The role of Ser-(Arg-Ser-Arg-Ser-GlcNAc)₁₉-GlcNAc *Fasciola gigantica* glycoprotein in the diagnosis of prepatent fasciolosis in rabbits

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Abstract In the present study, the carbohydrate structures associated with *Fasciola gigantica* adult worm were identified by indirect hemagglutination inhibition test. Glucose was found to be the main monosaccharide associated with the fluke. According to indirect hemagglutination inhibition results, purification of glycoprotein fractions from worm crude extract was carried out by affinity chromatography immobilized glucose agarose gel and Con-A lectin columns. The isolated glycoprotein fractions, FI and FII, were characterized by SDS-PAGE which revealed one band in FI of 26 kDa and another one band of 19.5 kDa in FII compared with 12 bands associated with whole worm extract. Both fractions were also characterized by isoelectric focusing technique which proved that both bands were acidic in nature with pIs 6.4 and 6.5 respectively. The comparative diagnostic evaluation of the two isolated glycoprotein fractions and crude extract of experimental fasciolosis in rabbits by ELISA revealed that FII was more potent in the diagnosis during prepatent (first week post infection) and patent periods (10 weeks post infection) than FI and crude extract. Moreover, infected rabbit sera at ten weeks post infection identified both bands; 26 and 19.5 kDa in western blot analysis confirming its immunodiagnostic activities which was

proved previously by ELISA. FII proved potency in diagnosis of fasciolosis in 200 buffalo serum samples of different ages and sexes using ELISA which recorded 95 % positive and 5 % negative samples. Moreover, the detailed structural analyses of the most potent fraction, FII, using mass spectrum was made and elucidated chemical structure; O-glycan [Ser-(Arg-Ser-Arg-Ser-GlcNAc)₁₉-GlcNAc]. The present result introduces GlcNAc rich fraction of *F. gigantica* that can be used successfully in the diagnosis of acute and chronic fasciolosis.

Keywords *Fasciola gigantica* · GlcNAc · Affinity column chromatography · Diagnosis · ELISA

Introduction

In Egypt, fasciolosis is highly affecting cattle, sheep, goats, buffaloes and humans (Haridy et al. 2002; Mazyad and El-Nemr, Mazyad and El-Nemr 2004; El-Shazly et al. 2005; Morsy et al. 2005; Haridy et al. 2006). It is an emerging disease which is already considered a serious health problem in Egypt (Curtale et al. 2000, 2005; Hussein and Khalifa Hussein and Khalifa 2010a, b). The early diagnosis of fasciolosis during prepatent period using antibody detection tests is an essential method for arresting its negative impact on productivity and preventing economic losses (Sanchez-Andrade et al. 2000; Dixit et al. 2004; Kumar et al. 2008). ELISA-based antibody detection systems using different protein antigens are excellent for the early diagnosis of fasciolosis (El Ridi et al. 2007; Espinoza et al. 2007; Mezo et al. 2007; Charlier et al. 2008; Kumar et al. 2008).

Because some of the isolated protein antigens were not effective in diagnosis against parasitic helminthes,

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attention is increasingly focused on the nature and role of carbohydrate antigens. Much information is accumulating on the structural characterization of these antigens and in some cases; there is evidence for their role in host immunity. It is now apparent that antibodies directed against helminthes carbohydrate moieties or glycoproteins are implicated in protecting hosts against infection, which indicates their potential as targets for vaccine and diagnosis of parasites. Several detailed reviews have been published which provide examples of the complex structure of parasite glycans and their role in immunity (Khoo and Dell 2001; Newton and Meeusen 2003; Nyame et al. 2004). However, little is known about the structural basis of carbohydrate-mediated host–parasite interactions. This is largely because the structures of relatively few helminthes carbohydrates have been rigorously defined and even fewer are available in quantities that permit immunological functional investigations. High-sensitivity mass spectrometry (MS) provides means of directly addressing the both problems (Haslam et al. 2001). Detailed structural analysis of carbohydrates is now possible through a combination of techniques including enzymatic or chemical degradation, separation by electrophoresis or chromatography and analysis using mass spectrometry (Dell 1987; Dell et al. 1994; Haslam et al. 2001; Hirabayashi and Kasai 2002; Chalkley et al., 2009). Moreover the potentials of glycoproteins (Ghosh et al. 2005a, b; McAllister et al. 2011; Georgieva et al. 2012; Young et al. 2012) or even glycolipids (Wuhrer et al. 2003; Wuhrer et al. 2004) in the diagnosis of fasciolosis are proved.

