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Monoclonal antibody against *Fasciola gigantica* glutathione peroxidase and their immunodiagnosis potential for fasciolosis

Pornanan Kueakhai[a,b], Kulathida Chaithirayanon[c], Athit Chaiwichien[a,b], Tepparit Samrit[a,b], Supawadee Osotprasit[a,b], Phawiya Suksomboon[a,b], Wipaphorn Jaikua[a,b], Prasert Sobhon[c], Narin Changklungmoa[a,b,*]

[a] Faculty of Allied Health Sciences, Burapha University, Long-Hard Bangsaen Road, Mueang District, Chonburi 20131, Thailand
[b] Research unit of vaccine and diagnosis of parasitic diseases, Burapha University, Long-Hard Bangsaen Road, Mueang District, Chonburi 20131, Thailand
[c] Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Rd, Bangkok 10400, Thailand

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ABSTRACT

Glutathione peroxidases (GPx), major antioxidant enzymes, secreted by *Fasciola* spp., are important for the parasite evasion and protection against the host's immune responses. In the present study, a monoclonal antibody (MoAb) against recombinant *F. gigantica* glutathione peroxidase (rFgGPx) was produced by hybridoma technique using spleen cells from BALB/c mice immunized with rFgGPx. This MoAb (named 7B8) is IgG1 with κ light chains, and it reacted specifically with rFgGPx at a molecular weight 19 kDa as shown by immunoblotting, and reacted with the native FgGPx in the extracts of whole body (WB), metacercariae, newly excysted juveniles (NEJs), 4 week-old juveniles and adult *F. gigantica* as shown by indirect ELISA. It did not cross react with antigens in WB fractions from other adult trematodes, including *Fischoederius cobboldi, Paramphistomum cervi, Setaria labiato-papillosa, Eurytrema pancreaticum*, *Gastrothylax crumenifer* and *Gigantocotyle explanatum*. By immunolocalization, MoAb against rFgGPx reacted with the native protein in the tegument, vitelline cells, and eggs of adult *F. gigantica*. In addition, the sera from mice experimentally infected with *F. gigantica* were tested positive by this indirect sandwich ELISA. This result indicated that FgGPx is an abundantly expressed parasite protein that is secreted into the tegumental antigens (TA), therefore, FgGPx and its MoAb may be used for immunodiagnosis of both early and late fasciolosis gigantica in animals and humans.

1. Introduction

Fasciolosis is a zoonotic disease that is caused by infection with *Fasciola hepatica* or *F. gigantica*. It is a serious health problem for both domesticated animals and humans in the tropical and subtropical regions of Africa, Middle East, and Asia. During penetration and migration, the juvenile parasites come into contact with and are damaged by reactive oxygen species (ROS) released by the host's immune cells, such as macrophages and other inflammatory cells. Therefore, the parasites must defend themselves by posing anti-oxidation mechanism which, in *F. gigantica,* consists of a family of antioxidant enzymes, including superoxide dismutase (SOD, Jaikua et al., 2016), peroxiredoxin (Prx, Chaithirayanon and Sobhon, 2010; Sangpairoj et al., 2014), thioredoxin (Trx, Gupta et al., 2015; Changklungmoa et al., 2014), thioredoxin glutathione reductase (TGR, Kalita et al., 2018; Changklungmoa et al., 2015), glutathione S-transferase (GST, Preyavichyapugdee et al., 2008) and GPx (Changklungmoa et al., 2018). These enzymes co-ordinate in neutralizing oxidative stress. GPx comprises both intracellular and extracellular isoforms (Ottaviano et al., 2009), and the latter is released from the young as well as adult parasites to protect their exteriors from the host's damaging immune reactions (Changklungmoa et al., 2018). Therefore, GPx is one of key targets for immunodiagnosis and vaccination.

Nowadays, the diagnosis of fasciolosis is based on identification of eggs in fecal samples by stool examination. The technique is cumbersome and needs expert for definite examination. On the other hand, the eggs appear in feces only when mature parasites start laying eggs, and pass them into bile ducts and feces, therefore early infection (less than 10 weeks) cannot be diagnosed. Circulating antigen detection is more specific in reflecting the current infection than circulating antibody detection (Cordova et al., 1999). FgGPx is abundantly expressed in both early and late stages and could be detected in tegument antigens (TA)

* Corresponding author at: Faculty of Allied Health Sciences, Burapha University, Long-Hard Bangsaen Road, Saen Sook Sub-district, Mueang District, Chonburi 20131, Thailand.

E-mail address: narinchang@go.buu.ac.th (N. Changklungmoa).

