UDC 575.
https://doi.org/10.2298/GENSR2103239K
Original scientific article

RAPID IMMUNODETECTION ASSAY BASED ON SOMATIC AND EXCRETORY SECRETORY ANTIGEN OF *Fasciola* SPECIES IN LARGE RUMINANTS

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Komal M., K. Afshan, S. Zafar, M. A. Khan, S. Firasat, M. Qayyum (2021). *Rapid immunodetection assay based on somatic and excretory secretory antigen of Fasciola species in large ruminants*. - Genetika, Vol 53, No.3, 1239-1251.
Fasciolosis, caused by liver fluke species of the genus *Fasciola,* are well recognized because of its high veterinary impact. Stool examination for *Fasciola* eggs is not a sensitive method, and limited efforts to find a reliable and cheaper means of detection are available. The present study aimed to develop rapid diagnostic ELISA test against fasciolosis. The excretory/secretory (ES) and somatic (SA) products of *Fasciola* helminths were analyzed using polyacrylamide gel electrophoresis (PAGE). Immunogenicity was evaluated by immunoblotting using hyperimmune sera raised in rabbits and seroprevalence was determined by indirect ELISA. The results of SA antigen of *Fasciola* species showed polypeptide bands ranged from 10kDa-100kDa, while ES antigen of *Fasciola* showed bands of 15kDa-55kDa. The immunoblotting results showed the most prominent bands against ES antibodies were 25, 35, 55-70, 100 and 250 kDa and SA antigens showed 10, 15-25, 35, 70, 100 and 250 kDa polypeptide bands. The sensitivity and specificity of developed indirect ELISA for SA antigens was 95.45% and 87.1%, while for ES antigens was 100% and 77.42% respectively. The overall seroprevalence recorded for fascioliasis based on SA antigen was 39.8% and 29.8% for ES antigen. The fasciolosis did not show significant association with host type, sex, and age groups of examined animals, however significantly higher infection was found in months of September and October. The result provides sensitive in house immunodetection assay for diagnosis of fasciolosis alternative to commercial kits with high import cost.

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Key words: Fasciolosis; Indirect ELISA; Somatic and excretory secretory antigen; Immunoblotting; Pakistan

INTRODUCTION

Fascioliasis is a worldwide neglected parasitic disease of domestic animals and human (WHO, 2021). The digenetic trematode *Fasciola hepatica* and *Fasciola gigantica* are parasites of great agricultural and economic importance not only in Pakistan but also in other tropic and sub tropic regions of the world (AFSHAN *et al.,* 2013). The human fascioliasis has been increasing in the last decades in a few countries and the disease is considered as an important human parasitic infection in several countries of the world, including Europe, Africa, America, Asia and Oceania (MAS-COMA *et al.,* 2014). *F. gigantica* along with *F. hepatica* incur huge economic losses that are about USD 3.2 billion worldwide (MEHMOOD *et al.,* 2017). The two species of *Fasciola* are usually different in morphological features, such as body length and width. However, because of variations in size of these two species and the presence of intermediate forms, morphometric features are not appropriate characters for distinguishing of these two species of *Fasciola.* Nowadays, molecular approaches have been considered as suitable method for discrimination of the *Fasciola* species (SHAFIEI *et al.,* 2013; 2014).

The control of fascioliasis was limited due to lack of early diagnostic test. Several investigations were performed on development of early immunological tests against fascioliasis in bovine (MEZO *et al.,* 2007; PHIRI *et al.,* 2006). Serological diagnosis of helminth parasites has been used for the detection of large number of samples at the same time than the detection of eggs. Serological detection methods use the circulating antigens, excretory secretory products (ES) in the blood of infected animal or somatic antigens and purified recombinant antigens (DEMERDASH *et al.,* 2011; SRIVENY *et al.,* 2006). Enzyme linked Immunosorbent Assay (ELISA) and Western blots are the two main techniques which have been extensively used in serological diagnosis of fascioliasis (ACICI *et al.,* 2017). However, no successful early diagnostic test is available at commercial level for animals harboring a smaller number of flukes, and with 100 percent sensitivity and specificity (BROCKWELL *et al.,* 2013). Moreover, these identified protective proteins are ineffective in other geographical areas due to existence of antigenic variation among *Fasciola* strains (BROCKWELL *et al.,* 2013; ULLAH *et al.,* 2017). To avoid these limitations, there is rapid need for developing prototype diagnostic kits against local strain for early detection of infection in Pakistan.

Due to existing research gaps on antigenic variation among *Fasciola* species in the country as compared to world, there is need to conduct studies on search of novel immunogenic proteins from local strains of *Fasciola spp*. for the development of prototype diagnostic assays. The proposed study was aimed to evaluate the potential of metabolic and tegumental /somatic protein extract in the serodiagnosis of animal fascioliasis.