However, little attempts have been applied concerning the identification of sugar structures associated with mature flukes and its diagnostic values. Consequently the objective of the current study is to identify the novel role of glycoprotein(s) in diagnosis and its assessment in acute and chronic fasciolosis.

Materials and methods

Parasites

Fasciola gigantica adult worms were collected from naturally infected livers of buffaloes slaughtered at Shebin-Elkome abattoir. Worms were washed thoroughly with PBS, pH7.2, identified morphologically according to (Periago et al. 2008) and kept frozen -20°C until use.

Fasciola gigantica metacercariae were purchased from Schistosoma Biological Supply Program, Theodor Bilharz Research institute, Giza, Egypt. Viability of metacercariae was checked by microscopy on arrival.

Animals

Native breed rabbits (1.5–2.0 kg each), pathogens free as proved by fecal examination and ELISA, were used for experimental infection and preparation of hyperimmune sera.

The current study was approved by the Animal Care and Ethics Committee of National Research Center, Egypt, Giza.

Experimental infection

Six rabbits were infected orally with 30 ± 5 metacercariae each (Muro et al. 1997). Blood samples were taken weekly for 10 weeks after infection from ear vein. Serum samples were stored at -20°C for serological analysis. Three non infected rabbits were used as negative control.

Preparation of *Fasciola gigantica* crude worm antigen

The crude worm antigen was prepared as the method reported by Timanova et al. (2003). Briefly; adult flukes were homogenized in 0.02 M Tris–HCl buffer pH 7.4 with glass homogenizer at 4°C . Homogenate was then centrifuged at 14,000 rpm for 30 min at 4°C , then supernatant was collected and assayed for protein content by the method of Lowry et al. (1951). The supernatant was aliquoted and stored at -20°C until use.

Collection of buffalo serum samples

A total of 200 blood samples from buffaloes of different ages and sexes were collected from Shebin-Elkome slaughter house. Blood samples were centrifuged in the laboratory at 2,000 rpm for 20 min and sera were separated, labeled and stored at -20°C until use.

Negative sera were donated as gift from Dr. Alaa Ghazy Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Center, Dokki, Giza, Egypt. Positive control samples were selected based on fecal and post mortem examinations.

Preparation of hyper immune sera

100 mg (Gubadia and Fagbemi 1997) of *F. gigantica* adult worm antigen was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously into each of 3 rabbits. The first booster dose was administered 2 weeks post the initial dose after emulsification in Freund's incomplete adjuvant. Second and third booster doses were given at 21 and 28 days respectively without adjuvant according to the method of Fagbemi et al. (1995). Sera were collected 4 days post last injection from ear vein.

Indirect haemagglutination inhibition test

Identification of sugar structures associated with adult worm antigen was performed using indirect haemagglutination inhibition test. Sheep erythrocytes (SRBCs) were collected from Shebin Elkome abattoir. Aliquots of SRBCs were formalin-fixed, tanned and coated with *Fasciola* antigen according to standard procedures described by Csizmas (1960). Different sugars were used to competitively inhibit the binding activities between *Fasciola* antigen and hyperimmune serum raised in rabbit against *Fasciola* antigen.