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and host's circulation by using polyclonal antibodies (PoAb) (Changklungmoa et al., 2018); therefore, FgGPx should be among the most easily and specifically detected antigens in the host's blood by its corresponding monoclonal antibody (MoAb). In this report, we had successfully produced and characterized a MoAb specifically for circulating FgGPx that may have diagnostic potential for fasciolosis by *F. gigantica*.

2. Methodology

2.1. Preparations of parasites and parasite antigens

The *F. gigantica* metacercariae were collected from *Lymnea* spp. snails infected with miracidia. About 4 weeks after infection period, cercariae were shed from the snails and transformed into metacercariae after settled on the plastic papers. The metacercariae were collected from the plastic papers and washed several times with distilled H_2O. The NEJs were produced by activating the excystment of metacercariae by incubated in a solution containing 0.2 % taurocholic acid, 0.02 % M sodium dithionite, 1 % $NaHCO_3$, 0.85 % NaCl and 0.5 % HCl at 37 °C for 30 min as previously published (Kueakhai et al., 2011, 2013). The 4 week-old juvenile parasites were obtained from the livers of hamsters infected with metacercariae via stomach tube at the 4th weeks post infection. Adult *F. gigantica*, *Fischoederius cobboldi, Paramphistomum cervi, Setaria labiato-papillosa, Eurytrema pancreaticum*, *Gastrothylax crumenifer* and *Gigantocotyle explanatum* were collected from infected cattle killed at a local abattoir Phetchaburi Province, Thailand. All parasites were subsequently washed with phosphate buffer saline (PBS) and used immediately for subsequent experiments.

Whole body (WB) extracts of various stages of *F. gigantica* (metacercariae, NEJ, 4 week-old juneniles, and adult), and other adult trematodes (*F. cobboldi, P. cervi, S. labiato-papillosa, E. pancreaticum*, *G. crumenifer* and *G. explanatum*) were prepared as previously published (Changklungmoa et al., 2018; Kueakhai et al., 2011). Adult *F. gigantica* tegumental antigens (TA) and excretory-secretory (ES) were prepared as described earlier (Changklungmoa et al., 2018).

2.2. Expression and purification of recombinant proteins

Then FgGPx cDNA, cloned by Polymerase chain reaction (PCR) technique as described earlier (Changklungmoa et al., 2018), was inserted into bacterial expression vector (pET-30b) (Novagen). The pET-30b recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3). The expression of the rFgGPx protein coupled with His-tag at N-terminus was induced with Isopropyl β-D-1-thiogalactopyranoside (IPTG) at 1.0 mM final concentration. The rFgGPx was purified by Ni-NTA affinity-chromatography under native conditions as described earlier (Changklungmoa et al., 2018). Each recombinant protein was eluted with 250.0 mM imidazole. The purity of the rFgGPx was analyzed by 12.5 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and kept at −20 °C for subsequent experiments.

2.3. Production and characterization of MoAbs against rFgGPx

The rFgGPx protein was used for immunization of eight week-old female BALB/c mice by subcutaneous injection. The mouse was primed by injection of 25 μg rFgGPx protein mixed with Freund′s complete adjuvant (FCA; Sigma–Aldrich Inc.). The first, second and third boosts were given with 25 μg of the rFgGPx mixed with Freund′s incomplete adjuvant (FIA; Sigma–Aldrich Inc.) at 2-week intervals by subcutaneous route. Blood samples from the immunized mice were collected, and the antibody titers (anti-rFgGPx) were estimated by indirect ELISA. B cells from immunized BALB/c mice were fused with myeloma cells (P3-X63-Ag8.653), using polyethylene glycol (PEG) (Sigma–Aldrich Inc.) (Kueakhai et al., 2013). The MoAbs against rFgGPx produced by the hybridoma clones were screened by indirect ELISA. The culture fluid of positive hybridoma clones that produced high antibody titer of MoAbs against rFgGPx were collected, and the immunoglobulin isotypes were determined by indirect ELISA using the SBA Clonotyping™ System/HRP (Southern Biotech).

2.4. Production of rabbit polyclonal antibody (PoAb) against rFgGPx

A New Zealand White rabbit was injected with 250 μg of purified rFgGPx diluted in sterile PBS mix with FCA for first immunization. The second and third immunizations were injected with 125 μg of purified rFgGPx diluted in sterile PBS mix with FIA. Blood sample was collected at day 10 after the third immunizations, centrifuged, and the serum was collected and kept frozen at -20ºC for subsequent experiments.

