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CROSS-REACTION: A COMMON TRAIT AMONG HELMINTHES

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Abstract

Cross-reaction between three important zoonotic helminths, *Fasciola gigantica*, *Trichinella spiralis* and *Echinococcus granulosus*, was proved by ELISA. Cross-binding activities in the prepared antisera were strongly directed towards protoscolices and hydatid fluid antigens of *E. granulosus* rather than to *F. gigantica* and *T. spiralis* antigens. Two sets of polypeptides were identified in each antigen by immunoblot; species-specific and cross-reactive. Cross-reactive components in *F. gigantica* antigen were 205 KD, 178 KD, 166 KD, 106 KD, 100 KD, 65 KD, 45 KD and 32 KD. While, cross-reactive molecules in *T. spiralis* antigen were 205 KD, 191 KD, 166 KD, 148 KD, 132 KD, and 32 KD. In protoscolices antigen six cross-reactive components were identified, 205 KD, 191 KD, 149 KD, 106 KD, 45 and 32 KD. Moreover, 205 KD, 190 KD, 177 KD, 149 KD, 103 KD and 33 KD were detected in hydatid fluid antigen by heterologous antisera. Interestingly, three polypeptides of 205 KD, 149 KD and 32 KD showed broad immunogenicity with the developed antisera raising the prospect of being putative common immunoprophylactic components.

Introduction

Analysis of the intriguing phenomenon of cross-reactivity among helminthes of different species, genera and even phyla represents a difficult challenge. The analysis of this pheno-

menon is of importance to understand the evolutionary conservation of antigens and for the development of sensitive and specific serodiagnostic reagents (Aronstein *et al.*, 1986). Although the cross-reactive components have their drawbacks in the field of immunodiagnosis, it can be successfully utilized in cross-protection against multiple infections (El-Azzouni and Hegazi, 1993; Hillyer, 1995; Roger *et al.*, 2000). Extensive previous studies proved cross-reactivity between helminthes of different phyla. Sera from experimentally infected animals with *Schistosoma sp., Fasciola hepatica, Trichinella spiralis, Taenia solium, Echinococcus granulosus* or *Paragonimus westermani* cross-react in diagnostic assays with antigens derived from Schistosomes (Tsang *et al.*, 1981). Moreover, a glycoprotein cross-reactive among *S. mansoni, T. spiralis* and *F. hepatica* was identified and characterized by use of monoclonal antibodies prepared against *S. mansoni* glycoproteins (Aronstein *et al.*, 1986). Choy *et al.* (1991) used a panel of monoclonal antibodies to examine the structure of the muscle larvae of *T. spiralis.* In addition to reaction of these antibodies with the cuticle of the *T. spiralis,* one of them also reacted with extracts of *Fasciolopsis buski* and *Ascaris suum.* Immunochemical and ultrastructure gold labelling techniques proved cross-reaction between *Moniezia expansa, S. mansoni* and *F. hepatica.* This cross-reaction was attributed to the contents of secretory vesicles in the axons and somatic cytoplasm of neurons (Marks *et al.*, 1995). Phosphocholine (PC) epitope was suggested to be responsible for cross-reaction between *Brugia malayi* nematode and *F. gigantica.* It was suggested that detection of PC antigen may provide a valuable immunodiagnostic tool for detection of helminthes infection in animals (Rao *et al.*, 1996). Cross-reaction was also observed between *Opisthorchis viverrini* and *Schistosoma sp., Taenia sp., T. spiralis, Strongyloides stercoralis,* hook worms, *Ascaris lumbricoides, Trichuris trichiura* using ELISA. While, western blot analysis determined the cross-reactive components to be ranged from 15.5 to 144 KDa (Sakolvaree *et al.*, 1997). Bossaert *et al.* (2000) proved cross-reaction between *F. hepatica, E. granulosus, Cysticercus tenuicollis* (*Taenia hydatigena*) and *C. ovis* by ELISA. Sato and Kamiya (2000) proved that intermediate filaments in helminth