Affinity chromatography

Glucose-agarose column

Prepacked glucose-agarose column obtained from Sigma Chem. Co. St. Louis was utilized for isolation of glycoprotein fraction from *Fasciola* worms. Crude *Fasciola* antigen was applied to the column and the bound fraction was eluted with 0.5 M α -glucose (Fukuda et al. 1993). The fraction was checked for protein content by the method of Lowry et al. (1951) and designated as FI.

Lectin affinity chromatography

Prepacked *Concanavalin ensiformis* (Con A) column obtained from Sigma Chem. Co. St. Louis was used for isolation of glycoprotein fraction as previously described. The fraction was checked for protein content by the method of Lowry et al. (1951) and designated as FII.

Characterization of isolated glycoproteins

SDS-PAGE

The technique was performed according to the procedures described by Laemmli (1970) for characterization of adult *F. gigantica* crude antigen and the two isolated glycoprotein fractions (FI and FII). Molecular weight standards (14–220 KDa) were electrophoresed in the same gel for comparative purposes.

Immunoblot

The immunogenicity of the isolated fractions was assessed by immunoblotting assay. The protocol described by Towbin et al. (1979) was used for the immunoblotting of the two purified glycoproteins onto nitrocellulose membrane blotting system. Subsequent detection of proteins was performed using rabbit antisera at 10 weeks post infection, Horse-radish peroxidase conjugated goat-anti-

rabbit IgG was added. ECL western blotting detection reagent (Amersham, UK) was utilized to visualize the immunoreactive bands.

Isoelectric focusing

IEF of *F. gigantica* crude and isolated fractions was performed as described by O'Farrell (1975) in slab gels supplemented with urea and ampholine. Gels were stained with Commassie blue and photographed wet. Isoelectric point (PI) of a particular protein can be determined by running a mixture of proteins of known isoelectric points on the same gel (IEF-USB, USA; 4.6–9.3).

Chemical elucidation

The analytical sample was homogenous by thin-layer chromatography (TLC), which was performed on EM silica gel 60 F sheet (0.2 mm) with $\text{CHCl}_3/\text{CH}_3\text{OH}$ (9:1, v/v); as the developing eluent, rate of follow (R_f) = 0.2. The spot was detected with U.V model UVGL-58 as a violet spot. EI Mass spectra recorded on a Varian MAT 311A spectrometer.

ELISA

The assay was utilized to evaluate the diagnostic potentials of the crude and isolated fractions in experimental fasciolosis in rabbits. The assay was also used to assess the potency of the potent fraction in the diagnosis of natural fasciolosis in buffaloes. The test was carried out as described by Oldham (1983) and the optimal concentration of the antigen and dilution of serum and conjugate were determined by checkerboard titration. Anti-rabbit and anti-bovine IgG horse radish peroxidase were used and the cutoff point of optical density values was determined as described by Almazan et al. (2001).

Data of ELISA were analyzed for the means and standard deviation according to Snedecor and Cochran (1982). Significance of the results was evaluated using one way ANOVA (F value and Duncan) using statistical package for social science (SPSS) computer programs (2002).

Results

Protein content of prepared antigens

The protein content of crude antigen of *F. gigantica* was 1,800 $\mu\text{g}/\text{ml}$. While the protein content of FI was 236 $\mu\text{g}/\text{ml}$ and that of FII was 316 $\mu\text{g}/\text{ml}$.

Indirect haemagglutination test

Erythrocytes were agglutinated in the presence of specific antiserum for the adsorbed antigen. The best results were obtained when erythrocytes were coated with 25 µg/ml antigen.

Indirect haemagglutination inhibition test

Lactose, the disaccharide in which galactose and glucose are linked in an 1-4 linkage, showed the highest inhibition percentage of the binding activities at both high (90 % at 200 mM) and low (60 % at 25 mM) sugar concentrations as shown in (Fig. 1 and Table 1). While glucose, a monosaccharide, gave 80 % inhibition at 200 mM, galactose which is one constituent of lactose showed only 25 % inhibition at high concentration (200 mM). While, L-fucose showed 60 % inhibition at 200 mM. Mannose showed mild inhibition percentage (50 %) at high concentration (200 mM) as shown in (Fig. 1 and Table 1).