2.5. Purification of MoAb against rFgGPx (clone 7B8) and rabbit PoAb against rFgGPx

The MoAb against rFgGPx and rabbit PoAb against rFgGPx were purified by using Aff-Prep protein A (Bio-Rad, USA) as previously published (Kueakhai et al., 2015). Elution of the IgG against rFgGPx bound to protein A was carried out using an elution buffer (Bio-Rad, USA), pH 3.0, and followed by neutralization of the eluent with 1 M Tris HCl, pH 9.0. The eluted fractions containing IgG against rFgGPx (MoAb and PoAb) were concentrated, and stored at 4 °C for subsequent experiments.

2.6. Characterization of MoAb against rFgGPx by immunoblotting analysis

The rFgGPx and WB, TA, ES of adult *F. gigantica* were separated in a 12.5 % SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad) for immunoblotting analysis by MoAb against rFgGPx (7B8). Nonspecific binding was blocked with 4 % (w/v) skim milk in PBS at room temperature (RT) for 1 h, and subsequently by incubation in the culture fluid of MoAb 7B8 at 4 °C overnight. The membranes were washed three times with PBS containing 0.1 % Tween-20 (PBST) and then incubated with goat anti-mouse IgG conjugated with alkaline phosphatase (AP) (Southern Biotech) diluted at 1:2000 in PBS for 1 h. The membranes were washed three times and then the color was developed in the dark using the substrates nitro-blue tetrazolium chloride/5-bromo-4-chloro-3-indodyl phosphate (NBT/BCIP). Finally, the reactions were stopped by adding a stop buffer (10 mM Tris−HCl and 1 mM EDTA).

2.7. Characterization of MoAb against rFgGPx by indirect ELISA

The immunoglobulin isotypes of MoAb against rFgGPx were determined by using indirect ELISA. The plates were coated with 100 μl of rFgGPx (1 μg/ml) in coating buffer as previously published (Kueakhai et al., 2015) at 4 °C overnight. The coated plates were washed three times with 0.05 % PBST, and nonspecific binding was blocked by adding 200 μl of 1 % BSA in PBS per well, incubated at RT for 1 h with shaking. Then the coated plates were washed three times with 0.05 % PBST, and 100 μl of culture fluids from hybridoma clone 7B8 and myeloma cells (negative control) were added and incubated at 4 °C overnight. The plates were washed three times and incubated for 1 h, at RT in 100 μl of goat anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgA, IgM conjugated with HRP (Southern Biotech) diluted in PBS at 1:5000 for heavy chain, and κ, λ light chain identifications. The plates were washed three times, and 100 μl of the substrate 2,2′-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS; Southern Biotech) were added per well and incubated for 15 min in dark at RT. The optical densities (OD) were measured at 405 nm in an automatic VersaMax Microplate Reader (Molecular Devices, CA, USA).

The reactivity of the MoAb against rFgGPx (7B8) and native proteins from WB of metacecariae, NEJs, 4-week-old juveniles, adult, and TA, ES of adult *F. gigantica* and WB of other adult trematodes (including, *E. pancreaticum, S. labiato-papillosa, G. crumenifer, G. explanatum, F. cobboldi* and *C. cotylophorum*) were determined by indirect ELISA. The plates were coated with 100 µl of 10 µg/ml WB of metacercariae, NEJs, 4-week-old juveniles, adult ES and TA, and WB of other adult trematodes in coating buffer at 4 °C overnight. The coated plates were washed three times with 0.05 % PBST and the nonspecific binding was blocked by adding 200 µl 1 % BSA in PBS per well at RT for 1 h with shaking. After that the coated plates were washed three times with 0.05 % PBST, and 100 µl of culture fluid of hybridoma clone 7B8 were added and incubated at 4 °C overnight. The plates were washed three times with 0.05 % PBST and incubated in goat anti-mouse IgG conjugated with HRP (Southern Biotech) diluted in PBS at 1:5000 for 1 h, at RT. Then the plates were washed three times with 0.05 % PBST, and 100 µl per well of 3,3′,5,5′-Tetramethylbenzidine (TMB) (KPL, Gaithersburg, USA) was added and incubated 15 min in dark at RT. Finally, reactions were stopped by adding 100 µl of 1 N HCl per well. The ODs were measured at 450 nm in an automatic VersaMax Microplate Reader (Molecular Devices, CA, USA).

