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*Short communications*

# *Echinococcus multilocularis*: antigenic variance between different parasite isolates*

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Immunodiagnosis of alveolar echinococcosis in humans has been significantly improved by the use of purified *Echinococcus multilocularis* metacestode antigen fractions (Gottstein et al. 1983, 1986) or antigenic polypeptides (Gottstein 1985). Similar approaches aimed at obtaining an increase in specificity have been performed by Western blot visualization of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-resolved crude *E. multilocularis* metacestode antigens (Furuya et al. 1989) or excreted/secreted antigens produced by *E. multilocularis* protoscolices maintained in vitro (Auer et al. 1987). Müller et al. (1989) recently described a promising procedure for the bacterial synthesis of a recombinant *E. multilocularis* antigen that shows good immunodiagnostic operating characteristics and direct applicability in enzyme-linked immunosorbent assay (ELISA). Methods for the production or purification of *E. multilocularis* metacestode antigens all rely upon an initial parasite isolate that is usually derived from a naturally infected intermediate host (rodent or human patient) and subsequently maintained in laboratory rodents. Uniform and comparable immunodiagnostic results require the standardization of techniques and characterization of antigens. Techniques such as Em2 ELISA have previously been standardized (WHO 1988), including the generation of standard reference Em2 sera that are made available upon request (Jacquier and Gottstein, personal communication).

Generally, the quality of crude antigens, antigen fractions or antigenic polypeptides derived from *E. multilocularis* metacestodes may be affected by genetically based differences, including polymorphism related to different parasite isolates. To investigate such putative antigenic variance, we carried out a comparative immuno-analytical study testing five different *E. multilocularis* isolates for variation in the pattern shown by antigen components shared by *E. multilocularis* and *E. granulosus* (previously designated as the Em1 fraction; Gottstein et al. 1983). Such variation may result in different immunodiagnostic antigen (crude or fractionated) qualities, such as different degrees of cross-reactivity or sensitivity.

The five *E. multilocularis* isolates investigated in the present study were of following origins: CH1, CH6, CH10 and CH11 isolates, all initially isolated from Swiss human patients with hepatic alveolar echinococcosis; the B isolate was originally obtained from a naturally infected fox (*Vulpes vulpes*) in southern Germany by Prof. W. Frank and Dr. E. Zeyhle (University of Stuttgart-Hohenheim). All laboratory infections with metacestodes were established and subsequently maintained in jirds (*Meriones unguiculatus*) by serial passages and intraperitoneal transplantations (Eckert and Pohlenz 1976). Several months after transplantation, metacestodes were isolated from the peritoneal cavities of the infected animals at necropsy, freed of host tissue as much as possible, and further processed to obtain a crude antigen extract as previously described (Gottstein et al. 1983). All metacestode isolates showed protoscolex formation and macroscopically similar morphological structures. Parasite metacestode material obtained from 5–15 infected animals were pooled for each isolate prior to processing. The parasite tissues were recovered at sequentially different times of transplantation (i.e. different passages) per isolate.

All crude antigen extracts were fractionated by affinity chromatography in parallel runs for isolation of the Em1 fraction (Gottstein et al. 1983) using equal amounts of soluble parasite proteins and solid-phase (CNBr-activated Sepharose 4B) rabbit anti-*E. granulosus* (hydatid fluid) antibodies. By definition, the Em1 fractions of the five isolates individually contained all of the antigenic components that are shared by *E. multilocularis* and *E. granulosus* species. In a first step, the comparison of the five *E. multilocularis* isolates was done by resolving the soluble crude protein extracts using analytical isoelectric focussing (IEF) on thin-layer polyacrylamide