tissue is responsible for cross-reaction between *Trichinella britovi, S. mansoni* and *E. multilocularis*. Besides, cross-reactivity between *F. gigantica, Toxocara vitulorum* and *Moniez expansa* was proved using ELISA, immunoblot and immuno-fluorescence assay (Abdel-Rahman and Abdel-Megeed, 2000; Abdel-Rahman *et al.*, 2000 and Hassanain *et al.*, 2000). This antigenic cross-reactivity between helminths has provided the basis for suggesting that cross-reactive components may be useful reagents for the development of a defined polyvalent vaccine. This suggestion was extensively studied and proved between *F. hepatica* and *S. mansoni* (Hillyer, 1995). Moreover, the protective role of *Fasciola* tegument against *T. spiralis* infections was studied by El-Azzouni and Hegazi (1993). Roger *et al.* (2000) proved that cross-reactive antigens between *Theileria annulata* and *Th. parva* mutually confer a degree of cross-species protection raising the prospect of common vaccine in the future.

The current research is an attempt to prove cross-reaction between *F. gigantica, T. spiralis* and *E. granulosus* and to identify those antigens responsible for this cross-reaction. The importance of these antigens reside in their putative value as immunoprophylactic molecules against helminthes.

Material and Methods

Fasciola gigantica antigen (F_gA): Adult worms were collected from condemned livers of buffaloes slaughtered in Cairo governomental abattoir. Worms were washed thoroughly with distilled water and homogenized in 0.15 M phosphate buffer saline (PBS), pH 7.5 supplemented with 2 mM phenyl methyl sulphonyl fluoride (PMSF) and 0.02% NaN_3 in a Ten Broeck tissue grinder. The homogenate was sonicated and then centrifuged at 18.000 rpm for 1 h at 4°C. Clear supernatant was collected and stored at -20°C until use (Abdel-Rahman and Abdel-Megeed, 2000).

Larval antigen of *Trichinella spiralis* (T_sA): Meat from *T. spiralis* infected pigs, examined by trichinoscope in Cairo abattoir, were minced and digested with pepsin over night at 37°C. Larvae were collected and washed extensively with water.

Larvae in 0.15 M PBS containing 2 mM PMSF were homogenized, sonicated and then centrifuged at 18.000 rpm for 1 h at 4°C. Larval antigen was aliquoted and stored at -20°C until use (Ruitenberg *et al.*, 1975).

Protoscoleces antigen of *Echinococcus granulosus* (PA): Protoscoleces were obtained from fertile hydatid cysts recovered from the lungs of naturally infected camels slaughtered in Cairo abattoir. Protoscolices were checked for its viability by eosin test (Himonas *et al.*, 1994) and sonicated in 0.15 M PBS containing PMSF. Sonicate was centrifuged and supernatant was aliquoted and stored at -20°C until use.

Hydatid cyst fluid antigen of *E. granulosus* (H_fA): Cystic fluids were collected from cysts of infected camel lungs by syringe, pooled and centrifuged at 10.000 rpm for 10 min at 4°C. The supernatant was dialyzed against distilled water for 3 days and then centrifuged at 10.000 rpm for 30 min at 4°C. Supernatant was lyophilized and lyophilized materials were reconstituted in physiological saline at use (El-Zayyat *et al.*, 1999).

Protein content of different prepared extracts was determined by the method of Lowery *et al.* (1951).

Raising hyperimmune sera: Antisera against each prepared extract were raised in rabbits according to Fagbemi *et al.* (1995). Rabbits were immunized subcutaneously with protein of each extract in Freund's complete adjuvant. A booster dose of extract in Freund's incomplete adjuvant was injected on day 14. Second and third booster doses were given on day 21 and 28 respectively and blood samples were collected 4 days post last injection. Rabbit anti F_gA (RAF_gA), anti T_sA (RAT_sA), anti PA (RAPA) and anti H_fA (RAH_fA) antisera were evaluated by ELISA using their corresponding antigens, aliquoted and stored at -20°C until use.

Enzyme linked immunosorbent assay (ELISA): Specific and cross-reactive antigenic activities of the four extracts against raised hyperimmune antisera were monitored by ELISA. The assay was performed according to Santiago *et al.* (1986) with little modifications and the cut off point of optical density values was determined by the method of Allan *et al.* (1992).

