandwich ELISA is the first experiment using two different anti-*Fasciola* IgG from two different sources. IgG obtained from the rabbit and mouse immunized with ESP was used for coating and capturing these antigens in naturally infected cattle. The sandwich ELISA has 94.55% sensitivity and 100% specificity with 100% positive predictive value, 97.39% negative predictive value, 0% false positive rate, 5.50% false negative rate and 98.2% accuracy. These results were in accordance with (Sánchez-Andrade *et al.*, 2000) who reported that Sensitivity and specificity values for indirect-ELISA and sandwich-ELISA were 92 and 94.4%, and 86 and 100%, respectively. The sensitivity, specificity, positive and negative predictive values for somatic antigen were 91.0%, 96.2%, 95.2% and 92.7% respectively, while these parameters for ES antigen were 95.2%, 98.0%, 97.5% and 96.2%, correspondingly (Rokni *et al.*, 2014). A commercial ELISA assay detected the liver fluke infection in sheep and cattle within 2 weeks with high degrees of sensitivity and specificity (Anuracpreeda *et al.*, 2006; Carnevale *et al.*, 2015). (El Ridi *et al.*, 2007) indicated that ESP-based ELISA reached nearly 100% sensitivity and specificity in the immunodiagnosis of sheep *Fasciolosis*.

In order to verify if antigens from other parasites could be detected by the assay, using rabbit anti- *F. gigantica* IgG for Coating and rabbit anti-*F. gigantica* IgG Conjugated with peroxidase as conjugate. Using different parasite antigens such as *S. mansoni* (n=16), Hydatid (n=20), Hook Worm (n=12) and Trichostrongyloid (n= 15). All of these parasites give 100% negativity except *S. mansoni* which 2 samples from 16 samples were positive and this is due to the cross-reactivity between *Fasciola* and *Schistosoma*.

Immunodiagnosis for Human fasciolosis has emerged as an important zoonotic disease in the last decade (Sarkari and Khabisi, 2017). The availability of an effective immunological method for confirmation of clinical and imaging diagnosis would improve diagnostic accuracy and help assess the epidemiological situation (Anuracpreeda *et al.*, 2006). Dot-BLOT using excretory-secretory antigens could be regarded as a cheap, rapid, antigen and serum-conservative diagnostic method in diagnosing fasciolosis (Arafa *et al.*, 1999; Demerdash *et al.*, 2011; Sarkari and Khabisi, 2017). This assay could be the alternative to ELISA, which used a paper matrix onto which the antigen is spotted (Vera-

Cabrera *et al.*, 1999). (Acici *et al.*, 2017) concluded that diagnosis of ovine fasciolosis can be accomplished using serodiagnostic assays based on E/S antigens of liver flukes and using ELISA and Western blot assays as screening and confirmational tests, respectively.

In this study, all patients infected with fasciolosis gave positive results the appearance of reddish-brown dots on nitrocellulose with all samples, while healthy control individuals showed disappearance of this color. These results could prove the ability of anti-rabbit IgG raised against ESP antigens of *F. gigantica* to bind with ESP of patients infected with fasciolosis. But in healthy individuals, there is no ESP in the sera so anti-rabbit IgG cannot bind and remove by washing and nitrocellulose paper is still colorless. These are important results because this assay is simple to perform, needs less test time and the least laboratory facilities, nitrocellulose membrane is very sensitive and efficient for this assay, it allows direct detection of the Antigen in a test sample, and the full analysis can be completed in just a few hours thus saves time, Nitrocellulose papers spotted with antigen are stable for at least three months at -20°C, all incubation steps are performed at room temperature, and the results can be read with the naked eye. Only 5/μ of serum is required for the dot-blot, a distinct advantage when serum is difficult to be obtained (Santos *et al.*, 2018). The test is applicable to diagnose fasciolosis in the field setting as well as in laboratories that are not well equipped and dot blot is simpler and allows the testing of multiple samples at the same time (Sarkari and Khabisi, 2017).

#### **Conclusion:**

The present results proved that the sandwich ELISA and Dot blot assays were more reliable tools for early serodiagnosis of fasciolosis as they were rapid, simple, economical and useful methods with high specificity and sensitivity in both humans and cattle. This study used for the first time, fasciola Anti-IgG polyclonal antibodies from two different animal species and this could facilitate the immunodiagnosis. Further studies were required for evaluating this assay in a large-scale number of infected animals in addition to humans.

#### **Ethical Approval:**

This study was performed in accordance with the ethical committee of Beni Sueif University, Egypt.

**Acknowledgements:** Not applicable.

**Conflict of Interests:** The authors declare that they have no conflict of interest.

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