2.8. Characterization of MoAb against rFgGPx by immunolocalization in adult F. gigantica

Adult *F. gigantica* were fixed in 4 % paraformaldehyde (w/v) in PBS, pH 7.4 at 4 °C for 3 h with shaking. Paraffin sections of adult *F. gigantica*, cut at 5 µm-thick, were deparaffinized and rehydrated through a serial concentrations of ethyl alcohol. The adult *F. gigantica* sections were treated with citrate buffer (10 mM citric acid, pH 6.0), in a microwave oven at 700 W, three times for 5 min. Nonspecific binding was blocked by incubation in 4 % (w/v) BSA in PBS for 1 h. The adult *F. gigantica* sections were then incubated in the culture fluid of hybridoma clone 7B8, at 4 °C overnight. Sections were then washed three times with 0.1 % PBST and incubated in goat anti-mouse IgG conjugated with AP (Southern Biotech), diluted to 1:2000 in PBS at RT, for 1 h. After that, the sections were washed three times with 0.1 % PBST and incubated in AP buffer, pH 9.5 at RT, for 10 min. The signals development was carried out using NBT/BCIP substrates (Roche) in the dark. Finally, the reactions were stopped by adding stop buffer (10 mM Tris – HCl and 1 mM EDTA, pH 8.0). The sections were examined and photographed under a light microscope (Olympus, CX33).

2.9. Demonstration of circulating FgGPx in mouse sera

Infected mouse sera were collected at 1 and 4 weeks after being infected with *F. gigantica*. Uninfected healthy mice sera were used as a negative control. The circulating FgGPx in mouse sera were detected by using indirect sandwich ELISA. The purified MoAb against rFgGPx (7B8) was used as capturing antibody. The healthy mice, 1-week post infected-, and 4-week post infected mouse sera were added 50 µl and incubated for overnight at 4 °C, and then 50 µl of purified rabbit PoAb against rFgGPx conjugated with biotin were added as detecting antibody. The 50 µl of streptavidin-conjugated HRP (Southern Biotech) was added, and then the plates were incubated with 50 µl of TMB (KPL, Gaithersburg, USA) for 15 min at RT. Finally, the enzymatic reactions were stopped by adding 50 µl of 1 N HCl. The ODs were measured at 450 nm in an automatic VersaMax Microplate Reader (Molecular Devices, CA, USA).

2.10. Statistical analysis

Differences between the reactivities of the MoAb against rFgGPx (7B8) to the culture fluid of myeloma cells, and native FgGPx proteins from WB of metacercariae, NEJ, 4- week-old juveniles, adult, and ES, TA of adult *F. gigantica* and other adult trematodes were tested using the Independent Samples t-test. A p-value lower than 0.05 was considered statistically significant.

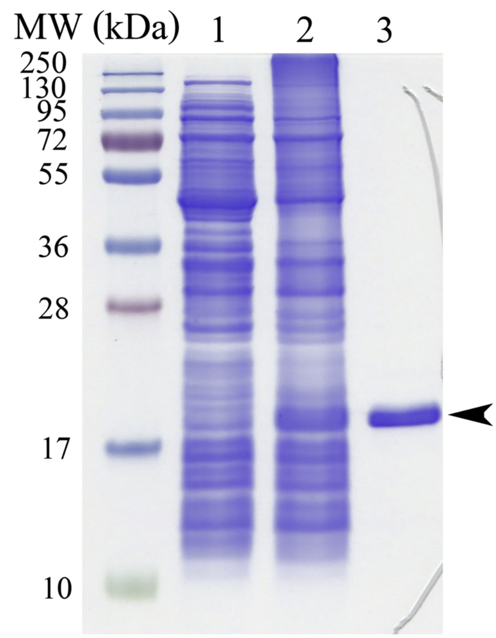

Fig. 1. The Ni-NTA affinity-purified rFgGPx were analysed by SDS-PAGE, and stained with Coomassie Blue. Lane 1, the whole bacterial lysate from the non-induced condition; lane 2, the whole lysate after induction by IPTG; lane 3, the purified recombinant FgGPx. MW markers are shown on the left side. Single arrow head indicates the MW of rFgGPx.

2.11. Ethics statement

Mice and rabbit were kept in steel cages in an air-conditioned room with a light–dark cycle of 12:12 h, at 22–25∘C, and 50–60 % humidity, and animal protocols were approved by The Animal Care and Use Committee (MUSC-ACUC), Faculty of Science, Mahidol University, Thailand.

3. Results

3.1. Expression of rFgGPx

The rFgGPx protein coupled with His-tag at N-terminus was expressed and purified by Ni-NTA chromatography. The purified rFgGPx was analyzed by SDS-PAGE and stained with Coomassie Blue. The rFgGPx protein was resolved as single band at approximately 19 kDa on SDS-PAGE (Fig. 1).

3.2. MoAb against rFgGPx

The rFgGPx was used to detect specific MoAb against rFgGPx by indirect ELISA and immunoblotting analysis. It was found that the culture fluid from hybridoma clone 7B8 specifically binds to rFgGPx and native FgGPx from adult WB and TA (Fig. 2). The immunoglobulin isotype of MoAb against rFgGPx in the culture fluid of hybridoma clone 7B8 is IgG1 with κ-light chain.

