Parasitology Research (2019) 118:453–460
https://doi.org/10.1007/s00436-018-6169-z

GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Proteomic analysis of *Fasciola gigantica* excretory and secretory products (FgESPs) interacting with buffalo serum of different infection periods by shotgun LC-MS/MS

Si-Yang Huang[1] · Dong-Mei Yue[2,3] · Jun-Ling Hou[2] · Xiao-Xuan Zhang[3] · Fu-kai Zhang[2] · Chun-Ren Wang[3] · Xing-Quan Zhu[1,2]

Received: 26 April 2018 / Accepted: 29 November 2018 / Published online: 18 December 2018

Abstract

Fasciolosis, caused by *Fasciola hepatica* and *Fasciola gigantica*, is an important zoonotic disease in the world. It affects livestock, especially for sheep and cattle, causing major economic loss due to morbidity and mortality. Although the excretory and secretory products (ESPs) of *F. hepatica* have been relatively well studied, little is known about the interaction between the ESP and host, and the mechanism of the key proteins involved in interaction. In this study, buffaloes were infected by *Fasciola gigantica*, and infection serum was collected at three different periods (42dpi, 70dpi, and 98dpi). The interaction proteins were pulled down with three different period serum by Co-IP assay, respectively, and then identified by LC-MS/MS analysis. A number of proteins were identified; some of them related to the biological function of the parasite, while most of them the functions were unknown. For the annotated proteins, 13, 5, and 7 proteins were pulled down by the infected serum in 42dpi, 70dpi, and 98dpi, respectively, and 18 proteins could be detected in all three periods. Among them, 13 belong to the cathepsin family, 4 proteins related to glutathione S-transferase, and 3 proteins are calcium-binding protein; other proteins related to catalytic activity and cellular process. This study could provide new insights into the central role played by ESPs in the protection of *F. gigantica* from the host immune response. At the same time, our research provided material for further studies about the interaction between *F. gigantica* and host.

Keywords *Fasciola gigantica* · Excretory and secretory products (ESPs) · Infection · Co-IP · Mass spectrometry

Introduction

Fasciolosis is a serious zoonotic disease caused by liver flukes of the genus *Fasciola* (*Fasciola hepatica* and *Fasciola gigantica*), which primarily affects domestic livestock such as sheep and cattle. It is estimated to cause annual economic losses > US $3 billion dollars in the world (Alvarez Rojas et al. 2014). Fasciolosis has a substantial adverse impact on farm profitability and can jeopardize sustainable food production, and has a serious economic impact on agricultural

Handling Editor: Julia Walochnik

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-018-6169-z) contains supplementary material, which is available to authorized users.

✉ Si-Yang Huang
siyang.huang@hotmail.com

✉ Xing-Quan Zhu
xingquanzhu1@hotmail.com

[1] Institute of Comparative Medicine, Yangzhou University, and Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonosis, and Jiangsu Key Laboratory of Zoonosis, Yangzhou 225009, Jiangsu Province, People's Republic of China

[2] State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, Gansu Province, People's Republic of China

[3] College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, Heilongjiang Province, People's Republic of China

industry. Livestock production losses occurring due to *Fasciola* infection include lower milk production in cattle, decreased growth rates, lower fertility and decreased wool growth in sheep (Spithill et al. 1999). Humans are also at risk from fasciolosis with about 2.4 million people infected, and more than 90 million people being at risk of infection in the world (Keiser and Utzinger 2005).

