ELECTROPHORETIC CHARACTERIZATION OF THE ANTIGENIC PROPERTY OF FASCIOLA GIGANTICA AND PARAMPHISTOMUM MICROBOTHRIUM

N. M. El-Masry and S. A. Abo El-Kheir

Animal Health Research Institute (Mansoura Lab.)

ABSTRACT

Sodium dodecyl-Sulphate polyacrelamide gel electrophoresis (SDS-PAGE) is used for characterization of the antigenic properties of crude and excretory secretory (E/S) antigens of Fasciola gigantica and Paraphistomum microbothrium. The crude antigen of F. gigantica showed two specific bands while four bands are found specific for P. microbothrium. Six bands are found common beteween the crude antigen of both parasites. E/S antigen of F. gigantica showed one specific band migrating below 97 KDa M.wt., whereas E/S antigen of P. microbothrium revealed three specific bands of which one band below 14KDa and the other two bands migrating above 97KDa M.wt. four bands are found common among E/S antigen of both parasite species.

INTRODUCTION

Parasitic infestation, particularly with the helminth parasites, is considered one of the most debilitating factors leading to either direct or indirect economic losses in all animal species.

In endemic areas, faecal examinastion for detection of eggs is the most reliable method for diagnosis of such infestations. On the other hand, this method lacks the required sensitivity due to either mild infestation or relatively long prepatent period Hillyer et al (1985) and Fagbemi and Ouobadia (1995).

Nowadays, diagnosis of helminth infestation is directed towards the detection of parasitic antigens, either in the form of whole worm antigen (crude antigen) or circulating excretory-secretory antigen (E/S antigen). The later being of considerable interest because it has been shown to stimulate host protective immunity in several cases (Rivera Marrero, et al. 1988).

So the present work aimed to identify the common as well as the specific protein constituent of Fasciola gigantica and Paramphistomum microbothrium, the most common digenetic trematodes which cause sever losses in the animal performance. In this regard, the antigenic proper-

ties of E/S and crude antigens of both parasites are characterised using SDS-PAGE (Sodium dodecyl sulphate polyachrylamide gel electrophoresis).

MATERIAL AND METHODS

Fasciola gigantica and Paramphistomum microbothrium were obtained from naturally infested cows slaughtered at Mansoura abattoir and identified as described by **Soulsby (1982).**

I- Preparation of F. gigantica and P. microbothrium crude antigens :

Crude antigen was prepared according to **Voller et al (1976)** as follows:

Freshly collected adult worms were washed several times in 0.01 M.phosphate buffer saline (PBS) pH 7.4. The worms were then homogenized with PBS 7.4 until uniform suspension was obtained, centrifugated at 10000 r.p.m. for 30 minutes at 4°c and the supernatant collected and stored at 70°c till used. The protein concentration of this supernatant was determined according to **Bradford (1976).**

II- Preparation of excretory-secretory antigens (E/S) of Fasciola gigantica and Paramphistomum microbothrium :

Excretory secretory antigens were prepared according to **Rivera Marrero et al (1988)** as follow:

Intact adult worms of F. gigantica and P. microbothrium were obtained and washed several times in PBS pH 7.4 at room temperature. The worms incubated at 37°c for 3 hours one worm/ 5ml 0.01 MPBS pH 7.4 was used. After incubation the worms were removed and the supernatant fluid was collected and centrifuged at 10.000 r.p.m. for one hour at 4°c. The supernatant was separated and designated as E/S antigen. The protein concentration was measured and the antigens stored at -70°c until use.

III- Fractionation of different examined antigens by using Sodium dodecyl Sulphate SDS Poly-acrylamide gel electrophpresis (SDS-PAGE):

The examined antigens were analysed by (SDS-PAGE) according to **Laemmli (1970).**

RESULTS AND DISCUSSION

In this study analysis of E/S and crude antigens of Fasciola gigantica and Paramphistomum microbothrium by SDS-PAGE (Fig. 1 and Diagram 1) reaveled several polypeptide bands migrating between molecular weight just below 14 to above 97 KDa.

The protien bandig pattern of P. microbothrium E/S antigen showed seven different bands, of which three were at molecular weight of 21,31 and below 66KDa, two bands over 97 KDa, one band just above 45 KDa and one band below14 KDa.

The protein profile of P. microbothrium crude antigen reaveled a total number of 10 bands of which three were migrating at molecular weight above 97 KDa, one at 97 KDa, one below 97 KDa, one at 45 KDa, one above 31 KDa, one band migrating between 21 and 31 KDa, one at 21 KDa and the last band was at 14 KDa.

On the other hand, a total number of 5 protein bands were obtained during the analysis of F. gigantica E/S antigen. This protein banding pattern showed 3 bands migrating at molecular weights of 21,31 and 45 KDa, one band below 66 KDa and one band belw 97 KDa.

