J Parasit Appl Anim Biol, Vol. 1 Number 1, January 1992, pp. 21-25

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BIOCHEMICAL CHARACTERIZATION OF SOME DIGENETIC TREMATODES USING PROTEIN PROFILES AS A PARAMETER

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MS received: 25 October 1991

ABSTRACT In order to unravel intraspecific strain variations, if any, electrophoretic study were performed using protein profile as a parameter on four species of trematodes, each originating from more than one host. The protein pattern of **Gastrothylax crumenifer** from buffalo, cattle and goat and **Explanatum explanatum**, **Homalogaster paloniae** and **Fasciola gigantica** of buffalo and cattle origin were found to be significantly distinct from one another and are suggestive of strain variations of the same species in different host types.

Key words: Biochemical Characterization, Digenea, Protein profiles

INTRODUCTION

Specimens of the same species collected from different host species often exhibit slight morphological differences amongst them. These differences are usually regarded as an expression of intraspecific variability (De Buron *et al*, 1986). However, variations at physiological and biochemical levels may not be expressed in morphological features at all (Bryant and Flockhart, 1986). Enzyme polymorphism and metabolism characteristics have proved to be of great use in recognition of intraspecific variants in the past few years. For systematic purposes, most polymorphism are detected for proteins in general or for specific enzyme activities among nematodes (Stockdale *et al*, 1974; Fenglin *et al*, 1980; Flockhart, 1982; Sood and Kapur, 1982) and cestodes (Bylund and Djupsund, 1977; LeRiche and Sewell, 1978 a&b; Bursey *et al*, 1980; Thompson, 1979; Macpherson and McManus, 1982).

Protein patterns using polyacrylamide gel electrophoresis (PAGE) technique have also been found to be of systematic value for various digenetic species of the genera *Schistosoma* (Yoshimura, 1968; Ruff *et al*, 1973; Fletcher *et al*, 1980), *Paragonimus* (Yoshimura, 1969 a&b; Yoshimura *et al*, 1969; Agatsuma and Suzuki, 1980a; Habe *et al*, 1983), *Fasciola* (Agatsuma and Suzuki, 1980b) and *Echinostoma* (Vasilev, 1984).

During the survey of digenean parasites from livestock in North- East India, some species were recovered from more than one host type. Many a time, morphological variations amongst the specimens of the same species, collected from cattle and other ruminant host, was evident. In order to ascertain their strain variations, if any, the present study was carried out on four such species, namely, *Gastrothylax crumenifer*, *Homalogaster paloniae, Explanatum explanatum* and *Fasciola gigantica* originating from different hosts. Electrophoretic profiles of proteins were used as a parameter for this purpose.

METHODOLOGY

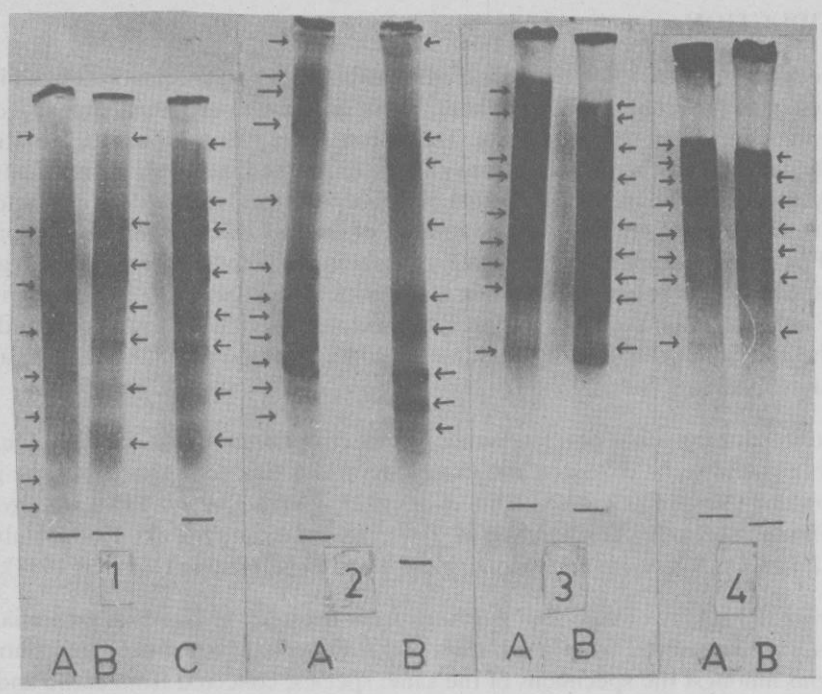
Mature specimens (uterus full of egg) of *G. crumenifer* were collected from buffaloes, cattle and goats, and those of *H. paloniae*, *E. explanatum* and *F. gigantica* were collected both from buffaloes and cattle in 0.9% saline solution and brought to laboratory. Extracts were prepared by homogenizing 0.5 g of worm in 5 ml of 1M phosphate buffer (pH 7.0) solution. The homogenate was centrifuged at 15,000 rpm for 30 min at 4° C. The clear supernatant was immediately used for protein estimation and disc gel electrophoresis. Total protein content of the extract was determined by the method of Lowry *et al* (1951), using Beckman's DV-26 Spectrophotometer.