Helminths are masterful immunoregulators. The excretory/secretory products (ESPs) released by live parasites are known to interact with every aspect of host immunity from initial recognition to chronic stage effector mechanisms (Hewitson et al. 2009). Recently, the protein composition of ESP has been characterized in several species by proteomics approaches based on mass spectrometry, such as *Haemonchus contortus*, *Heligmosoides polygyrus*, *Ascaris suum*, and *Nippostrongylus brasiliensis* (Chehayeb et al. 2014; Gadahi et al. 2016; Sotillo et al. 2014). The excretory/secretory products (ESPs) of parasites play important roles because they are exposed to the host's immune system directly (Liu et al. 2017; Smallwood et al. 2017). Previous investigations have indicated that *F. gigantica* excretory-secretory products (FgESPs) and *F. hepatica* excretory-secretory products (FhESPs) function in modulating host immune responses (Zhang et al. 2005). FhESPs have immuno-suppressive and modulatory effects in lymphocytes of rat and sheep, respectively (Goose 1978; Jefferies et al. 1996). Proteomics analysis of *F. hepatica* secreted proteins has been carried out for various lifecycle stages, these different proteins secreted by infective larvae, immature flukes, and adult *F. hepatica* indicated that these proteases are developmentally regulated and correlate with the passage of the parasite through host tissues and its encounters with different host macromolecules (Robinson et al. 2009) Previous studies indicated that the protein composition of parasites' ES products is simple and cyclic. It has better sensitivity and specificity compared with the flukes' antigen, and the antibody levels are positively correlated with severity of infection by the flukes in hosts (Gupta and Yadav 1994; Rotmans et al. 1981). A global information on the components, proportion, and relative abundance of FhESPs were analyzed (Jefferies et al. 2001). Unfortunately, this information is still limited for FgESPs, since parasites in different developmental stages can excrete or secrete different antigens during their development in the hosts, and it is quite difficult to obtain the parasites of different developmental stages. They have numbers of effects on the host immune system, either by evasion (Berasain et al. 2000) or defense against the immune system (Cervi et al. 1996), and the immune reactions of host were also changed with the development of parasites. The recent report of the *Fasciola* genome and associated transcriptome data sets (Cwiklinski et al. 2015a) has now allowed us to address these limitations and perform a definitive characterization of the total antigenic targets of adult *F. gigantica* in different infection periods.

The objectives of this study were to identify the antigenic targets in FgESPs using an immunoproteomic approach. We infected buffaloes with *F. gigantica,* and obtained the serum at 42 days, 70 days, and 98 days post infection (dpi), respectively. The target proteins that interacted with positive serum of different infection stages were pulled down by co-immunoprecipitation (Co-IP), respectively. The immunoprecipitates were analyzed and characterized by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Materials and methods

FgESPs preparation

FgESPs were prepared according to the standard procedure described previously (Novobilsky et al. 2007). Briefly, adults of *F. gigantica* were separated from the bile ducts of infected buffaloes, washed three times with warm PBS, and then incubated in RPMI 1640 medium at 37 °C for 1 h. The parasites were moved to new medium and incubated at 37 °C for 12 h. Then, the supernatant was obtained, centrifuged (2500×*g* for 20 min), filter-sterilized (0.2 μm), concentrated, and freeze-dried into powder and stored at − 80 °C. When needed, the powder was dissolved in deionized water and the protein concentration was determined by Pierce BCA protein assay kit.

Preparation of buffalo serum of different infection periods

The buffaloes were purchased from a farm in Guangxi Zhuang Autonomous Region, PR China. To exclude any prior infection of buffaloes with *Fasciola* spp., fecal examination and ELISA testing of IgM and IgG antibodies against *Fasciola* spp. were conducted before the buffaloes were selected. Finally, six buffaloes 8–9-month-old that were negative for *Fasciola* spp. were selected for this study, and then they were randomly divided into two groups: (i) three as the non-infected control group and (ii) three as the infected group. Three buffaloes were each infected orally with 500 viable metacercariae, while control buffaloes were mock-inoculated with 0.85% *w/v* NaCl solution without metacercariae. At 42, 70, and 98 days post-infection (dpi), blood sample was taken from each animal aseptically into tubes without anticoagulant and serum was separated by centrifugation and preserved at − 80°C for further use (Zhang et al. 2017).