MATERIALS AND METHODS

Adult Fluke and Blood Collection

Active and mature *Fasciola* worms were collected from the bile duct of cattle and buffaloes slaughtered at the local abattoirs. The study was conducted by following the ethical guideline approved by the Ethical Committee of Quaid-i-Azam University Islamabad, Pakistan.

The collected *Fasciola* helminths were washed several times with 0.85% NaCl solution and used immediately. After the identification by SOULSBY (1982) key, these helminths were divided into two groups for collection of excretory secretory (ES) and somatic antigens (SA). Blood samples was obtained from the jugular vein of animals in non-EDTA coated vacutainers, centrifuged at 3000 rpm for 15 min and sera was separated and kept at −20 °C until used for antibodies detection.

Preparation of Excretory-Secretory (ES) and Somatic Antigens

Briefly, ES product was prepared by incubating freshly collected, living adult helminths in 0.01M PBS (1 helminth / 5 ml) for 24 hr. After incubation, the suspensions were collected and centrifuged at 10,000 rpm for 10 min. The supernatant was collected, filtered and protein solution stored at -70°C until used. SA antigen was obtained from helminths homogenized in 0.1M Tris HCl, sonicated for a few seconds and centrifuged at 10,000g. The resultant supernatant was stored at -70°C and the protein concentrations of the SA as well as ES product were determined through the process described by BRADFORD (1976).

Production of Polyclonal Antibodies

Eight male rabbits, weighing 1.5–2 kg, were allocated in two groups and each four rabbits were subcutaneously immunized with ES and SA antigens and hyperimmune sera were raised (ALMAZAN *et al.,* 2001). Subsequently, two booster doses were given at an interval of 10 days and rabbit was bled for sera separation.

SDS-PAGE and Western Blotting Assay

For analyses of protein profiles, SDS-PAGE was performed according to method described by LAEMMLI (1970). The immunoblotting followed for the transfer was according to HONGBAO (2006). Briefly, separated antigens were transferred to nitrocellulose membrane, after blocking incubated overnight at 4 °C with primary antibody (1:500) obtained from immunized rabbits and naturally infected animals. The incubated membrane was washed with Tris-buffered saline with 0.1% Tween and treated with secondary antibody (Anti-goat antibody) for 2 hours. After washing substrate (BCIP/NBT) was added and stored in dark and incubated for 15 minutes until the bands became visible.

Enzyme Linked Immunosorbent Assay (ELISA)

ELISA was performed according to method described by GUOBADIA and FAGBEMI (1997). Each eluted antigen was mixed with coating buffer in equal proportion (1:1) and 100µl was added to each well of microtiter plate and incubated overnight at 4°C. After washing with 0.05% PBS-Tween 20 blocked with 0.05% BSA for 2 hours at room temperature. Then washed three times and 100 µl of sera from infected cattle and buffaloes was added to each well and incubated for 2 hours at 37°C. The plates were washed three times and 100 µL/well goat anti-bovine IgG secondary antibodies (1:10.000), conjugated with alkaline phosphatase (InvitrogenTM Cat.nos. WP20006, WP20007) was incubated for 1 h at room temperature. After washing the plates 100 µL of the substrate para-Nitrophenyle Phosphate (pNPP) was added in each well and plate was incubated in the dark for 15 minutes. The reaction was stopped by

adding 50 µL stopping solution of 3N NaOH. The OD values were determined by ELISA reader at 405 nm.

Evaluation of ELISA using Naturally Infected Cattle and Buffalo Sera

Developed ELISA was validated with 53 blood samples collected from positive and control cattle/buffaloes confirmed with fecal/postmortem examination. Blood samples were taken from cattle/buffaloes positive for *Fasciola* species (n=22) and cross reactivity was tested with control sera collected from cattle/buffaloes positive for *Gigantocotyl eexplanatum* (n=10) and *Paramphistomum spp.* (n=10). Negative control sera (n=11) were obtained from 2 week old kids born to herd having history of stall feeding. The sensitivity and specificity of indirect ELISA using somatic and ES antigens of *Fasciola* species was computed in comparison to fecal/postmortem examination by formulae described by (MANDAL *et al.,* 1998).

Sensitivity= true positive/ (true positive + false negative) ×100

where 'true positive' is animals number positive by ELISA and fecal/postmortem examination, while 'false negative' is animals positive by fecal/postmortem examination but negative by ELISA.

Specificity= true negative/ (false positive + true negative) ×100

where 'true negative is animals negative by ELISA and fecal/postmortem examination, while 'false positive' is animals negative by fecal/postmortem examination but positive by ELISA.

Field Implementation of ELISA

After evaluation and standardization of ELISA test, 500 blood samples were collected from randomly selected cattle and buffaloes to determine the prevalence of fasciolosis.