Fig. 1 Carbohydrate inhibition of binding activities of rabbit hyper immune sera to *F. gigantica* crude adult extract coated SRBCs. Each point on the curve is the mean value of series of assays

Table 1 Sugar inhibition of the binding activities in hyper immune sera toward *F. gigantica* crude extract

Sugar inhibitor	Inhibition percentage			
	Inhibitor concentration (mM)			
	200 mM (%)	100 mM (%)	50 mM (%)	25 mM (%)
α-Glucose	80	70	60	50
L-Fucose	60	50	41	25
D-Mannose	50	0	0	0
D-Galactose	25	25	25	25
α-Lactose	90	80	70	60

Concentrations of sugars are in mM and the percent competitive inhibition was measured by IHA assays

Fasciola gigantica glycoprotein isolated by monosaccharide affinity column

The crude *F. gigantica* extract was separately applied to glucose-agarose and Con-A lectin columns chromatography, after washing the bound glycoprotein fractions was eluted with 0.5 M glucose. The yielded bound fractions were designated as F1 and F11 respectively.

Characterization of isolated glycoproteins

SDS-PAGE

The electrophoretic profile of the three *F. gigantica* antigens (crude extract and two isolated fractions) demonstrated that the crude antigen showed multiple bands with molecular weights ranging from 15–220 kDa (Fig. 2). The glycoprotein fraction F1 resolved into one band with molecular weight 26 kDa, and F11 showed also one band but with molecular weight 19.5 kDa (Fig. 3).

Fig. 2 SDS-polyacrylamide gel electrophoretic profile stained with Coomassie blue R-250 of *F. gigantica* crude extract lane (A), lane (S); molecular weight standards in kDa

Fig. 4 Isoelectric focusing of the isolated fractions. Lane A FI, Lane B FII and lane S isoelectric focusing standards

Fig. 3 Electrophoretic profile of the isolated fractions from *Fasciola gigantica* crude worm extract. Lane A FI, Lane B FII and Lane S molecular weight standards in kDa

Fig. 5 Immunoreactive bands of two isolated fractions identified by *F. gigantica* positive rabbit sera (10 weeks post infection) using immunoblot assay. Lane A FI, Lane B FII and Lane S molecular weight standards in kDa

Isoelectric points of isolated fractions

For further characterization of FI and FII, Isoelectric focusing technique was utilized. The PI of FI band was 6.4. While that of FII was 6.5. The two glycoproteins are in acidic range as shown in (Fig. 4).

Western blot analysis of *F. gigantica* isolated glycoproteins

Western blot analysis was adopted to evaluate the immunogenicity of the isolated band in each fraction using serum collected from rabbits at 10 weeks post experimental infection with fasciolosis. The serum identified both bands. FI showed one immunogenic band of molecular weight 26 kDa and FII showed also one immunogenic band but of 19.5 kDa (Fig. 5).

Chemical structure of isolated glycoprotein FII

The Thin Layer Chromatography (TLC) gave one spot in MeOH/CHCl₃ (80:20 v/v) as an eluent. By immersing the TLC sheet in 5 % H₂SO₄/MeOH solution and heating it gave burning red color for GlucNAc sugar moiety. The mass spectrum of the monomer $m/z = 1,000$ [M+3H]. Ser-Arg-Ser-Arg-Ser-GlucNAc-GlucNAc. The fragmentation or peak at $m/z = 106$ is an indication for the Serine (Ser) residue [M+H]. The fragmentation or peak at $m/z = 170$ is an indication for the Arginine (Arg) residue [M-4H]. The fragmentation or peak at $m/z = 223$ is an indication for the GlucNAc residue [M+2H]. The fragmentation or peak at $m/z = 424$ is an indication for the GlucNAc-GlucNAc residue [M]. The fragmentation or peak at $m/z = 792$ is an indication for the [monomer-OglucNAc]. The electrophoresis give a value at 19.5 kD which mean that the

Fig. 6 Chemical structure of isolated glycoprotein FII

glucopeptide polymer is: *O*-glycan Ser-(Arg-Ser-Arg-Ser-GlucNAc)₁₉-GlucNAc. EI-Mass: (m/z %) = 1000 ($M+3H$, 50) (Figs. 6, 7).