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE): After treatment with reducing sample buffer different extracts were separately electrophoresed on SDS polyacrylamide slab gels according to Laemmli (1970). Bio-Rad high and low molecular weight markers were included in each gel. After separation, gels were fixed in 50% methanol and stained with silver stain according to Wray *et al.* (1981).

Immunoblotting: The four extracts were separated by SDS-PAGE and then electroblotted onto nitrocellulose papers (Towbin *et al.*, 1979) in a blotting system. Nitrocellulose papers were incubated with the four diluted antisera raised in rabbits. After washing, papers were incubated with alkaline phosphatase conjugated anti-rabbit IgG. Then exposed to substrate solution for 30 min. Nitrocellulose sheets rinsed with distilled water to stop the reaction. Molecular weight of the detected polypeptides was calculated according to the software programme Gel-Pro Analyzer V. 3.0.

Results

Detection of antibody binding activities in the prepared antisera: The binding activities of the different antisera either toward their respective antigens or different ones, as measured by ELISA were depicted in Figs 1,2,3 and 4. These figures showed the presence of cross-reactive binding capacities in the four antisera in addition to specific ones.

Specific binding: The binding of each antisera with its corresponding antigen showed different patterns where specific binding of T_sA (Fig 2) and F_gA (Fig 1) to their respective sera is lower than that of H_fA (Fig 4) and PA (Fig 3) to its homologous sera as reflected by OD values. This observation was also confirmed particularly at high dilution of antisera where the activity was still strong in the specific assays of H_fA and PA in contrary to that observed at the same dilution of antisera in the specific assay of T_sA and F_gA.

Cross-reactive binding: Figures 1,2,3 and 4 depicted the corss-reaction between the four antigens. Cross-reactive binding activities towards F_gA in RAH_fA and RAT_sA (Fig 1) are comparable to those of RAF_gA, RAH_fA and RAPA to T_sA (Fig

2). These reactivates were apparently low than the specific ones in RAF_gA (Fig 1) and RAT_sA (Fig 2). On the other hand, the cross-binding activities of RAH_fA, RAF_gA and RAT_sA towards PA (Fig 3) and of RAPA, RAF_gA and RAT_sA towards H_fA (Fig 4) were more potent than the previous ones particularly at low sera dilution but still low than specific assays except with RAPA towards H_fA (Fig 4). It is worthy to note that the cross binding activities in RAH_fA towards PA is more potant than in RAT_sA and RAFgH towards the same target specially at high sera dilution (Fig. 3). Moreover, cross-reactivity in RAF_gA to H_fA was higher than that detected in RAT_sA towards H_fA (Fig. 4).

Electrophoretic make up of the four antigens: Fig. 5 (lanes A,B,C and D) demonstrated complex separation pattern of antigens and also showed extensive electrophoretic similarity between the four extracts specially in terms of 205 KD, 191 KD and 98 KD. Nevertheless exclusive molecules to each antigen were detected as 62 KD selectively expressed by PA Fig 5 lane C and 103 KD associated only with H_fA Fig 5 lane D.

Identification of antigenic targets recognized by the different antisera: Specific targets: These targets were probably significant contributors to the observed specific binding. RAF_gA recognized seven polypeptides in F_gA of molecular weight 205 KD, 190 KD, 178 KD, 132 KD, 100 KD, 78 KD and 32 KD (Fig 6 lane A). While RAT_sA identified six bands in T_sA with 205 KD, 191 KD, 166 KD, 106 KD, 77 KD and 32 KD (Fig 7 lane B). RAPA detected seven polypeptides of 205 KD, 177 KD, 149 KD, 118 KD, 98 KD, 78 KD and 45 KD in PA (Fig 8 lane C). Eight specific molecules were recognized in H_fA by RAH_fA. These molecules were 205 KD, 190 KD, 149 KD, 118 KD, 103 KD, 98 KD, 64 KD and 33 KD (Fig 9 lane D).