3.3. Purification of MoAb and PoAb against rFgGPx

SDS-PAGE analysis (Fig.3) of the purified rFgGPx-immunized rabbit serum IgG and purified MoAb against rFgGPx showed immunoglobulin heavy and light chain bands at MW 50 and 25 kDa, respectively for the MoAb and PoAb anti-rFgGPx. This analysis indicated that IgG was pure.

3.4. Estimation of native FgGPx in F. gigantica and other adult trematodes

Relative levels of the immunoreactivities of the MoAb against

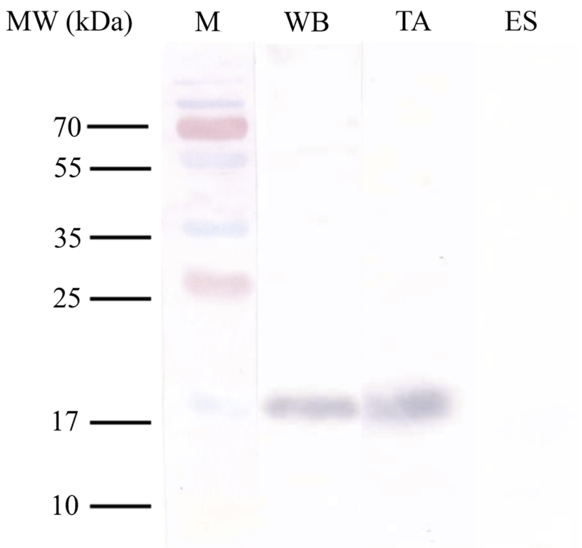

Fig. 2. Immunoblots of WB, TA and ES of adult *F. gigantica* with 7B8 MoAb against rFgGPx shows positive bands at 19 kDa. Lane 1 (WB): WB of adult *F. gigantica*. Lane 2 (TA): TA of adult *F. gigantica*. Lane 3 (ES): ES of adult *F. gigantica*. MW markers are shown on the left side.

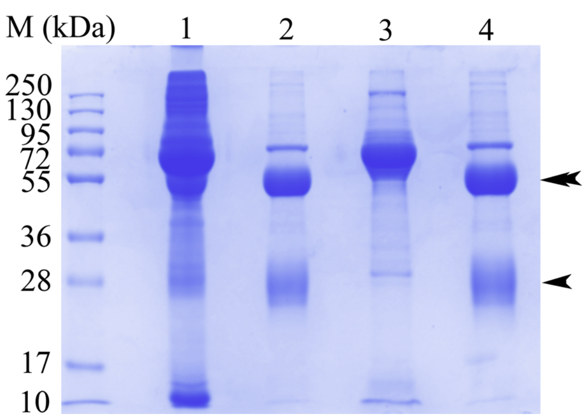

Fig. 3. The purity of MoAb IgG1 and PoAb IgG after purification by Affi-Gel® protein A MAPS® II Kit was determined by 12.5 % SDS–PAGE. Lane 1: Rabbit antiserum (containing all serum proteins including PoAb against rFgGPx). Lane 2: Purified IgG of PoAb against rFgGPx. Lane 3: culture fluid of MoAb (7B8). Lane 4: Purified MoAb against rFgGPx. Single and double arrow heads indicate molecular weight of light chain and heavy chain, respectively. The MW markers are on the left side.

rFgGPx (7B8) with WB of metacercariae, NEJ, 4-week old juveniles and adult, TA, and ES of adult *F. gigantica* and WB of other adult trematodes, were estimated by indirect ELISA. The results showed that the levels of immunoreactivities of native FgGPx in WB of all stages and TA of adult with the MoAb against rFgGPx (represented by OD values) were significantly higher when compared with culture fluid of control myeloma cells (Fig. 4), while there was no significant difference between the OD levels of ES of adult *F. gigantica* and WB of other adult trematodes, when compared with the control (Fig. 4, Fig. 5).

3.5. Immunolocalization of FgGPx

Immunolocalization of FgGPx in adult *F. gigantica* sections was performed with MoAb against rFgGPx 7B8. The culture fluid of myeloma cells was used as a negative control. Strong positive signal was detected in the tegument, egg, and vitelline cells of adult *F. gigantica* but not in caecal epithelial cells (Fig. 6).

3.6. Detection of circulating FgGPx in sera of infected mice

The mouse sera at 1 and 4 weeks post infection with *F. gigantica* were analyzed by indirect sandwich ELISA. The circulating FgGPx was detected in sera of infected mice with high levels at both 1 and 4 weeks post infection when compared with uninfected mouse sera (Fig. 7).