Co-immunoprecipitation of FgESPs-antibody binding proteins

The Protein A/G plus-agarose immunoprecipitation kit (Santa Cruz Biotechnology, USA) was used for Co-Immunoprecipitation (Co-IP) according to the manufacturer's instructions. Nine hundred micrograms FgESPs were precleared by incubation with 1000 μL buffalo normal serum (negative for *F. gigantica*) and 20 μL Protein A/G plus-agarose beads at 4 °C for 2 h. After pelleting the beads by centrifugation at 1000×*g* for 5 min at 4 °C, the supernatant was transferred to a fresh tube, and averagely divided to three tubes, 500 μL buffalo positive serum (42 dpi, 70 dpi, and 98 dpi)were added to the tubes, respectively and 20 μL Protein A/G plus-agarose beads was added to each tube and incubated overnight at 4 °C. The pellet was collected by centrifugation at 1000×*g* for 5 min at 4 °C and the supernatant was discarded carefully; the pellet was washed by PBS buffer for 3 times and resuspended by 50 μL 1 × SDS-loading buffer after final washing. The sample was boiled at 100 °C for 10 min. Ten microliters of the sample was analyzed by SDS-PAGE (Liu et al. 2017). The other 40 μL sample was left for mass spectrometry identification.

In-solution trypsin digestion

The digestion of proteins was conducted as previously described: briefly, the concentration of proteins was measured by nanodrop after extracted from gel, and 30 μg sample was boiled with 30 μL of SDT buffer for 5 min, 200 μL of UA buffer (8 M urea, 150 mM Tris-HCl, pH 8.5) was added, and then the samples were loaded into the MicroconUltracel YM-30 filtration devices (Millipore, Billerica, MA, USA) and centrifuged (5000×*g* for 2 min), discarded the filtrate, 100 μL iodoacetamide (100 mM IAA in UA buffer) was added, and then oscillated for 1 min; each sample was incubated for 30 min at RT in the dark, centrifuged again and discarded the filtrate. Then, added 100 μL of UA buffer and centrifuged twice, added 100 μL of 25 mM NH_4HCO_3 and centrifuged twice. The mixture of peptide was digested with 40 μL trypsin (Promega) overnight at 37 °C, The digestions were stopped by the addition of trifluoroacetic acid (TFA) to a final concentration of 0.1% and the samples was freeze-dried into powder in a vacuum centrifuge.

LC-MS/MS analysis

Peptides were dissolved in 40 μL of 0.1% *v*/*v* formic acid (FA) and analyzed by HPLC/MS. MS data was acquired with Q Exactive (ThermoFinnigan, San Jose, CA). Briefly, loading peptides onto a reverse phase trap column (Thermo Scientific Acclaim PepMap100, 100 μm*2 cm, nano Viper C18) that was pre-equilibrated with 0.1% *v*/*v* FA. An analytical column (Thermo Scientific Easy Column, 10 cm long, 75 μm inner diameter, 3 μm resin) was used for separation, with a linear gradient of buffer B (84% *v*/*v* acetonitrile and 0.1% *v*/*v* Formic acid) at a flow rate of 300 nL per min controlled by Intelli Flow technology over 60 min (liquid linear gradient of solution B: 4–50% (0–50 min), 50–100% (50–54 min), B was maintainedat100%). The mass spectrometer was operated in positive ion mode. MS data was obtained using a data-dependent top10 method as previously descripted (Liu et al. 2017).

Data analysis

MS/MS spectra were analyzed with Proteome Discoverer 1.4 by MASCOT engine (Matrix Science, London, UK; version 2.2) according to the UniProt *Fasciola* database (20170108). For protein identification, the following parameters were set: Peptide mass tolerance was 15 ppm, MS/MS tolerance was 0.1 Da, enzyme was trypsin, missed cleavage was 2, fixed modification was carbamidomethyl (C), and oxidation (M) was searched as a variable modification. The cut-off of false discovery rate (FDR) was set to ≤ 0.01. For MASCOT searches, a molecular weight search (MOWSE) score should > 20.