Cross-reactive antigenic determinants: Cross-reactive components in F_gA that recognized by RAT_sA are 205 K 178 KD and 100 KD (Fig. 7 lane A), while those recognize by RAPA are 178 KD, 106 KD and 65 KD (Fig 8 lane A). PAH_fA identified four polypeptides of molecular weight 205 KD, 166 KD, 45 KD and 32 KD in the same antigen (Fig 9 lane A). Mean while RAF_gA recognized three bands in T_sA of molecular weight 191 KD, 166 KD and 132 KD (Fig. 6 lane B). In the same extract RAPA detected 3 components of 205 KD, 148 KD and 32 KD (Fig. 8

lane B). Furthermore, RAH_fA identified only two cross-reactive molecules in T_sA of molecular weight 205 KD and 166 KD (Fig. 9 lane B). PA cross-reactive determinants that identified by RAF_gA are 205 KD and 32 KD (Fig 6 lane C). While those recognized by RAT_sA are 205 KD, 149 KD and 106 KD (Fig. 7 lane C). Three polypeptides of PA were detected by PAH_fA of molecular weight 191 KD, 149 KD and 45 KD (Fig. 9 lane C). Two cross-reactive components of H_fA were recognized by RAF_gA, 205 KD and 177 KD (Fig. 6 lane D). Two cross-reactive polypeptides of H_fA identified by RAT_sA were 205 KD and 103 KD (Fig. 7 lane D). Finally, RAPA reacted with 3 polypeptides of 190 KD, 149 KD and 33 KD in H_fA (Fig. 8 lane D). These cross-reactive components were mainly responsible for the proved cross-reaction between the four antigens.

Discussion

This study proved cross-reaction between three economically important zoonotic helminthes belong to different phyla. This cross-reaction was fundamentally observed by ELISA in which antisera raised against one antigen cross-reacted with the other antigens. The specific reactions of F_gA, T_sA and PA to their homologous antisera were stronger than cross-reactions towards different antisera which are in accordance with that of Smith *et al.* (1983) who observed high reactivity of ascarid antigens with their respective antisera than heterologous ones. It was also agreed with the observations of Abdel-Rahman and Abdel-Megeed (2000) in a study concerning cross-reaction between *F. gigantica*, *T. vitulorum* and *M. expansa* using ELISA. However, the cross-reactive binding activities in RAF_gA and RAT_sA towards PA and RAPA towards H_fA were higher than their specific activities observed in the current research. This observation is in agreement with that of Cuêllar *et al.* (1995) who recorded intense corss-reaction between *Toxascaris leonina*, *Toxocara canis* and *A. suum* where heterologous assays recorded high values than homologous ones. This discrepancies between theses observations may be attributed to differences in the immunogenicity between specific and cross-reactive components.