Statistical Analysis

The online software 'QuickCalcs' was used to calculate the Kappa value of developed ELISA. The cut-off value was set at 0.6. The cut-off was calculated by the mean optical density (OD) of the negative reference serum, plus three times standard deviations (0.36+3*0.074=0.582). The prevalence percentages and their association with risk factors were determined by Chi-square analysis by using SPSS version 20.

RESULTS AND DISCUSSION

SDS-PAGE

The measured values of protein concentration of SA and ES antigens were calculated by comparing with standard graph of known values of bovine serum albumin. The value of R^2 calculated from linear regression equation was 0.8515 and 0.773 for SA (Fig 1a) and ES (Fig 1b) antigens respectively. The antigenic profile of *Fasciola* specie was analysed by 10% and 12 % SDS-PAGE. The polypeptide bands of SA antigen ranged from 10kDa-130kDa, with most prominent bands of 10kDa, 15kDa, 25kDa, 35kDa, 55kDa and 70kDa. MORALES and ESPINO (2012) separated 40 polypeptides bands from the *F. hepatica* tegument antigens ranged 10-150 kDa. SRIASIH and MUNJIZUN (2021) separated 14 bands of the somatic extracts with an estimated molecular weight ranging from 8 to 105 kDa. DAR *et al.* (2019) revealed 20 distinct bands from crude somatic fraction by SDS-PAGE. These differences in molecular weight of polypeptides

may be due to the isolation methods of somatic extracts. This may be explained with presence of different strains of flukes, collected from different host species or it may be because of geographic variations (ABBAS *et al.,* 2017).

The ES antigen of *Fasciola* was separated between 15kDa-55kDa. The most prominent bands were at 15kDa, 25kDa, 35-55 kDa. These results were in accordance with previously reported 6 protein bands ranging from 15 to 42 kDa for both species of *Fasciola* (MESHGI *et al.,* 2008). KHAN *et al.,* (2017) reported the polypeptide profile of ES products that a total of 24 polypeptides out of 12 were immunogenic polypeptides identified by SDS-PAGE followed by western blotting. The 27 kDa is characterized as an immunodominant protein band of ES and somatic antigens of *Fasciola* species (DAR *et al.,* 2019). The results were consistent with another study evaluated that antigen ranging from 45-65, 14-60, 25 to 30kDa of ES were important in detecting antibodies against fascioliasis (KAMEL *et al.,* 2013).

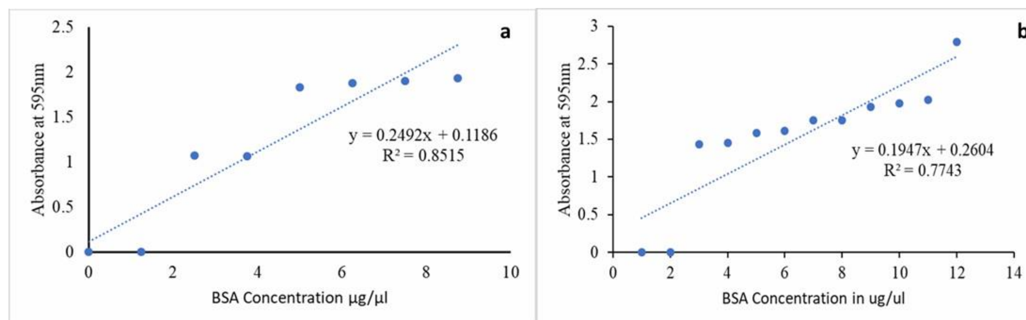

Figure 1. a) Standard bovine serum albumin (BSA) graph used to calculate somatic protein concentration and b) for finding ES proteins concentration.

Western Blotting

Polyclonal antibodies raised in rabbits against *Fasciola* species SA antigen detected 6 bands ranging from 10 to 250kDa, whereas polyclonal antibodies to *Fasciola* species ES antigen detected 5 bands, ranging from 25 to 250kDa. The most prominent detected bands against somatic antibodies were of 10kDa, 15-25kDa, 70kDa, 100kDa and 250kDa (Fig. 2a), while bands detected for ES antibodies were 55-70kDa, 100kDa and 250kDa (Fig. 2b). MESHGI *et al*. (2008) showed the presence of 8 (ranging from 18 to 62 kDs) protein bands in somatic antigens of *F. hepatica*. DE ALMEIDA *et al*. (2007) reported 17 protein bands (ranging from 2 to 80 kDa) in *F. hepatica* somatic antigen and 19 protein bands (ranging from 2 to 80 kDa) for ES antigen. SRIASIH and MUNJIZUN (2021) revealed that western blotting analysis using sera of fasciolosis positive cattle exhibited several antigenic proteins with a molecular weight ranged 8-47 kDa. Differences in number and molecular weight of reported protein bands of ES or somatic antigens of *Fasciola* spp. in various studies might be related to differences in the adult worms which have been isolated from different hosts. A study on serum of infected sheep with *F. hepatica* reacted with protein bands of 24, 33, 35, 44–55, and 66 kDa somatic antigen, and protein bands of 24,

33, 39.5, 42 and 44–55 kDa of ES antigen (GONENCE *et al.,* 2004). The differences in the reported bands with present study might be due to source of infection.