Diagnostic potentials of isolated glycoprotein fractions and crude extract in rabbit experimental fasciolosis

The optimal concentration of *F. gigantica* crude antigen and dilution of serum as determined by checkerboard titration in ELISA were 10 µg/ml and 1:100 respectively. While the isolated glycoprotein fractions (FI and FII) were used at concentration of 2.5 µg/ml. Serum samples were collected weekly from first weeks post infection till ten weeks post rabbits infection. Obtained results are presented in (Table 2; Fig. 8).

There is a significant increase in mean OD level in FII compared with FI and crude antigen in all weeks post infection, but the most significant increase was at first and 10 weeks post infection. It is clear that FII is the best for early diagnosis of fasciolosis than FI and crude antigen. FII is of choice for diagnosis of fasciolosis during prepatent and patent periods.

Diagnosis of natural fasciolosis in buffaloes

FII proved further potentials in the diagnosis of natural fasciolosis in buffaloes using ELISA. The results are shown in Fig. 9 demonstrating 190 positive samples with a percentage of 95 %, however, the negative samples were 10 recording a percentage of 5 %. The positive absorbance values of ELISA were taken to be those exceeding the cut-off value (0.194). Mean OD value of positive control was $0.777 \pm .0120$.

Discussion

The current results proved that carbohydrate structures associated with *F. gigantica* adult worm are mainly glucose. The presence of glucose in *Fasciola* fluke was previously

recorded by Hillyer and Sagramosa de Ateca (1979); Hanna (1980); Hrzenjak et al. (1984); Wuhner et al. (2001; 2004). Furthermore, the results presented in this paper constitute the first detailed structural analysis of isolated glycoprotein fraction (FII) using mass spectrum and elucidate chemical structure: *O*-glycan Ser-(Arg-Ser-Arg-Ser-GlucNAc)₁₉-GlucNAc. This result agrees with that of Wuhner et al. (2003) who presented the structural characterization of *F. hepatica* acidic (glyco) lipids, one of which exhibits unique structural features, such as GlcNAc linked via a phosphodiester to ceramide monohexoside (CMH). Also, similar to Leverly et al. (1992) who observed the fucosyllactose epitope is a series of terminal fucose –linked α 1–3 and 1–4 to *N*-acetylglucosamine as well as internally substituted fucose with *N*-acetylgalactosamine-glucose ceramide backbone which has been characterized with highly selective monoclonal antibody 128 C3/3 and showed agglutinability with *Fasciola* miracidial extract coated SRBCs.

Compared with other parasitic helminthes, *F. gigantica* glycan profiles are both similar and distinct. They are similar to *Schistosoma* (Wuhner et al. 2006a; Hokke et al., 2007), *Haemonchus contortus* (Haslam et al. 1996, 1998), and *Taenia solium* (Haslam et al. 2003). High level of *N*-acetylglucosamine (GlcNAc) as a major component of the total glycan pool has been described for other helminthes. However, some common structures reported in several parasitic helminthes, such as the LeX epitope, mannose and multi-fucosylated termini (Wisniewski et al. 1993; Haslam et al. 1996, 1998, 2003; Wuhner et al. 2006b and Hokke et al. 2007), were not found in the current isolated glycoprotein fraction. The interpretation of this observation confirms that at the level of structural characterization, there is a common compound between some parasites as GlcNAc but not necessarily occurred in all helminthes which proved existence of unique structure (van Die and Cummings, 2010) of *Fasciola* [Ser-(Arg-Ser-Arg-Ser-GlucNAc)₁₉-GlucNAc] not necessarily exist in other helminthes.