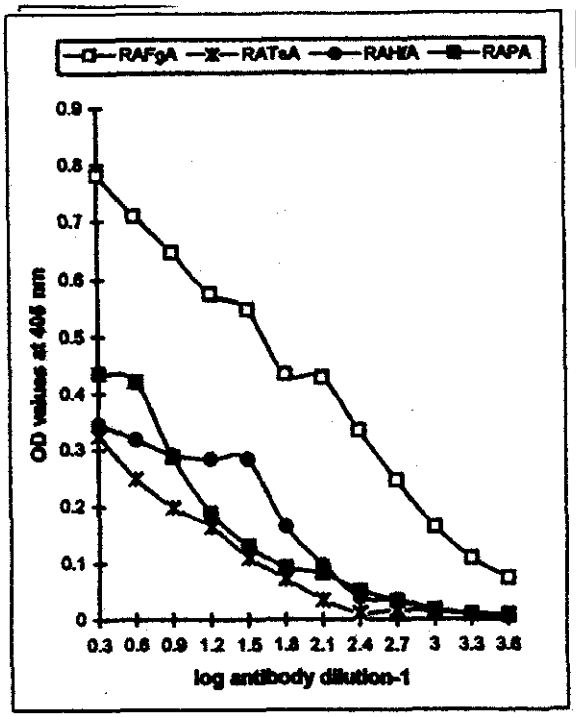

Fig. 1: The binding activities of RAF_gA, RAT_sA, RAPA and RAH_fA towards FgA

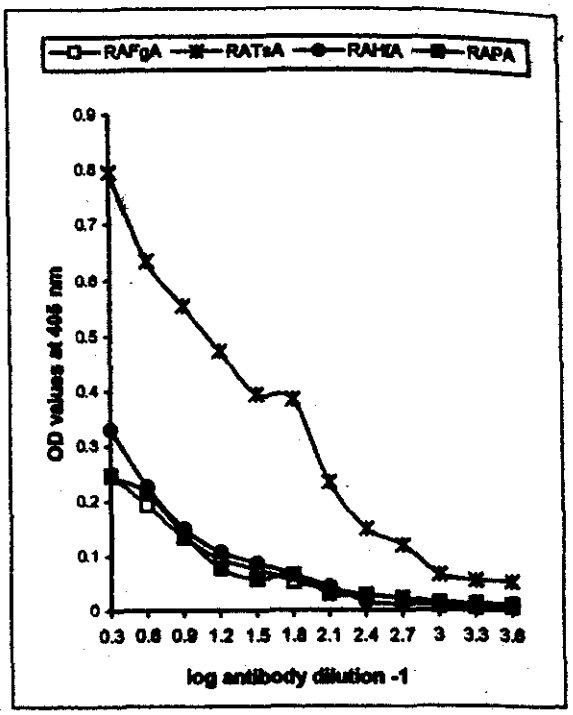

Fig. 2: The binding activities of RAF_gA, RAT_sA, RAPA and RAH_fA towards TsA.