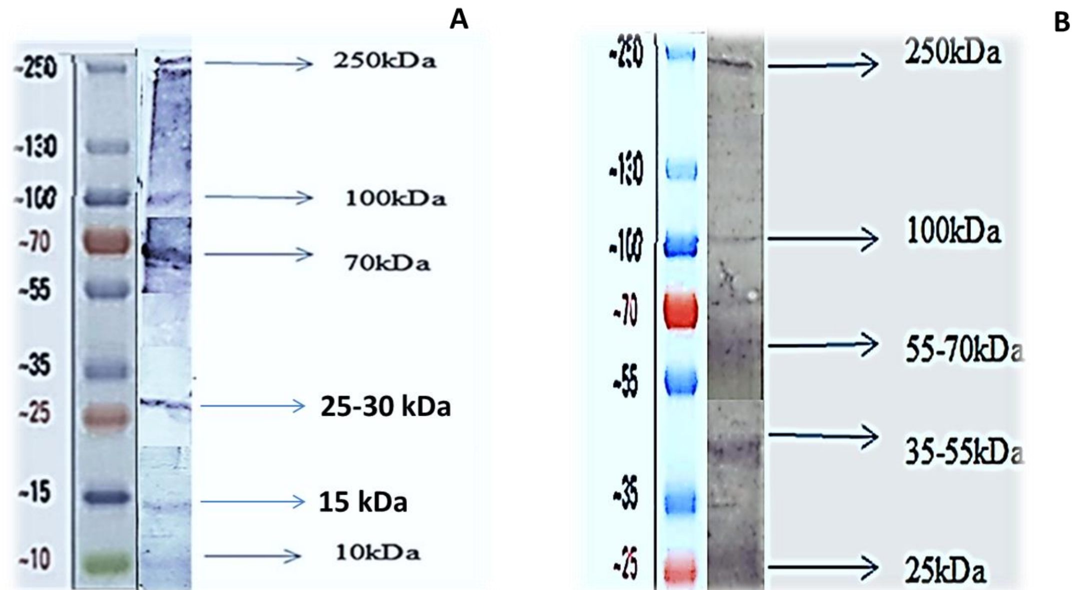

Figure 2. The immunogenic polypeptides in Western blot profile of the SA (A) and ES (B) products of *Fasciola* using anti-*Fasciola* polyclonal antibodies, respectively. Std: standard molecular weight marker and the prominent bands have been shown with arrows.

Several studies pointed out that a 27 kDa protein band is an immunodominant protein band of ES and somatic antigens of *Fasciola* (KAMEL *et al.,* 2013). However, in few studies, a 29 kDa protein band has been suggested as a principal protein band of *Fasciola* ES antigen for diagnosis of fascioliasis (MEZO *et al.,* 2010). In our study, all produced polyclonal antibodies, against ES and somatic antigens of *Fasciola* species commonly reacted with the 27 kDa protein band in western blotting. In addition, a 25-kDa protein band was commonly detected in both ES and somatic antigens by all produced polyclonal antibodies. Taken together, in the present study rabbit polyclonal antibodies, raised against *Fasciola* species ES antigen reacted with main five protein bands and polyclonal antibodies raised against somatic antigens reacted with three protein bands. Accordingly, the 25, 27 and 72 kDa protein bands may serve as immunodominant antigens that can be considered for serodiagnosis of fasciolosis. Previously, the circulating 66 kDa protein was also identified as tegumental antigen and has been used in the immunodiagnosis of *F. hepatica* (DAR *et al.,* 2019).

ELISA

Isolation of helminths from bile duct of slaughtered animals was considered as gold standard. The sensitivity and specificity of diagnostic test for SA antigens were 95.45% (95% CI: 77.16%- 99.88%) and 87.1% (95% CI: 70.17%- 96.37%), respectively. Kappa value of test was calculated 0.809 with 95% CI 0.652% to 0.967% (Table 1). The results of Kappa value revealed that the strength of agreement is very 'good'. The cross reactivity with *G. explanatum* positive sera was 20% and 10% was recorded for *Paramphistomum* infected sera. The sensitivity and specificity of the diagnostic test for *Fasciola species* ES antigens were 100% and 77.42% respectively. The kappa value calculated for the test 0.746 (Table 2) and revealed that the strength of agreement 'good'.

Table 1. Diagnostic efficacy of ELISA established for Fasciola somatic antigen with faecal/postmortem examination

Test	ELISA Test			Sensitivity	Specificity	KAPPA
Fecal/Postmortem Examination	Positive	Negative	Total	95% CI	95% CI	
Positive	21	1	22	95.45 (77.16-99.88)	87.1 (70.17-9.37)	Kappa= 0.809
Negative	4	27	31			SE of kappa = 0.081
			53			95% confidence interval: 0.652 to 0.967.