4. Discussion

The gold standard diagnosis of fasciolosis is usually performed by detecting parasite eggs in a fecal sample. This technique can detect only late infection as the eggs can only be found in the fecal sample after the mature parasites move into bile ducts and start laying eggs (Mas-Coma et al., 2005). Immunodetection, based on indirect ELISA to detect antibodies in the sera of infected animals and humans by using either crude extracts of parasites, purified proteins from their ES products and also recombinant proteins (Chaouadi et al., 2019; Aguayo et al., 2018; Mirzadeh et al., 2018; Figueroa-Santiago et al., 2011; O'Neill et al., 1998). However, this method could detect only circulating antibody that may not describe the current infection and parasite load. A better method is an immunoassay that is capable of detecting circulating antigens by either PoAb or MoAb, with the latter proven to be more specific (Cordova et al., 1999). Several MoAbs against specific *F. gigantica* antigens have been used in immunodiagnosis for fasciolosis, including recombinant cathepsin L1H (Wongwairot et al., 2015), recombinant cathepsin B3 (Anuracpreeda et al., 2011), 66 kDa tegumental antigens (TA) (Krailas et al., 1999), 28.5 kDa TA antigen (Chaithirayanon et al., 2002), recombinant *F. gigantica* saposin-like protein-2 (Kueakhai et al., 2013), *F. hepatica* ES antigens (Abdolahi

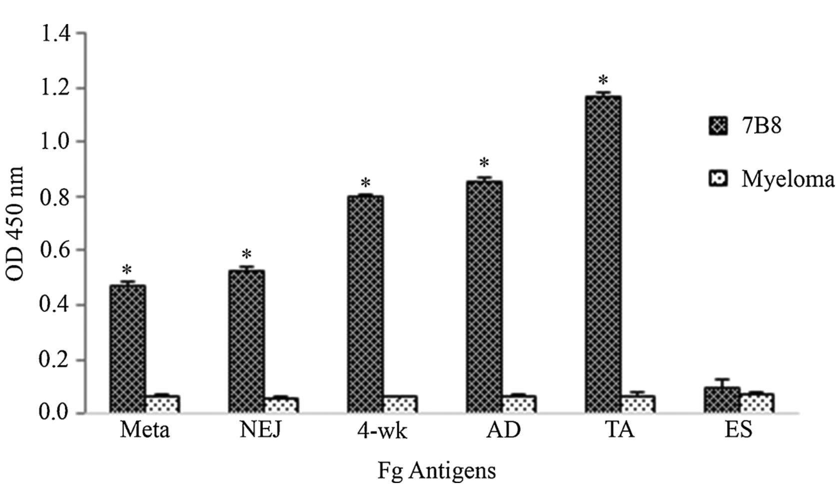

Fig. 4. Semi-quantitation of the levels of expression of native FgGPx in WB of metacercariae, NEJ, 4 week-old juveniles, adult, and ES, TA of adult *F. gigantica* by estimating the reactivities with MoAb 7B8 (black bar) using indirect ELISA. Significant increases of ODs were shown in WB from all developmental stages, and TA of adult *F. gigantica* when compared with culture fluid of control myeloma cells (white bar), denoted by * ($p < 0.05$). No significantly increase was shown in ES.

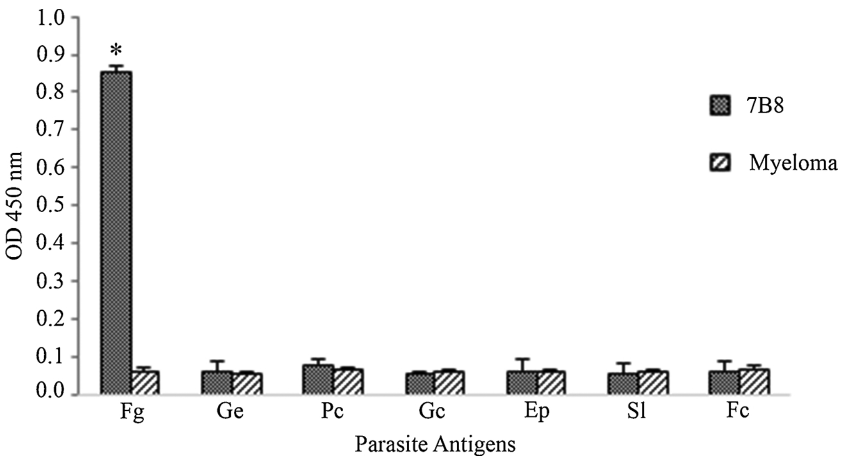

Fig. 5. Semi-quantitation of the levels of expression of native FgGPx in WB extracts of *F. gigantica* (Fg), *C. cotylophorum* (Cc), *S. labiato-papillosa* (Sl), *G. explanatum* (Ge), *G. crumenifer* (Gc), *E. pancreaticum* (Ep), and *F. cobboldi* (Fc) by estimating the reactivities with MoAb 7B8 (black bar) using indirect ELISA. Significant increases of ODs were shown in WB of adult *F. gigantica* when compared with culture fluid of control myeloma cells (white bar), denoted by * ($p < 0.05$). No significantly increase was shown in WB of other trematodes.