Fig. 7 Mass spectrum of isolated glycoprotein FII **Table 2** Mean optical density (OD) of antibody levels in infected rabbits against crude antigen and isolated glycoproteins detected by ELISA

Antigens	OD values					
	Weeks post infection					
	1	2	4	6	8	10
FII	0.84 ^a ± 0.01	0.59 ^a ± 0.06	0.6 ^a ± 0.05	0.86 ^a ± 0.02	0.56 ^a ± 0.12	1.19 ^a ± 0.005
FI	0.73 ^b ± 0.01	0.38 ^{bc} ± 0.03	0.29 ^b ± 0.017	0.43 ^b ± 0.02	0.34 ^b ± 0.01	1.09 ^{bc} ± 0.005
Crude	0.616 ^c ± 0.02	0.36 ^{bc} ± 0.03	0.43 ^c ± 0.08	0.72 ^c ± 0.04	0.14 ^c ± 0.005	1.06 ^{bc} ± 0.03
F value	0.000***	0.002**	0.001***	0.000***	0.001***	0.000***

Cut off values are mean O.D. of the control negative serum ± 2 standard deviation

Difference letter means significant; same letter means no significant

** Highly significant $p \leq 0.01$

*** Very highly significant $p \leq 0.001$

Fasciola shares several glycosylation features with the mammalian host, such as terminal Gal, GlcNAc, Galb1-4GlcNAc or Gal-NAcb1-4GlcNAc structures (Haslam et al. 2001). The similarity between these simple *F. gigantica* glycan termini to some termini of host glycans may offer protection from host immune surveillance through “molecular mimicry,” in which molecules are either derived from the pathogen or acquired from the host to evade recognition by the host immune system (Damian 1965, 1997), or through “glycan gimmickry,” in which parasites use their glycans to neutralize glycan binding proteins (GBPs) of the host immune system (van Die and Cummings 2010).

In the present study FII band has a molecular weight of 19.5 kDa at pI 6.48, while that of FI has a molecular weight 26 kDa at pI 6.3. Abdul-Salam and Mansour (2000) isolated, using *Lymana* hemolymph lectin affinity column,

three fucosyllactose bearing glycoproteins from *F. gigantica* of 38 kDa, 33 kDa at pI 6.5–7.4 in addition to a 44 kDa with pI rang of 5.4–6.5 theses differences between isolated glycoproteins in both studies probably attributed to the adoption of different purification tools Ghosh et al. (2005a, b). Isolated acidic glycoprotein with molecular weight similar to that of FII and using the same method, Con-A affinity chromatography. These results together with that observed in the current research confirmed the existence of acidic glycoprotein antigens in *Fasciola* fluke that contain glucose and have molecular weight of 26–27 kDa.

ELISA was selected in the current study for evaluation of the diagnostic potency of the isolated glycoproteins based on its high sensitivity and possibility of processing many serum samples simultaneously (Cornelissen et al. 2001; Charlier et al. 2008). In the present study, it was

Fig. 8 Comparative diagnostic potentials of crude antigen and isolated glycoproteins in rabbit experimental fasciolosis using ELISA