Table 2. Diagnostic efficacy of ELISA established for Fasciola excretory secretory antigen with faecal/postmortem examination

Test	ELISA Test			Sensitivity	Specificity	KAPPA
Fecal/Postmortem Examination	Positive	Negative	Total	95% CI	95% CI	
Positive	22	0	22	100 (84.56-100.00)	77.42 (58.90-90.41)	Kappa= 0.746
Negative	7	24	31			SE of kappa = 0.088
			53			95% confidence interval: 0.567 to 0.913

Field Implementation of In-house Developed Sero ELISA

Overall Seroprevalence and Associated Risk Factors of Fasciolosis

The overall seroprevalence recorded for fascioliasis was 39.8% and 29.8% for somatic and ES antigen-based ELISA tests. Previously reported result in Punjab on seroprevalence was 37.24% (ANJUM *et al.,* 2014). Seroprevalence of this study was higher than previously recorded 25.46% for ES antigen (MEHMOOD *et al.,* 2017). In another study conducted in Khyber Pakhtunkhwa, the prevalence was 27% (ULLAH *et al.,* 2016). Fascioliasis reported from Nile

Delta Egypt was 9.77% (EL-TAHAWY *et al.,* 2017), 31.4% in the middle Black Sea region, Turkey (ACICI *et al.,* 2017), 52.2% in Australia and 3.28% were found to be infected in Iran (ISAH, 2019). These differences in prevalence in different regions are possibly because of variation of geographic region such as landscape, marshy or swampy areas, differences in agricultural and irrigation (ZAFAR *et al.,* 2019).

Table 3. Field implementation of in-house developed ELISA based on somatic and excretory secretory antigens with respect to associated risk factors

Characteristics	Somatic ELISA Positive n (%)	Negative n (%)	Chi-Square p-Value	ES ELISA Positive n (%)	Negative n (%)	Chi-Square p-Value
Host Type			2.675			0.964
Buffalo	124(24.8)	199(39.8)	0.26	93(18.6)	230(45.8)	0.61
Cattles	75(15)	102(20.4)		56(11.2)	121(24.2)	
Age (years)			3.912			5.72
2 - 4	28(5.6)	56(11.2)	0.27	18(3.6)	66(13.2)	0.45
5 - 7	48(9.6)	67(13.4)		36(7.2)	79(15.8)	
8 - 10	86(17.2)	110(22)		65(13)	131(26.2)	
11-13	37(7.4)	68(13.6)		30(6)	75(15)	
Sex			2.16			0.14
Female	118(23.6)	198(39.6)	0.14	92(18.4)	224(44.8)	0.66
Male	81(16.2)	103(20.6)		57(11.4)	127(25.4)	
Month			50.99			106.80
January	9(1.8)	41(8.2)	0.0001*	16(3.2)	34(6.8)	0.0001*
April	8(1.6)	42(8.4)		24(4.8)	26(5.2)	
May	3(0.6)	47(9.4)		0(0)	50(10)	
June	19(3.8)	31(6.2)		12 (2.4)	38(7.6)	
July	24(4.8)	26(5.2)		14(2.8)	36(7.2)	
August	18(3.6)	32(6.45)		24(4.8)	26(5.2)	
September	36(7.2)	14(2.8)		23(4.6)	27(5.4)	
October	39(7.8)	11(2.2)		10(2.0)	40(8.0)	
November	27(5.4)	23(4.6)		12(2.4)	39(7.8)	
December	16(3)	34(6.8)		14(2.8)	35(7.0)	
Total	199(39.8)	301(60.2)		149(29.8)	351(70.2)	

The seroprevalence of fascioliasis associated with host type, sex, age, and months are given in Table 3. However, no significant association was found with the host type, age, and gender with fascioliasis. In present somatic and ES antigen-based ELISA test highest number of infections was found in buffaloes 24.8%, 18.6% and lowest in cattle population 15%, 11.2%

respectively. The buffaloes were found to be more affected as compared to cattle that could be attributed to the fact they live in swampy areas (ZAFAR *et al.,* 2019). Age wise result of present investigation showed highest infection in 8-10 years of age group 17.2%, 13% while lowest was found in 2-4 year age group 5.6% and 3.6% for somatic and ES ELISA respectively. These results are in accordance with previous results in which adult cattle showed more infection as compare to younger one (ACICI *et al.,* 2017). Similarly, other studies recorded adult belonging to 13 years of age group bovine showed a greater number of positive infections as compared to adults belonging to 0-3yrs (MUFTI *et al.,* 2015). This could be due to fact that most of the farmers take animals of this age group for grazing, thus, there are more chances of interaction with an intermediate host (ACICI *et al.,* 2017; ISAH, 2019; ZAFAR *et al.,* 2019; MUFTI *et al.,* 2015). Similarly, the current results of both type of ELISA showed females were more susceptible to infection 23.6%, 18.4% compared to males 16.2%, 11.4%. This could be due to those females that have poor immunity because of breeding and milk productions and is considered as a stressful factor making females more vulnerable to infections (MUFTI *et al.,* 2015).