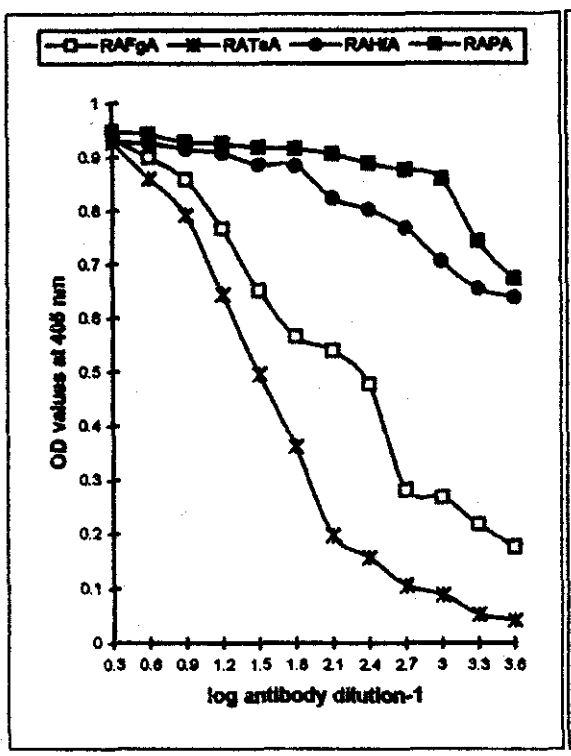

Fig. 3: The binding activities of RAF_gA, RAT_sA, RAPA and RAH_fA towards PA

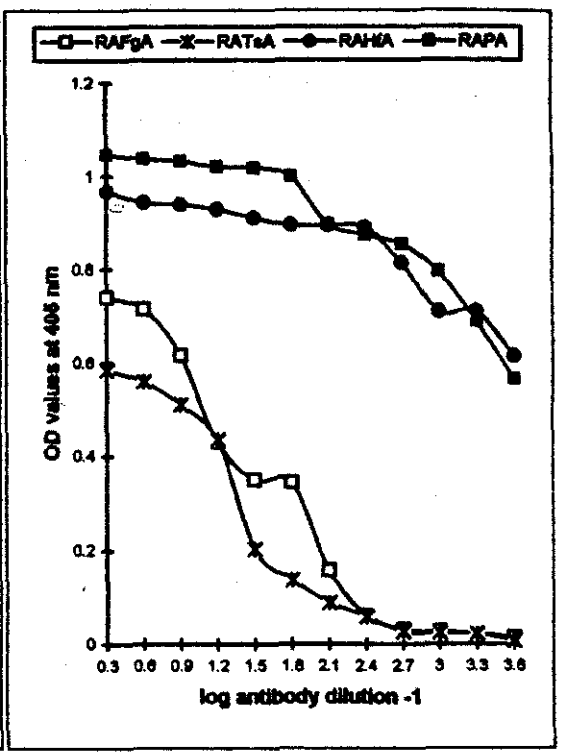

Fig. 4: The binding activities of RAF_gA, RAT_sA. RAPA and RAH_fA towards H_fA.

Fig. 3: The binding activities of RAF_gA, RAT_sA, RAPA and RAH_fA towards PA

Fig. 4: The binding activities of RAF_gA, RAT_sA, RAPA and RAH_fA towards H_fA.

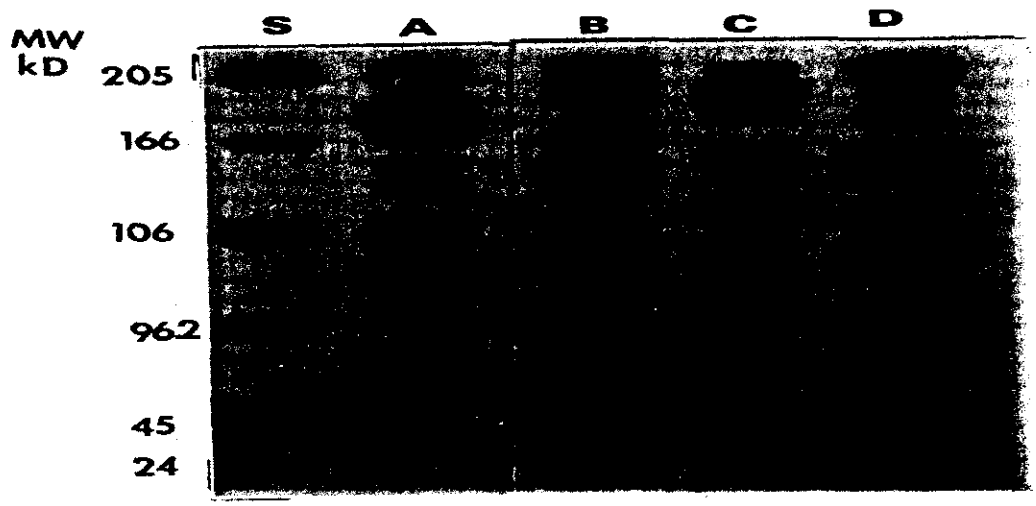

Fig. (5): Comparative SDS-PAGE pattern of four antigens. Lane A. F_gA, Lane B. T_sA, Lane C. PA and Lane D. H_fA. Molecular weight standards (Lane S).

In this report, the complex electrophoretic profile of the four antigens was described. This profile showed common components as 205 KD, 191 KD and 98 KD which possibly responsible for the proved cross-reaction. It also showed exclusive molecule to each antigen. Electrophoretic analysis was previously used to compare between protein composition of helminthes (Siles-Lucas and Bandera, 1996). The variations within the one species, or between different species of *E. granulosus* were probed by employing SDS-PAGE (Derbala, 1998). He introduced common as well as specific components of protoscoleces, hydatid fluids and mature worms of *E. granulosus* isolated from camel and equine origins. Furthermore, a comparative electrophoretic profile of four adult ascaridid nematodes was introduced by Ashour *et al.* (1995) using SDS-PAGE. This profile demonstrated 13 common bands among the four species in addition to species-specific bands. More recently a structural homology between eggs of *Toxocara vitulorum, F. gigantica* and *M. expansa* was proved by the use of SDS-PAGE (Abdel-Rahman *et al.*, 2000). Consequently, SDS-PAGE could be successfully utilized to determine common as well as specific components of helminth proteins. Identification of the components responsible for specific binding activities and cross-reactive ones was performed by immunoblotting. The immunoblotting assay revealed several cross-reactive components between the four antigens in addition