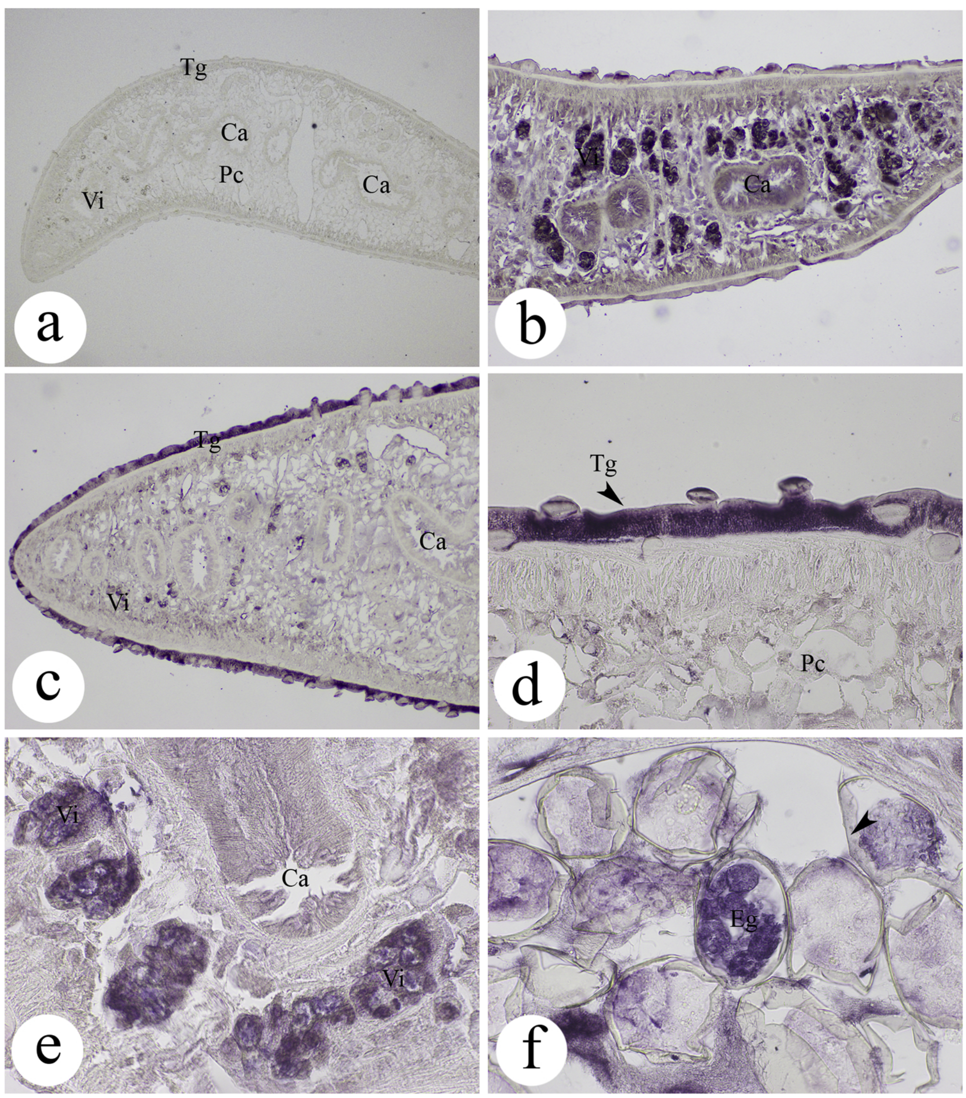

Fig. 6. Localization of native FgGPx in the tissues of adult *F. gigantica* by immunohistochemical detection using MoAb 7B8. (A) A section was probed with culture fluid of myeloma cell shows no staining. (B, C) Sections probed with culture fluid of 7B8 show strong positive signal in tegument (Tg) and cells of vitelline grands (Vi). (D) A section probed with culture fluid of 7B8 shows strong positive signal in the tegument (Tg). No signal was observed in the parenchymal cells (Pc). (E) A section shows strong positive signal only in the vitelline grands (Vi). (F) An adult *F. gigantica* section showing strong positive signal only in the egg (Eg).