The result showed significantly (p=0.0001) highest number of cases in the month of October (7.8%) and September (7.2%) for somatic ELISA, while higher number of positive cases with ES antigen-based ELISA were in the months of April (4.8%), August (4.8%) and September (4.6%). In our study the prevalence was observed throughout the year, and this could be attributed to the fact of availability of water, giving continued exposure to encysted metacercaria, our climatic condition of humidity and semi humid throughout the year and resistance of metacercerie to dissociate in shallow water (ISAH, 2019). The geographical distribution of fascioliasis is strongly linked to climate and environmental conditions (AFSHAN *et al.,* 2014). Previous study showed highest infection during September and lowest in January (ULLAH *et al.,* 2017). In accordance with present study highest prevalence in late rainy season from August to October and lowest at dry season was recorded (ISAH, 2019).

CONCLUSIONS

The SA and ES products of *Fasciola* under study contain multiple antigens of varied molecular weights and diverse origin. The laboratory raised polyclonal antibodies against the SA and ES antigen of the parasite was successfully used in detection of antigenic proteins. Further characterization of these immunodominant proteins from the SA and ES product of *Fasciola* species can be used for the development of early diagnostic kit and can also provide us some novel vaccine candidates.

ACKNOWLEDGMENT

The research work presented in this article is under funding provided by Higher Education Commission of Pakistan under the project grant No:7402/Federal/NRPU/R& D/HEC/2017. This research work is part of PhD dissertation of Maria Komal.

Received, September 27th, 2020

Accepted May 18th, 2021

REFERENCES

AFSHAN, K., C.A., FORTES-LIMA, P., ARTIGAS, M.A., VALERO, M., QAYYUM, S., MAS-COMA (2014): Impact of climate change and man-made irrigation systems on the transmission risk, long-term trend and seasonality of human and animal fascioliasis in Pakistan. Geospatial health, *8*(2): 317-334.

AFSHAN, K., M., QAYYUM, S.S.R., RIZVI, M., MUKHTAR, M., MUSHTAQ, J.E., MILLER (2013): Serological and coprological comparison for rapid diagnosis of *Fasciola hepatica* infection in small ruminants from sub- tropical area of Pakistan. Small Rumin. Res., *113*: 267-272.

ABBAS, A., Z., IQBAL, R.Z., ABBAS (2017): In vivo anticoccidial effects of *Beta vulgaris* (sugar beet) in broiler chickens. Microb Path., *111*: 139 – 44.

ACICI, M., O., BUYUKTANIR, C.S., BOLUKBAS, G.Z., PEKMEZCI, A.T., GURLER, S., UMUR (2017): Serologic detection of antibodies against *Fasciola hepatica* in sheep in the middle Black Sea region of Turkey. J. Microb. Imm. Infec., *50(3)*: 377-381.

AGUAYO, V., F.B.N., VALDÉS, M., RODRÍGUEZ-VALENTÍN, C., RUIZ-JIMÉNEZ, M.J., RAMOS-BENÍTEZ, L.B., MÉNDEZ, A.M., ESPINO (2019): *Fasciola hepatica* GST downregulates NF-κB pathway effectors and inflammatory cytokines while promoting survival in a mouse septic shock model. Sci. Rep., *9*: 2275.

ALMAZAN, C., G., AVILA, H., QUIROZ, F., IBARRA, P., OCHOA (2002): Effect of parasite burden on the detection of *F. hepatica* antigens in sera and feces of experimentally infected sheep. Vet. Parasitol., *97*:101e12.

ANJUM, R., M.S., KHAN, M.S., SAJID (2014): Frequency distribution of fascioliasis in small ruminants population at District Sargodha. Glob. Vet., *12*: 26-32.

BRADFORD, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem, *72(1-2)*: 248-254.

BROCKWELL, Y.M., T.W., SPITHILL, G.R., ANDERSON, V., GRILLO, N.C., SANGSTER (2013): Comparative kinetics of serological and coproantigen ELISA and faecal egg count in cattle experimentally infected with *Fasciola hepatica* and following treatment with triclabendazole. Vet. Parasitol, *196*: 417-26.

DAR, J.S., B.A., GANAI, R.A., SHAHARDAR, U.R., ZARGAR (2019): Molecular characterization and Immunodiagnostic potential of various antigenic proteins of *Fasciola gigantica* species isolated from sheep of Northwest Himalayan Region. Helminthologia, *56(2)*: 93-107.