Electrophoresis was performed on 7.5% polyacryalamide gels at 4° C, as described by Davis (1964). For analysis, 0.5 ml of extract containing approximately 50-60 μg of protein was layered on top of the gel and the apparatus was run at a current strength of 2 mA per gel tube for one hour using 0.025 M Tris-glycine (pH 8.3) buffer. Staining was done with 0.1% amidoblack solution. Relative mobility (Rm) valve was determined by the formula:

Rm = distance of protein band migrated/distance of indicator dye migrated

RESULTS AND DISCUSSION

The protein content per gram wet weight of the flukes originating from different animal hosts is presented in Table 1. In *G. crumenifer* from buffalo, 9 protein bands were



Comparison of disc electrophoresis patterns of protein extracts from *Gastrothylax crumenifer* (Fig.1), *Explanatum explanatum* (Fig.2) *Homalogaster paloniae* (Fig.3) and *Fasciola gigantica* (Fig.4) originating from different animal hosts (A: buffalo, B: cattle, C: goat)

observed (Fig. 1a), whereas the isolates of cattle and goat origin exhibited 7 and 8 bands respectively (Fig. 1 b-c). The central portion of gels in all the three cases had six bands, and each with identical mobilities and thickness for cattle and goat material; the mobility of these bands was found to be different in samples from buffalo (Table-2). The negative portion of the gel in the material of buffalo and cattle origin had only one band each, but that of goat origin had 2 bands. Two light bands observed near the positive terminal in gels for the buffalo material were not present in those of cattle or goat origin.

E. explanatum samples of buffalo and cattle origin had a protein comprising 12 and 9 bands respectively (Fig. 2a-b). The central portion of the gel for the former showed 5 dark bands and 2 light bands and the negative terminal portion had 5 bands; however, that of the latter had 4 dark bands and one light band in its central portion and only 4 near its negative terminal. The thick ness and mobility of most of the central bands were found to be similar in both samples. In *H. paloniae*, 9 protein bands were observed in samples from buffalo and cattle, with a single light band in each towards the positive terminal(Fig. 3 a-b) of the gels. However, the first 3 bands of the negative ends showed differences in their mobility and thickness. In *F. gigantica* the number of protein bands was 8 and 7 respectively for buffalo and cattle material. Excepting the first band nearing the negative terminal in the sample from buffalo, the banding pattern was similar with regard to thickness and mobility (Fig. 4 a-b).

The quantitative estimation of the protein content as carried out in the present study did not reveal a significant difference in *G. crumenifer* samples of buffalo, cattle and goat origins; however, a marked difference was seen in the case of *H. paloniae* and *F. gigantica* samples both from buffalo and cattle.

Considering dark bands on the gel as the prominent protein types, the difference between the protein profiles of the isolates of all these species from different hosts seems significant. In respect of *G. crumenifer*, while the isolates from buffalo showed a distinct dark fifth band, the corresponding band in those from cattle and goat was light and had a difference in mobility. In the case of E. explanatum, samples from cattle did not show any bands corresponding to the 2nd, 3rd, 5th and 7th dark bands as revealed in the material from buffalo. Likewise, the *H. paloniae* and *F. gigantica* isolates from buffalo had the dark first cathodal band, none corresponding to it was observed in the sample from cattle.

Qualitative and/or quantitative differences in protein profiles of samples of a species occurring in several different hosts are indicative of their being strain variants (Bryant and Flockhart, 1986). With the use of protein/enzyme polymorphism as a criterion, host-linked strain variations have been established in respect of several helminth species, eg. horse and sheep strains of *Echinococcus granulosus* (McManus and Smyth, 1978), hydatid cyst strains of sheep and cattle origin (Le Riche *et al.* 1982; Macpherson and McManus, 1982), and *Moniezia expansa* strains of cattle and sheep (Hermoso *et al.* 1982).

From a practical point of view, the total protein pattern seems to have an advantage over isoenzyme patterns, since freezing and thawing of worm tissue may cause a marked shift in enzyme band mobility (Ortec, 1973), whereas protein would not alter much during the process while shifting from field to laboratory. However, in order to authentically establish the differences in the protein pattern, it would be desirable to have densitometeric tracing curves of the gels.

ACKNOWLEDGEMENTS

This study was supported by a research grant from the North-Eastern Council, Govt. of India to VT. Laboratory facilities provided by the Head, Department of Zoology, NEHU are thankfully acknowledged.

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