Fig. 9 Scatter graph representing the potency of FII antigen in the diagnosis of natural fasciolosis in buffaloes

concluded that FII glycoprotein could detect experimental *F. gigantica* infection in rabbits as early as one week post infection with high significance than FI and crude extract. Also FII was more potent in diagnosis of fasciolosis in all weeks post infection. This fact is probably attributed to nature of the glycoprotein of the FII, which enhances processing, presentation, and recognition of glycan antigens by APCs and T/B-lymphocytes (Carbon and Gleeson 1997; Bachmann and Dyer 2004), leading to T cell-dependent and relatively strong IgG1 responses (Classon et al. 1991; Lee et al. 2002a, b). Another reason is probably the average density of glycan antigens on a cell is likely to be much greater than the density of a specific protein antigen, since a single glycoprotein may contain multiple glycan epitopes. Thus, it is possible that anti-glycan

antibodies will provide a higher level of bound antibodies and promote complement fixation or opsonization more quantitatively than antibodies to a specific protein. Among the previous trials concerned with the diagnostic potentials of glycoproteins in fasciolosis is that of Diaz et al. (1998) who indicated that *F. hepatica* ES antigens with molecular weights 23–28 kDa, which were glycoprotein in nature, have been identified by enzyme-linked immunotransfer blot and immunoprecipitation, as the major components recognized by sera of experimentally infected rabbits at 3 weeks post infection. While, the glycoprotein isolated in the current study diagnose the disease at 1 week post infection. Although Ghosh et al. (2005a, b) utilized the same purification approach and isolated 27-kDa glycoprotein, the fraction detected experimental fasciolosis in cattle at 2 weeks post infection which is latter than FII isolated in the present study. This difference between both results is probably attributed to variation in the used infected sera. This agree with Cornelissen et al. (1999) who reported that cattle, sheep and human showed enormous variation in immunogenicity against parasite antigens. For the diagnosis of fasciolosis in rabbits, Dalton et al. (1985) isolated 260 kDa glycoprotein associated with *F. hepatica* adult worm that reacted with rabbit serum 3 weeks post infection. Also isolated 38–25 kDa from mature *F. hepatica* immunoreactive with sera of rabbits infected for 3 and 9 weeks. Diagnosis of fasciolosis as early as 1 week post infection is by far better than late detection, an advantage associated with the current isolated glycoprotein FII and make it more potent than previously reviewed fractions (Santiago and Hillyer 1988; Yamasaki et al. 1989; Fagbemi and Guobadia 1995; Sampaio-Silva et al. 1996; Dixit et al. 2002; Molloy et al. 2005; Yadav et al. 2005; Raina et al. 2006; Sriveny et al. 2006; Jezek et al. 2007; Kumar et al. 2008).

In the present study, it is important to note that the diagnostic potentials of all three prepared antigens (FI, FII and crude) used in ELISA declined at the eighth week post infection, then elevated at the tenth week post infection. This observation is closely similar to Ghosh et al. (2005a, b). The reason behind this observation probably attributed to worm behavior where at 8 weeks post infection it begins to move into the bile duct, this mature migrating fluke secreted thiol-activated proteolytic enzyme that could cleave immunoglobulin in a papain-like manner (at a site within the hinge region), and they proposed that this action might protect the parasites from immune attack during their migration by separating the antigen-binding region from the FC portion of host antibodies (Chapman and Mitchell 1982; Dalton and Heffernan 1989; Spithill and Dalton 1998). At 10 weeks post infection the fluke reached to their final place in bile duct and become away from the circulation, so the antibodies level then elevated.

The important aim of the present study is the utilization of the isolated glycoprotein FII, 19.5 kDa, for serodiagnosis of bovine fasciolosis using ELISA. Positive result percentage of naturally infected buffaloes obtained by ELISA was 95 %; however, the negative percentage was 5 %. This high positive percentage, recorded in the present study, may be attributed to efficacy of isolated glycoprotein FII and sensitivity of ELISA. Collectively, proteins are not the only parasite molecules involved in *Fasciola*-host interactions. It is currently recognized that *Fasciola* produce a variety of complex glycans, which are expressed on lipids and proteins, and that these glycans can play important roles in induction and modulation of the host immune response as well as in the immunopathology of fasciolosis (Ghosh et al. 2005a, b; Wuhrer et al. 2003; Wuhrer et al. 2004). For this reason, *Fasciola* glycans and glycoconjugates are regarded as good immunodiagnostic targets.

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