DE ALMEIDA, M.A., M.B., FERREIRA, S., PLANCHART, A., TERASHIMA, V., MACO, L., MARCOS, E., GOTUZZO, E., SÁNCHEZ, C., NÁQUIRA, J.V., SCORZA, R.N., INCANI (2007): Preliminary antigenic characterisation of an adult worm vomit preparation of *Fasciola hepatica* by infected human sera. Rev. Inst. Med. Trop. São Paulo, *49(1)*: 31-35.

DEMERDASH, Z.A., T.M., DIAB, I.R., ALY, S.H., MOHAMED, F.S., MAHMOUD, M.K., ZOHEIRY, W.A., MANSOUR, M.E., ATTIA, A.E., EL-BASSIOUNY (2011): Diagnostic efficacy of monoclonal antibody-based sandwich enzyme linked immunosorbent assay (ELISA) for detection of *Fasciola gigantica* excretory/secretory antigens in both serum and stool. Parasites & vectors, *4*(1): 1-7.

EL-TAHAWY, A.S., E.K., BAZH, R.E., KHALAFALLA (2017): Epidemiology of bovine fascioliasis in the Nile Delta region of Egypt: Its prevalence, evaluation of risk factors, and its economic significance. Vet. World, *10(10)*: 1241.

GÖNENÇ, B., H.O., SARIMEHMETOĞLU, M., KARA, F., KIRCALI (2004): Comparison of crude and excretory/secretory antigens for the diagnosis of *Fasciola hepatica* in sheep by western blotting. Turk. J. Vet. Ani. Sci., *28(5)*: 943-949.

GUOBADIA, E.E., B.O., FABEMI (1997): The isolation of *Fasciola gigantica*-specific antigens and their use in the serodiagnosis of fascioliasis in sheep by the detection of circulating antigens. Vet. Parasitol., *68(3)*: 269-282.

HONGBAO, M. (2006): Western Blotting Method. Am. J Sci.,*2(2)*: 23-27.

ISAH, H. (2019): Spatial Species Distribution Models to Predict Liver Fluke Disease (F*asciola gigantica*) In Cattle: A case study of Sokoto State, Nigeria (Doctoral dissertation, Department of Geography).

KAMEL, H.H., G.A., SAAD, R.M., SARHAN (2013): Dot-blot immunoassay of *Fasciola gigantic* infection using 27 kDa and adult worm regurge antigens in Egyptian patients. Korean J. Parasitol.,*51*: 177–182.

KHAN, M.H., R., ULLAH, A., REHMAN, L., REHMAN, A.S., P.A, S.M.A., ABIDI (2017): Immunolocalization and immunodetection of the excretory/secretory (ES) antigens of *Fasciola gigantica*. Plos one, *12(10)*: e0185870.

LAEMMLI, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, *227*: 680.

MANDAL, S., S.C., YADAV, R.L., SHARMA (1998): Evaluation of *Fasciola gigantica* antigenic preparations in serodiagnosis of fasciolosis in sheep. J. Parasitic Dis., *22*: 25-29.

MAS-COMA S., M.D., BARGUES, M.A., VALERO (2014): Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitol.*, 141*(1):1918–1946.

MEHMOOD, K., H., ZHANG, A.J., SABIR (2017): A review on epidemiology, global prevalence and economical losses of fascioliasis in ruminants. Microb. Path., *109*: 253-62.

MESHGI, B., A., ESLAMI, F., HEMMATZADEH (2008): Determination of somatic and excretory-secretory antigens of *Fasciola hepatica* and *Fasciola gigantica* using SDS-PAGE. Iranian J. Vet. Res., *9(1)*: 77-80.

MEZO, M., M., GONZÁLEZ-WARLETA, J.A., CASTRO-HERMIDA, L., MUIÑO, F.M., UBEIRA (2010): Field evaluation of the MM3-SERO ELISA for detection of anti-*Fasciola* IgG antibodies in milk samples from individual cows and bulk milk tanks. Parasitol. Int., *59(4)*: 610-615.

MEZO, M., M., GONZA´ LEZ-WARLETA, F.M., UBEIRA (2007): The use of MM3 monoclonal antibody for the early immunodiagnosis of ovine fasciolosis. J. Parasitol., *93*: 65-72.

MORALES, A., A.M., ESPINO (2012): Evaluation and characterization of *Fasciola hepatica* tegument protein extract for serodiagnosis of human fascioliasis. *Clin.* Vaccine Immunol.*, 19(11)*: 1870-1878.

MUFTI, S., K., AFSHAN, I.A., KHAN, S., IRUM, I.Z., QURESHI, S.S.R., RIZVI, M., MUKHTAR, M., MUSHTAQ, Z., IQBAL, M., QAYYUM (2015): Serological and coprological studies of bovine fascioliasis in the Pothwar Region, Pakistan. Pak. Vet. J., *35(2)*: 178-182.

PHIRI, A.M., I.K., PHIRI, J., MONRAD (2006): Prevalence of amphistomiasis and its association with *Fasciola gigantica* infections in Zambian cattle from communal grazing areas. J. Helminthol., *80*: 65-68.