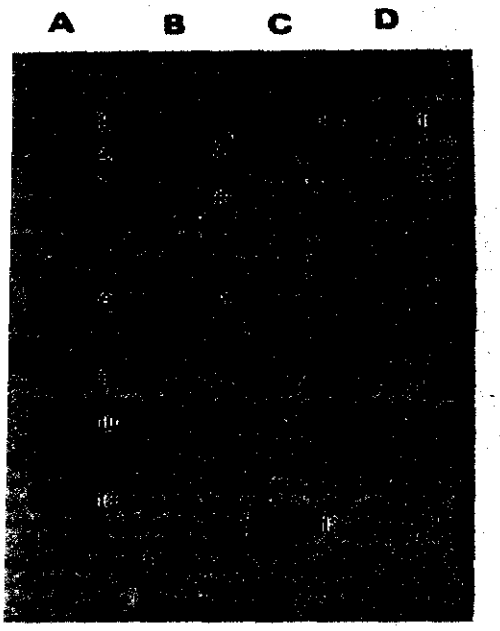

Fig. (6): Identification of antigenic targets recognized by RAF_gA in different extracts A: F_gA, B: T_sA, C: PA and D: H_fA.

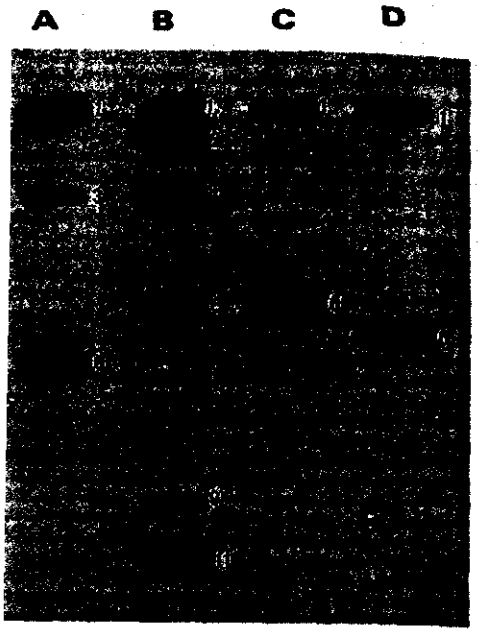

Fig. (7): Identification of antigenic targets Recognized by RAT_sA in different extracts A: F_gA, B: T_sA, C: PA and D: H_fA.

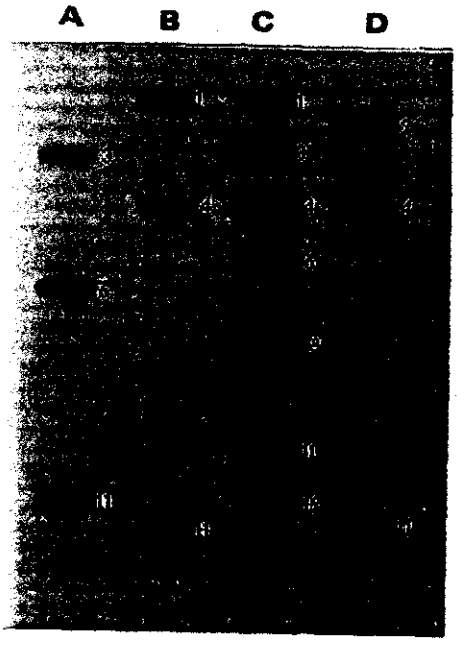

Fig. (8): Identification of antigenic targets recognized by RAPA in different extracts A: F_gA, B: T_sA, C: PA and D: H_fA.