Results and discussion

Parasites often cause diseases of major socioeconomic importance worldwide in animals, *Fasciola* species is one of the parasitic trematodes cause an importance of economic losses in production animals. There is a clear need and global interest, in development of improved methods for control fascioliasis. An immuno-proteomic approach was used to identify those proteins secreted by *F. gigantica*, specifically using infection sera to pull down the FgESPs that are likely to be involved in the host-parasite interaction. Using this method, we identified a number of proteins which were already reported by other researchers in *F. gigantica* and *F. hepatica*, and some proteins described here for the first time in *F. gigantica*. SDS-PAGE was used to confirm the results of Co-IP assay (Fig.1). The results indicated that the antibodies from serum of different infected period could recognize and pull down the specific proteins from FgESPs. The majority of proteins ranged in molecular weight from 25 to 170 kDa. From these results, we found that numbers of proteins were co-purified in all three periods, which indicated that these proteins might take part in the interaction between host and parasite during the whole infection, and some proteins can only be detected in the early stage (42 dpi), which may explain that these proteins caused the early response of the host.

In this study, 147 proteins were identified in 42 dpi, 119 proteins were identified in 70 d, and 130 proteins were

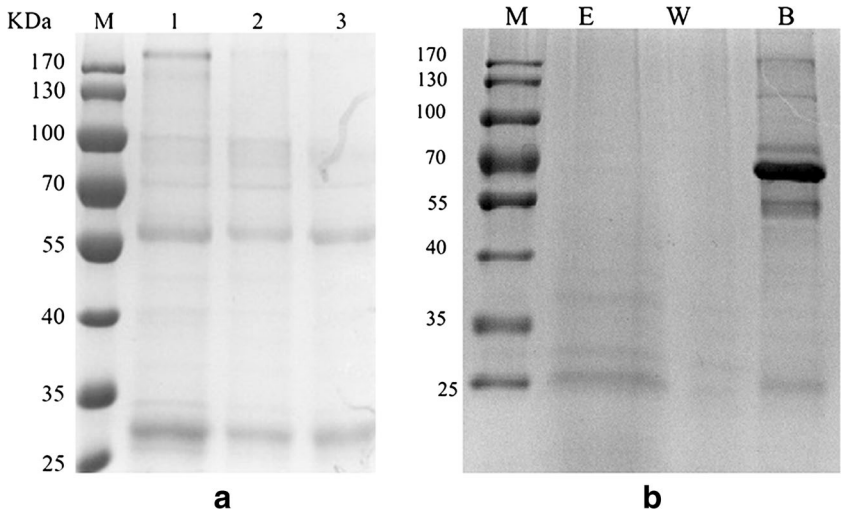

Fig. 1 **a** SDS-PAGE analysis of the protein samples with the buffalo serum of different infection period co-cultured with the FgESPs. Lane 1: the proteins pulled down by the buffalo serum of 42 days post-infection (dpi) with *Fasciola gigantica*. Line 2: the proteins pulled down by the buffalo serum of 70 dpi. Line 3: the proteins pulled down by the buffalo serum of 98 dpi. **b** Line E: the ESP prior to co-immunoprecipitation. Line W: the supernatant of incubated in RPMI 1640 medium at 37 °C for 1 h before ESP collection. Line B: the proteins pulled down by the buffalo negative control serum with *F. gigantica*

identified in 98 dpi according to LC-MS/MS analysis. Although protein sequences are identified in *F. hepatica* genomic data, most of them are not annotated currently. For the annotated proteins, there are 44, 32, and 31 were identified in 42 dpi, 70 dpi, and 98 dpi based on the information from UniProt, respectively. Among them, 13 proteins could only be detected in 42 dpi (Table 1); several proteins were represented by proteolysis related function, such as cathepsins B2, B4, and B5, and 3 tubulin proteins were found. Five different cathepsin Bs were only identified in 42 dpi, especially, cathepsin B4 was firstly reported in *F. gigantica*. These results indicated cathepsin Bs are important to the early invasion, thus they are thought to be vaccine candidates that can block the invasion and migration of the juvenile parasite. At the same time, these proteins could be good targets for detection of fascioliasis in the sera of early infected animals. Tubulin is the target of many parasite therapeutic drugs, and the subtle differences between the tubulins of the host and parasite are sufficient to provide selective toxicity for some agents and morphological studies have indicated that inhibition of microtubule-mediated functions occurs when flukes are exposed to triclabendazole (TCBZ), which suggested that tubulin may be its target. Previous study established that the adult fluke present in its mammalian definitive host expresses at