Analysis of F. gigantica crude antigen reaveled 8 polypeptide bands of which one band migrating above 97 KDa, one between 66 and 97 KDa, one at 66, one band at 45 KDa, one band between 31 and 45 KDa, one band just below 31 KDa and one band at molecular weight 21 and the other at 14 KDa.

Comparing the antigenic properties of the two helminthes, it was found that E/S antigen of P. microbothrium has 3 specific bands, one band below 14 KDa and the other 2 bands migrating above the molecular weight of 97 KDa whereas E/S antigen of F. gigantica showed one specific band migrating below 97 KDa. The remaining 4 bands were found common among E/S antigen of the two parasite species.

Regarding the crude antigen, P. microbothrium revealed four specific bands of which two bands migrating above 97 KDa, one band at 97 KDa and one band between 21 and 31 KDa. At the same time, only two specific bands were detected for F. gigantica crude antigen, of which one band at 66 KDa and the other band just below 31 KDa. The other six bands were found common between the crude antigen of both parasites

These results being coincided with **Hillyer and Serrano (1983)** who suggesteld the existence of a common antigen among the digenea. Also, **Aly (1993)** mentioned that there were several common protein bands in the different antigens.

The presence of specific bands for each parasite species agreed with **Santiago and Hillyer (1988)** who recorded specific bands at 13 -39 KDa for F. hepatica. **Mousa (1992)** found that the fraction of 12 - 39 KDa were useful for diagnosis of chronic fasciollasis. However, **Sahlab and Abdel-Aal (1998)** recorded that the polypeptide bands at 34.5, 48.5 and 105 KDa were appeared specific for Fasciola gigantica crude antigen while E/S antigen was detected at 14.5, 27.8 and cluster of bands at 56 3, 68 and 87.2KDa.

On the other hand, non-significant cross-reaction between Fasciola and Paramphistome parasites have been observed by **Larramendy and Pedroso (1984)** using specific antisera of cattle. The same observation has been detected by **Ashmawy et al. (1998)** between Fasciola gigantica and Gastrodiscus aegyptiacus on using quchterlony immuno-diffusion confirming the suggestion of **Hillyer and Serrano (1983)** about the existence of a common antigen among the Digenea.

Anyhow, in the present work, the detection of specific protein bands for each of crude and E/S antigens of Fasciola gigantica and Paramphistomum microbothrium, proved the diagnostic value of SDS-PAGE. It is recommended as an aid of diagnosis in order to avoid the cross-reactions and / or false positive results that may induced during the application of the serodiagnostic tests.

The specific antegenic bands of each parasite could be separated and used in an ELISA for developing sensitive diagnostic test to Fasciola gigantica and Paramphistomum microbothrium in infested animals.

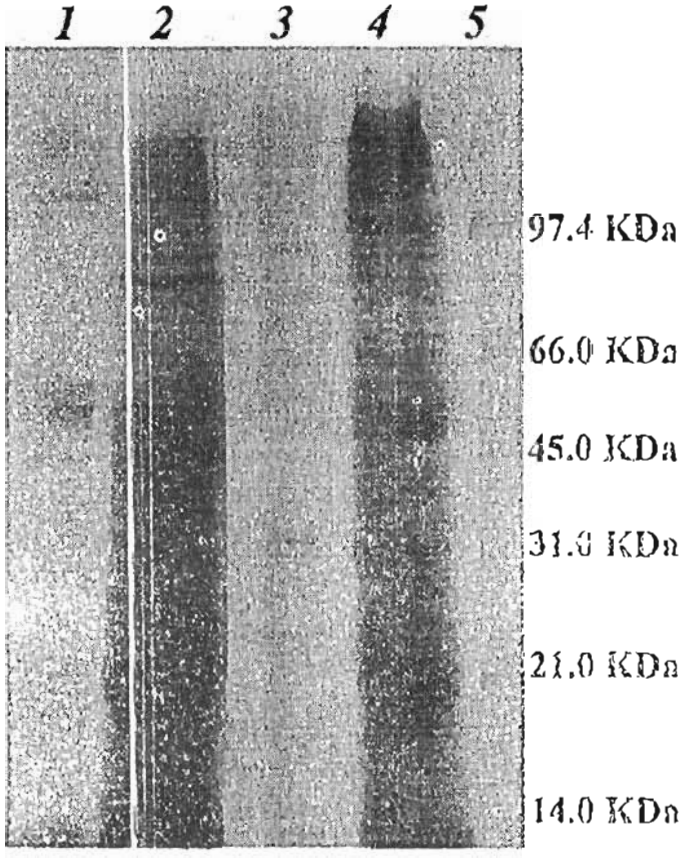

Fig. 1 : Protein Electrophoesis of Examind Antigen :

1- Paramphistomum microbothrium E/S antigen.