SHAFIEI, R., B., SARKARI, A., MOSHFE (2013): Consistent PCR-RFLP assay based on ITS-2 ribosomal DNA for differentiation of *Fasciola* species. Iran J. Basic Med. Sci.*,16:* 1266–1269.

SHAFIEI, R., B., SARKARI, S.M., SADJJADI, G.R., MOWLAVI, A., MOSHFE (2014): Molecular and morphological characterization of *Fasciola* spp. isolated from different host species in a newly emerging focus of human fascioliasis in Iran. Vet. Med. Int., 405740.

SOULSBY, E.J.L. (1982): Helminths. Arthropods and Protozoa of domesticated animals, 291.

SRIASIH, M., A., MUNJIZUN (2021): Identification of Somatic Antigens of Adult *Fasciola gigantica* Isolated from Bali Cattle. World, *11(1)*: 138-146.

SRIVENY, D., O.K., RAINA, S.C., YADAV, D., CHANDRA, A. K., JAYRAW, M., SINGH, R., VELUSAMY, B.P., SINGH (2006): Cathepsin L cysteine proteinase in the diagnosis of bovine *Fasciola gigantica* infection. Vet. Parasitol., *135*: 25-31.

ULLAH, I.M.F.N., A.A.K., JADOON, S., TABASSUM (2016): Prevalence of *Fasciola hepatica* in Domesticated Cattle of District Karak, Khyber Pakhtunkhwa, Pakistan. Asian J. Ani. Sci., *10*: 85-91.

ULLAH, R., A., REHMAN, M.F., ZAFEER, L., REHMAN, Y.A., KHAN, M.A.H., KHAN (2017): Anthelmintic Potential of Thymoquinone and Curcumin on *Fasciola gigantica*. PLoS one*, 12*: 2.

WHO: The "Negleted" Neglected Worms. Action against Worms. Available online: https://www.who.int/neglected_diseases/preventive_chemotherapy/ Newsletter, 10.pdf (accessed on 30 May 2021).

ZAFAR, A., M.K., KHAN, D., SINDHU, R.Z., ABBAS, S., MASOOD, Z., ABBAS, M.S., MAHMOOD, M.K., SALEEMI, J.A., KHAN, R., HUSSAIN, M.U., NASEER (2019): Seroprevalence of *Fasciola hepatica* in Small Ruminants of District Chakwal, Punjab, Pakistan. Pak. Vet. J., *39(1)*.

BRZA IMUNODETEKCIJSKA ANALIZA ZASNOVANA NA SOMATSKOM I EKSRETORNO SEKRETORNOM ANTIGENU VRSTE *Fasciola* KOD VELIKIH PREŽIVARA

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Izvod

Fasciolijaza, uzrokovana vrstama jetrenih metilja iz roda *Fasciola*, dobro je poznata zbog svog velikog veterinarskog uticaja. Ispitivanje stolice na prisustvo jaja *Fasciole* nije osetljiva metoda, a mogućnosti pronalaženja pouzdanog i jeftinijeg načina za detekciju su ograničene. Ova studija je imala za cilj razvoj brzog dijagnostičkog ELISA testa za detekciju fasciolijaze. Ekskretorni/sekretorni (ES) i somatski (SA) produkti crevne gliste *Fasciola* analizirani su primenom elektroforeze na poliakrilamidnom gelu (PAGE). Imunogenost je procenjena imunoblotingom korišćenjem hiperimunih seruma proizvedenih kod zečeva, a seroprevalencija je određena indirektnim ELISA testom. Rezultati SA antigena vrste *Fasciola* pokazali su polipeptidne trake u rasponu od 10kDa-100kDa, dok je ES antigen *Fasciola* pokazao trake od 15kDa-55kDa. Rezultati imunoblotinga su pokazali da su najistaknutije trake za ES antitela bile 25, 35, 55-70, 100 i 250 kDa, a SA antigeni su pokazali polipeptidne trake od 10, 15-25, 35, 70, 100 i 250 kDa. Osetljivost i specifičnost razvijene indirektne ELISA analize za SA antigene iznosila je 95,45% i 87,1%, dok je za ES antigene bila 100% i 77,42% respektivno. Ukupna seroprevalencija zabeležena za fasciolijazu zasnovanu na SA antigenu bila je 39,8% i 29,8% za ES antigen. Fasciolijaza nije pokazala značajnu povezanost sa tipom domaćina, polom i starosnim grupama ispitivanih životinja, ali je značajno veća infekcija utvrđena u mesecima septembru i oktobru. Dobijeni rezultati pružaju osetljivu kućnu imunodetekciju za dijagnozu fasciolijze koja je alternativa komercijalnim setovima sa visokim uvoznim troškovima.

Primljeno 27.IX.2020.
Odobreno 18.V.2021