Table 1 The annotated *Fasciola gigantica* excretory and secretory products (FgESPs) which were detected only by the buffalo serum of 42 days post-infection (dpi) with *Fasciola gigantica*

Protein description	Species	Genome mapping Gene ID[a]	Pep count (sequence)	Unique pep count	Cover percent	GeneBank Bank ID	Theor MW[b]	Theor PI[c]
Cathepsin B4	*F. hepatica*	BN1106_s9304B000006	2	3	11.83	AIS22046.1	10677.11	6.06
Alpha-tubulin	*F. hepatica*	BN1106_s925B000539	2	2	3.64%	CAO79604.1	48985.86	5
Beta-tubulin	*F. hepatica*	BN1106_s1153B000359	3	3	15.24	CAO79610.1	24259.32	5.1
Beta-tubulin2	*F. hepatica*	BN1106_s3488B000143	2	2	3.41	CAO79611.1	20293.06	5.92
Glucose transporter-2 protein	*F. gigantica*	BN1106_s584B000350	2	3	3.43%	AFD33411.1	56161.45	8.03
Glutathione S-transferase	*F. hepatica*	BN1106_s4479B000057	2	2	1.79	ADP09370.1	58252.69	6.56
Mu-glutathione transferase, partial	*F. hepatica*	BN1106_s4370B000168	3	3	3.77	AAA29139.1	27520.76	7.52
Mu-glutathione transferase, partial2	*F. hepatica*	BN1106_s7830B000018	2	2	2.74	AAA29140.1	38599.19	6.79
Phosphoglycerate kinase	*F. hepatica*	BN1106_s3033B000087	2	2	3.61	AAZ17561.2	44178.98	8.73
Pro-cathepsin B2, partial	*F. hepatica*	BN1106_s6570B000050	3	3	3.44	CAD32937.1	35969.42	7.08
Cathepsin B4 like	*F. gigantica*	BN1106_s5100B000033	3	4	4.21	AIS22047.1	29282.73	5.55
Cathepsin B5	*F. gigantica*	BN1106_s14651B000004	2	3	12.36	ALX81544.1	10031.21	5.59
Cathepsin b5like	*F. gigantica*	BN1106_s14666B000004	2	3	11.83	AAD11445.1	10515.79	6.83

[a] The genome mapping gene id relates only to *Fasciola hepatica*

[b] Theoretical molecular mass (kDa)

[c] Theoretical isoelectric point

Table 2 The annotated *Fasciola gigantica* excretory and secretory products (FgESPs) which were detected only by the buffalo serum of 70 days post-infection (dpi) with *Fasciola gigantica*

Protein description	Species	Genome mapping Gene ID[a]	Pep count (sequence)	Unique pep count	Cover percent	GeneBank Bank ID	Theor MW[b]	Theor PI[c]
Kunitz	*F. hepatica*	BN1106_s8826B000029	5	3	55.88%	PIS91154.2	7351.56	8.39
14-3-3 z protein	*F. gigantica*	BN1106_s3904B000042	2	2	3.28%	PIS82069.1	26776.77	8.77
Cathepsin L	*F. gigantica*	BN1106_s4636B000039	4	3	9.27%	AAF44676.1	45296.56	6.28
Glutathione S-transferase omega	*F. gigantica*	BN1106_s1029B000154	3	2	2.34%	AFX98104.1	33652.63	6.38
Taurocyamine kinase	*Fasciola* spp.	BN1106_s4261B000116	3	3	1.40%	AHH34783.1	80381.97	6.96

[a] The genome mapping gene id relates only to *Fasciola hepatica*

[b] Theoretical molecular mass (kDa)