2- Paramphistomum microbothium crude antigen.

3. Fasciola gigantica E/S antigen

4. Fasciola gigantica crude antigen.

5. Standard molecular weight.

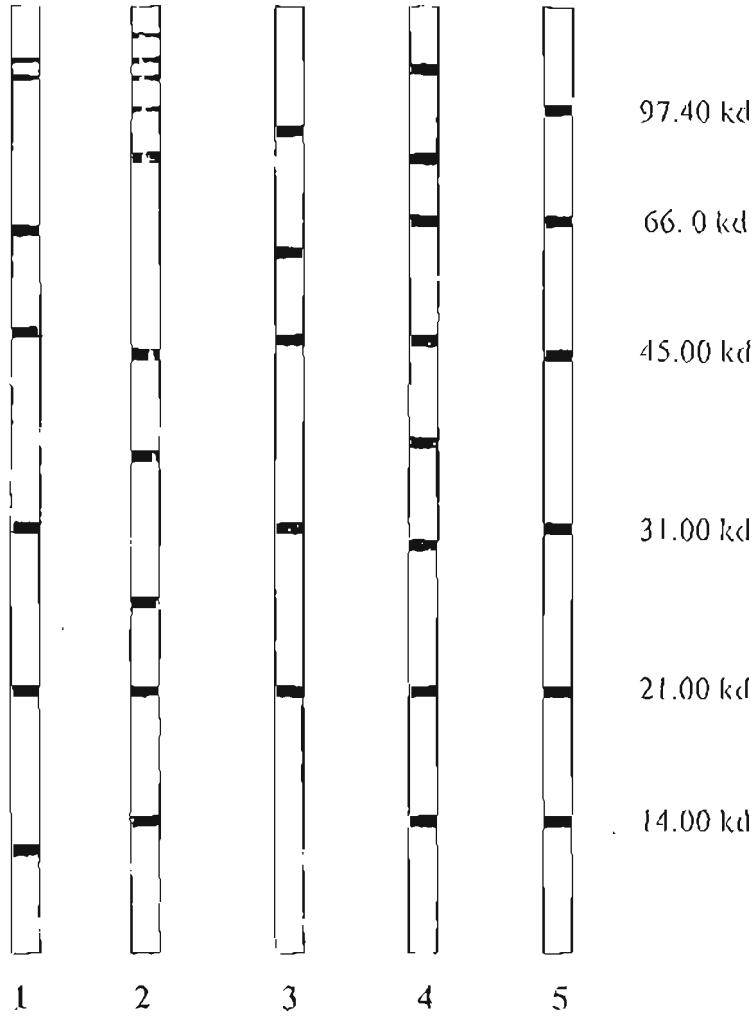

Diagram 1 : Electrophoesis pattern of examined antigens :

1. Paramphistomum microbothrium E/S antigen.
2. Paramphistomum microbothium crude antigen.
3. Fasciola gigantica E/S antigen.
4. Fasciola gigantica crude antigen.
5. Standard molecular weight.

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الملخص العربى

تحديد المواصفات الأنيجينة للدودة الكبدية والبارمفيستومم باستخدام التحليل الكهربى

المشتركون فى البحث

د/ نبيله محمود المصرى و د/ شعبان عبدربه أبوالخير

معهد بحوث صحة الحيوان - معمل المنصورة

فى هذه الدراسة تم إستخدام التحليل الكهربى فى البولى اكريلاميد جيل (SDS-PAGE) وذلك لتحليل الأنتيجينات المحضرة من الديدان الكاملة (Crude antigens) وكذلك الأنتيجينات المحضرة من التمثيل الغذائى (Excretory - Secretory) لكل من الديدان الكبدية وديدان البارامفيستومم.

وأثبتت الدراسة أنه على الرغم من وجود حزم بروتينية متماثلة فى أنتيجينات الديدان محل الدراسة إلا أنه توجد حزم بروتينية أخرى خاصة بكل نوع من الأنتيجينات لكل طفيل على حدة.

ففى الديدان الكبدية وجد عدد ٢ حزمة بروتينية خاصة بالأنتيجين المحضر من الدودة الكاملة بينما وجدت ٤ حزم بروتينية مختلفة خاصة بنفس الأنتيجين المحضر من ديدان البارمفيستومم الكاملة. ومن ناحية أخرى فإن الأنتيجين المحضر من التمثيل الغذائى للديدان الكبدية أثبت وجود حزمة بروتينية واحدة خاصة به بينما مثيله من ديدان البارمفيستومم أظهر ثلاثة حزم بروتينية خاصة به.

وقد خلصت الدراسة إلى التوصية بإمكانية إستخدام التحليل الكهربى فى البولى إكريلاميد جيل فى تشخيص الإصابة بهذه الطفيليات.