Khabisi et al., 2017) and 27 − 26 kDa ES antigens (Maleewong et al., 1999). Likewise, these MoAb could detect both early and late infection depending on the time in the life cycle that the corresponding proteins are expressed and released into the blood circulation.

In this study, we have produced and characterized the MoAb specifically against rFgGPx by using hybridoma technique. By

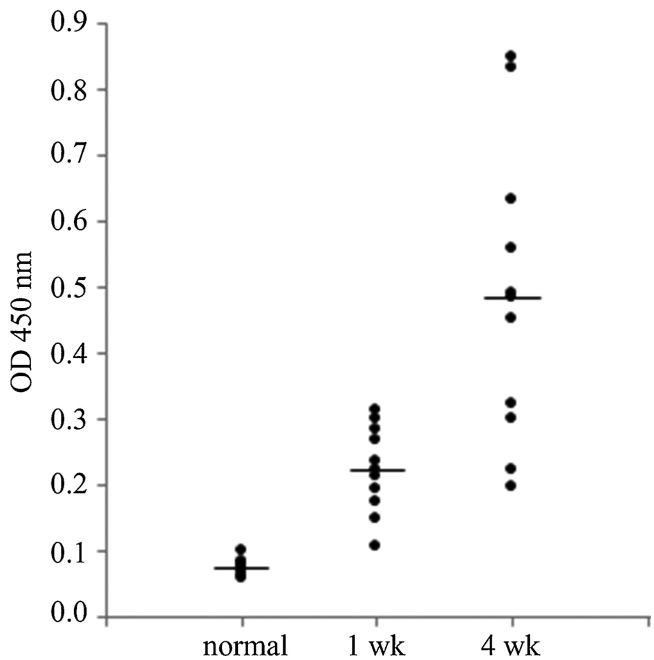

Fig. 7. A scattergram showing relative levels (represented by OD values) of circulating FgGPx in sera of mice infected with *F. gigantica* at 1 and 4 weeks post infection.

immunoblotting analysis, this MoAb was IgG1 with κ light chain which could react with rFgGPx and the native FgGPx with approximate MW of 19 kDa. When the levels of native FgGPx was estimated by indirect ELISA using the MoAb against rFgGPx (7B8), it was showed that the expression levels of FgGPx were high in WB of all developmental stages of *F. gigantica*, including metacercariae, NEJs, 4 week-old juveniles, adult and adult TA. Therefore, the reactive antigens were produced and predominantly released at high amount in all developmental stages and adult parasite TA (Changklungmoa et al., 2018, 2015, 2014; Jaikua et al., 2016). A similar finding was reported by our group using a polyclonal anti-rFgGPx (Changklungmoa et al., 2018). Furthermore, we have used immunoblotting analysis to show that FgGPx was expressed in all developmental stages and also expressed in TA of adult *F. gigantica* (Changklungmoa et al., 2018). The FgGPx expression in all developmental stages of *F. gigantica* is similar to that of *S. japonicum* (Changklungmoa et al., 2012; Zhang et al., 2015). For the indirect sandwich ELISA, rabbit anti-rFgGPx IgG and MoAb against rFgGPx had been produced and purified by protein-A-affinity as determining by SDS-PAGE analysis which showed heavy and light chains of IgG class at MW 50 and 25 kDa, respectively (Fig. 3). In this present study, protein A from *Staphylococcus aureus* was used as the precoat that could enhance the binding of IgG to the plate as previously described (Kueakhai et al., 2015), and hence also the binding of FgGPx antigen. Furthermore, the applications of MoAb and rabbit PoAb against rFgGPx both as antigen-capturing and antigen-detecting antibodies for detecting circulating FgGPx antigen in infected sera were 100 % positive at both 1 week and 4 week-post infection. Furthermore, while this MoAb (7B8) is specific for FgGPx there was no cross-reactivity with antigens from other trematodes, including *S. labiato-papillosa*, *G. explanatum*, *C. cotylophorum*, *F. cobboldi*, *G. crumenifer*, and *E. pancreaticum*. Thus, we expect that this MoAb can be used for as an immunodiagnosis of both early and late infections in human and ruminant fasciolosis. This assay will be developed in future study.

In conclusion, the MoAb against rFgGPx could react with FgGPx which were expressed at high levels in tegument of all developmental stages of *F. gigantica*, and in the TA, vitelline and egg of adult parasite as well as circulating FgGPx. This MoAb specifically reacted with only FgGPx, but not with antigens from other trematodes (*E. pancreaticum*, *S. labiato-papillosa*, *C. cotylophorum*, *G. crumenifer*, *F. cobboldi*, *G. explanatum*). This result strongly suggests that the MoAb against rFgGPx can be used for immunodiagnosis of both early and late fasciolosis in ruminants and humans, which is presently being validated.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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