[c] Theoretical isoelectric point

least six-tubulin isotypes designated (Fuchs et al. 2013; Ryan et al. 2008). Five proteins could only be detected in 70 dpi (Table 2); taurocyamine kinase was first reported in *F. gigantica*, which belongs to the family of transferases, specifically those transferring phosphorus-containing groups (phosphotransferases) with a nitrogenous group as acceptor. 14-3-3 protein was also identified in this period, which is a family of adaptor proteins with a wide range of functions in cell signaling. These proteins can only be detected in this stage, they may prevent the development of a protective Th1-type immune response against the parasite, thereby allowing the development of a chronic infection. Seven proteins could only be detected in 98 dpi (Table 3); calmodulin-like protein 2 was identified in this group, Calmodulin is a calcium ion-sensing signaling protein wide distributed in eukaryotics. Several calmodulin-like genes were reported in adult liver flukes, but little information about the functions was available (Russell et al. 2007), and SNF7 family protein was also found in this group, which has an additional role in activation of the transcription factor Rim101, it is quite important for gene expression, but the function in *Fasciola* is unknown (Lee et al. 2011). This protein could be located on the membrane of extracellular vesicles (EVs). Fasciola secretes at least two subpopulations of EVs (15 K and 120 K) that differ according to size, and at least 180 different EV proteins were identified (Cwiklinski et al. 2015b). EVs could be purified by differential centrifugation according to previous study (Marcilla et al. 2012), and contain most of the proteins identified as components of ESP. Some of these proteins play an important role in host parasite communication. In this study, isolation of EV was not carried out from *F. gigantica* ESP, and some EV proteins may be included. Thioredoxin glutathione reductase was also identified in this priod, and this protein can not confer protection against fasciolosis in cattle. These 7 proteins can only be detected in the chronic infection stage, and may have the immunomodulatory capacity of suppression of T cell responses. They might mediate the balance between the parasite and host; thus, the parasite can survive in host for a long period. A total of 17 proteins were found in all three periods (Table 4), and these proteins belonged to different protein families, such as cathepsin family, calcium-binding protein family, GST, and leucine amino peptidase family, which take part in the whole infection period. Although some members of this protein family only take part in specious

Table 3 The annotated *Fasciola gigantica* excretory and secretory products (FgESPs) which were detected only by the buffalo serum of 98 days post-infection (dpi) with *Fasciola gigantica*

Protein description	Species	Genome mapping Gene ID[a]	Pep count (sequence)	Unique pep count	Cover percent	GeneBank Bank ID	Theor MW[b]	Theor PI[c]
Calmodulin-like protein 2 (cam2)	*F. hepatica*	BN1106_s2277B000048	4	4	9.15%	CAL91033.1	16421.2	4.98
Cathepsin L1D like	*F. hepatica*	BN1106_s7456B000012	5	4	3.98%	ACJ12894.1	25465.46	6.24
CD59-like protein	*F. hepatica*	BN1106_s6720B000015	5	4	9.84%	AIE76460.1	13283.61	8.53
SNF7 family protein	*F. hepatica*	BN1106_s6543B000070	5	3	21.05%	PIS78662.1	23978.04	4.62
Cathepsin L1	*F. gigantica*	BN1106_s2303B000143	3	2	4.47%	AAL23917.1	20598.73	4.89
Cathepsin L2	*F. gigantica*	BN1106_s11179B000023	3	2	6.57%	AAM44832.1	16057.9	5.75
Thioredoxin-glutathione reductase 1	*F. gigantica*	BN1106_s3005B000095	4	3	4.98%	AKU75589.1	26040.68	9.52

[a] The genome mapping gene id relates only to *Fasciola hepatica*

[b] Theoretical molecular mass (kDa)

[c] Theoretical isoelectric point

Table 4 The annotated *Fasciola gigantica* excretory and secretory products (FgESPs) which were detected by all of the buffalo serum of 42, 70, and 98 days post-infection (dpi) with *Fasciola gigantica*