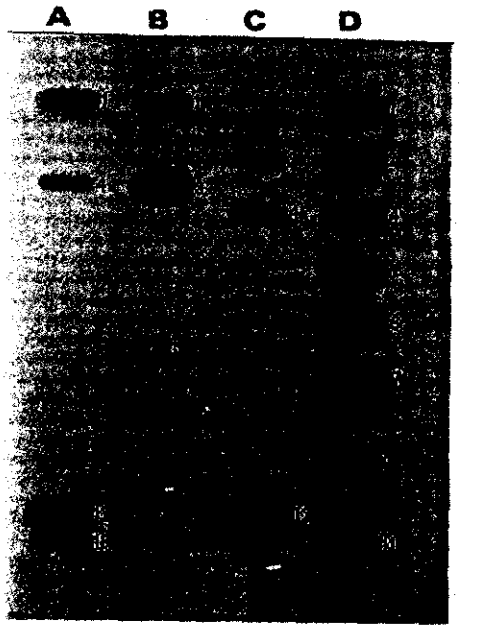

Fig. (9): Identification of antigenic targets Recognized by RAH_fA in different extracts A: F_gA, B: T_sA, C: PA and D: H_fA.

to those specific to each one. Of interest is a component of 205 KD which was identified in F_gA by RAF_gA, RAH_fA and RAT_sA and in T_sA by RAT_sA, RAH_fA and RAPA. Moreover the same component was recognized in PA by RAPA, RAF_gA, RAT_sA and in H_fA by RAH_fA, RAT_sA and RAF_gA. This broad immunogenicity of the molecule strongly supports its importance as a putative common vaccine candidates against the three helminthes. This hypothesis merits further investigations to evaluate its immunoprophylactic value. Abdel-Rahman and Abdel-Megeed (2000) identfied a cross-reactive component between *F. gigantica*, *T. vitulorum* and *M. expansa* mature flukes of molecular weight 210 KD. Besides, Abdel-Rahman *et al.* (2000) identified a cross-reactive component of 206 KD in the eggs of *F. gigantica*, *M. expansa* and *T. vitulorum* by different anti-adult antisera. These components have molecular weight close to the cross-reactive one of 205 KD described in the current study which adds to its value and encourage a strategy evaluating it as a multivalent vaccine. Another important molecule identified in the present research is that of 149 KD which was recognized in H_fA using RAH_fA and RAPA and also in PA employing RAPA, RAT_sA and RAH_fA. This component could be related to that of 143 KD recorded by Abdel-Rahman and Abdel-Megeed (2000) to be responsible for cross-reaction between *M. expansa* and *T. vitulorum* adult worms. Interestingly, 149 KD polypeptide was identified in PA and H_fA by the homologous antisera in addition to helerologous antisera which proved that a single immunogenic molecule might express both cross-reactive and higly specific epitopes (Aronstein *et al.*, 1986). A low molecular weight polypeptide of 32 KD was detected in F_gA by RAH_fA and in T_sA by RAPA and in PA by RAF_gA. Collectively, 205 KD, 149 KD and 32 KD components identified in the current research showed broad immunogenicity with the developed antisera and probably have an immunoprophylactic effect against the three infections.

In conclusion, the present study proved cross-reaction between three important zoonotic helminths and introduced 2 sets of polypeptides which are species-specific and cross-reactive. The broad immunogenicty of some molecules introduced in the current research raise the prospect of being

common immunoprophylactic candidates against multiple infections. Of relevance to this assumption is the work of El-Azzouni and Hegazi (1993) who utilized a tegumental antigen from *Fasciola* worm to protect animals against infection with *T. spiralis*. In addition to the observations of Hillyer (1985 and 1995) demonstrating that *F. hepatica* antigens which cross-reacted with *S. mansoni* conferred protection against challenge infection with *S. mansoni* cercariae in mice. While non-cross-reactive antigens did not exhibit that protection. Moreover, SDS-PAGE and western blotting analysis were employed to characterize a cross-protective component of *S. mansoni* (Sm_{14}) isolated from other different preparations of the same worm (Thaumaturgo *et al.*, 2001). Both assays were also utilized to characterize Sm_{14} related proteins in three different helminthes extracts, *A. suum, Echinostoma paracnsei* and *T. saginata* (Thaumaturgo *et al.*, 2002). The genomic sequence and the polymorphism of the same polypeptide (Sm_{14}) was characterized for the first time by Ramos *et al.* (2003). Investigations are ongoing to evaluate the protective value of some identified cross-reactive molecules against multiple helminth infections.

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