Protein description	Species	Genome mapping Gene ID[a]	Pep count (sequence)	Unique pep count	Cover percent	GeneBank Bank ID	Theor MW[b]	Theor PI[c]
Calcium-binding protein	*F. hepatica*	BN1106_s214B000741	2	2	7.94%	CAA06035.1	22259.27	5.88
CaBP1	*F. hepatica*	BN1106_s214B000742	5	5	29.48%	AML33332.1	20091.81	5.96
Enolase	*F. hepatica*	BN1106_s3227B000227	5	3	2.19%	CAK47550.1	60472.57	6.77
Heat shock protein 70	*F. hepatica*	BN1106_s309B000234	7	7	14.88%	ABS52704.1	70751.87	5.54
Kunitz	*F. hepatica*	BN1106_s318B000274	2	2	23.29%	PIS91154.1	8144.31	7.52
14-3-3 zeta protein	*F. gigantica*	BN1106_s3904B000042	5	4	15.03%	AVD68700.1	41881.65	5.46
Secreted cathepsin L2	*F. gigantica*	BN1106_s8098B000020	2	2	4.25%	ABQ95351.1	45326.82	5.3
Cathepsin L1D	*F. gigantica*	BN1106_s10332B000010	5	3	4.98%	ACJ12893.1	29932.45	5.59
Glutathione S-transferase 2	*F. gigantica*	BN1106_s1081B000242	3	3	14.96%	AFX98103.1	26931.97	8.5
Legumain-1	*F. gigantica*	BN1106_s4223B000091	4	4	8.72%	ABQ02437.1	41529.88	8.76
Leucine amino peptidase	*F. gigantica*	BN1106_s617B000567	2	2	19.31%	AAV59016.1	15715.86	6.39
Leucine amino peptidase 2	*F. gigantica*	BN1106_s617B000566	9	9	23.98%	ACS94976.1	42372.28	7.77
Multi-domain cystatin	*F. gigantica*	BN1106_s1612B000138	9	9	5.60%	AIT39701.1	173959.31	8.11
Secreted saposin-like protein (SAP-3)	*F. gigantica*	BN1106_s10326B000017	4	4	11.36%	ABC66278.1	9521.26	5.18
Calcium-binding EF-hand protein 4	*F. gigantica*	BN1106_s214B000744	5	4	25.13%	AEX92829.1	22250.22	5.04
Thioredoxin	*F. gigantica*	BN1106_s4026B000080	6	5	8.65%	2VIM_A	11683.31	5.86
Thioredoxin peroxidase	*F. gigantica*	BN1106_s1614B000280	3	2	11.54%	PIS86707.1	17463.03	8.84

[a] The genome mapping gene id relates only to *Fasciola hepatica*

[b] Theoretical molecular mass (kDa)

[c] Theoretical isoelectric point

infection period, but these proteins were quite different, they were existed in whole infection period and took part in the interaction between the parasite and host, thus, some of them could be good candidates for diagnosis markers. The accurate diagnosis of *Fasciola* infections is central to studying the surveillance of fascioliasis and control of this disease. These results provided plenty of candidates for further diagnosis study.

Comparison of our data sets for different infected period revealed that there is an overlap in protein expression between these samples, the relationship of these proteins pulled down from were summarized in Venn diagram (Fig. 2).

ESPs play an important role during the development of parasites, especially in the host-parasite interaction, lots of ESPs of *Fasciola* spp. have been proved to be able to

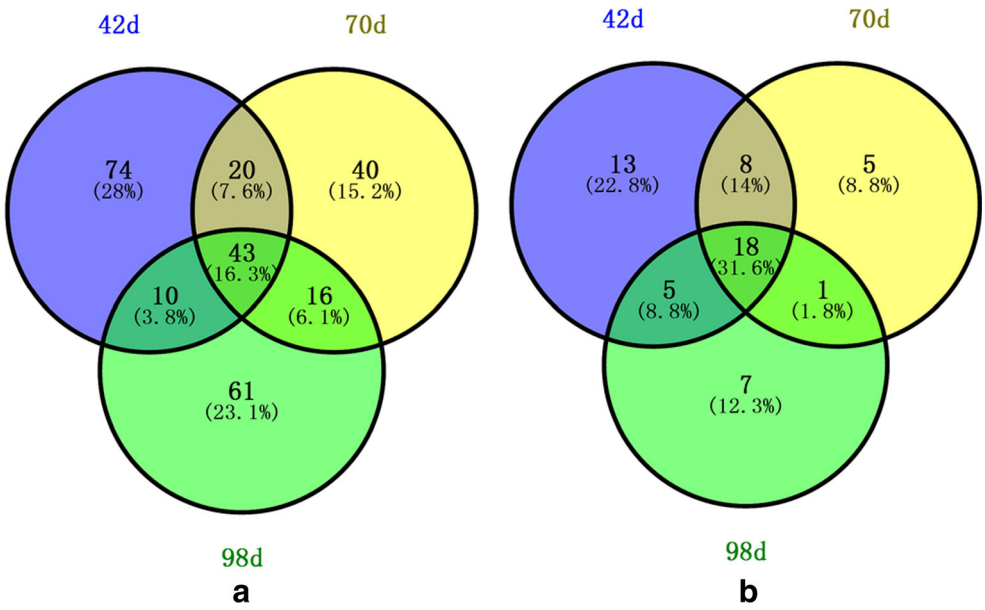

Fig. 2 **a** Total proteins identified binding to buffalo serum of 42 days post-infection (dpi) with *Fasciola gigantica* (blue), 70 dpi (yellow) and 98 dpi (green). **b** For the annotated proteins identified binding to buffalo serum of 42 dpi (blue), 70 dpi (yellow) and 98 dpi (green)

evade, defend against the host immune response (Jefferies et al. 2001). FhESPs can down modulate the proliferation of spleen mononuclear cells (Cervi and Masih 1997), and induce immunomodulatory effects on macrophages by inducing T cell activity via selective up-regulation of PD-L2 expression in a Dectin-1 dependent way (Guasconi et al. 2015). Proteases of the cathepsin L and cathepsin B families are expressed in newly excysted juveniles (NEJ), immatures, and adults, which are the major components of the ESPs (Law et al. 2003; Meemon et al. 2004; Smith et al. 1993). Cathepsin L1 and L2 are important for the parasites to migrate through the host as they have proteolytic activities against extracellular matrix (Berasain et al. 1997). A cathepsin B of NEJ in *F. hepatica* can against sheep immunoglobulin heavy chain and bovine serum albumin which has protease activity (Wilson et al. 1998). It has been reported that Glutathione S-transferases (GSTs) had catalytic activity and might be also involved in evasion of the host immune response (Cervi et al. 1999). From our result, we could find that our results were consistent with the previous studies, and the cathepsin family took part in all three infection periods, suggesting that this family is very important in the parasite's life cycle. Fasciolosis is divided into acute (1–6 weeks after infection) and chronic (from 7 to 8 weeks), comparing to chronic, more proteins were involved in the parasite-host interplay in acute (40d). Little is known about the mechanism underpinning these changes cellular biological level.

ESPs from *F. hepatica* are exposed directly to the host immune system, and widely used as antigens in serological assays. Cathepsin L, cathepsin L1, and rFh8, were used to detect anti-*Fasciola* IgG antibodies in sheep sera and showed good diagnostic sensitivity and specificity in ELISA (Muino et al. 2011; O'Neill et al. 1999; Silva et al. 2004). Lots of proteins were identified in this study, and the functions of most of them are unknown; the key genes involved in the interplay between host and parasites have not been identified. We suggest that this highlights the central role played by ESPs in the protection of *F. gigantica* from the host immune response. At the same time, this study provided material for further studies into the interaction between *F. gigantica* and host. These proteins could also be good candidates for diagnostic antigens in serological assays. These data now provide an excellent chance to explore host immune responses to *F. gigantica* infection at the molecular level.

Acknowledgments Project support was provided by the "National Key Basic Research Program (973 Program) of China" (Grant No. 2015CB150300), the National Key Research and Development Program of China (Grant No. 2017YFD0501200), and A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (Veterinary Medicine); The authors thank Shanghai Applied Protein Technology Co. Ltd., for technical assistance in LC-MS/MS analysis.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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