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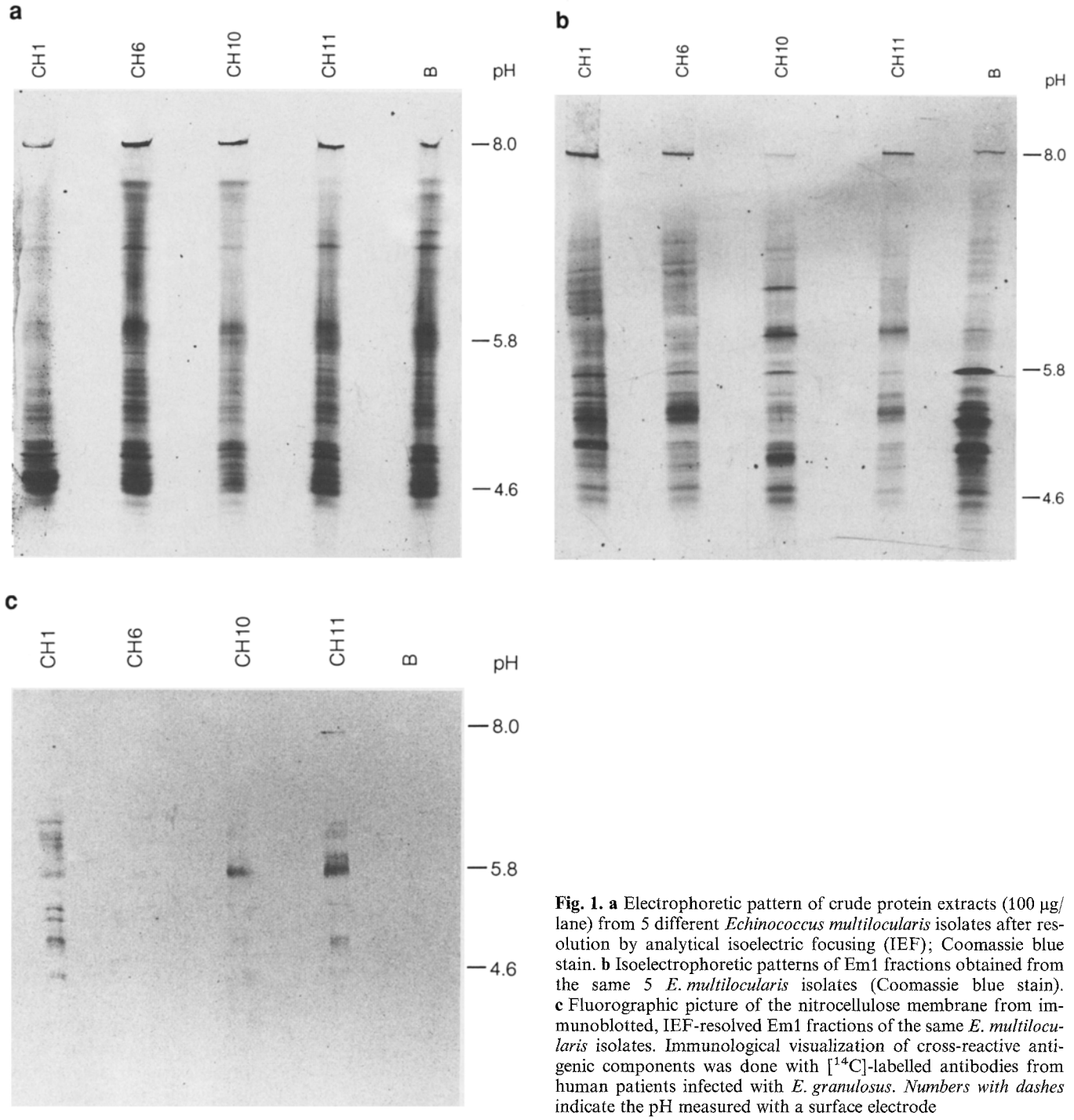


**Fig. 1. a** Electrophoretic pattern of crude protein extracts (100 µg/lane) from 5 different *Echinococcus multilocularis* isolates after resolution by analytical isoelectric focusing (IEF); Coomassie blue stain. **b** Isoelectrophoretic patterns of Em1 fractions obtained from the same 5 *E. multilocularis* isolates (Coomassie blue stain). **c** Fluorographic picture of the nitrocellulose membrane from immunoblotted, IEF-resolved Em1 fractions of the same *E. multilocularis* isolates. Immunological visualization of cross-reactive antigenic components was done with [$^{14}$C]-labelled antibodies from human patients infected with *E. granulosus*. *Numbers with dashes* indicate the pH measured with a surface electrode

gels as previously described (Gottstein 1985), followed by Coomassie blue staining. The comparative analysis of the Em1 fractions was carried out in the same way.

The results of these two investigations are shown in Fig. 1 (a, b). Crude antigen extracts of the five isolates demonstrated a relatively conserved homogeneity with regard to IEF-resolved and stained polypeptides (Fig. 1 a). The immunologically based fractionation of these crude protein extracts by affinity chromatography (solid-phase hyperimmune rabbit anti-*E. granulosus* antibodies) had resulted in a distinct variation between the protein-banding patterns of Em1 fractions from different isolates. Several of these differences may be related to quantitative differences in individual bands, although some isolates seemed to show a complete lack of individual bands. However, the most relevant approach to the determination of antigenic differences involved immunoblot analysis of IEF-resolved Em1 fractions that had been electrophoretically transferred to nitrocellulose according to Gottstein (1985). The subsequent immunological visualization of antigenic components shared by *E. multilocularis* and *E. granulosus* was

achieved by incubation of the nitrocellulose sheet with the same pool of serum (obtained from ten patients with clinically confirmed hepatic *E. granulosus* infection) that had previously been used for similar testing (Vogel et al. 1988). This serum pool was labelled with carbon 14 by reductive methylation according to the method of Jentoft and Dearborn (1979). The IEF bands indicating the binding of patients' antibodies in immunoblots were detected and visualized using enhanced (NEF-970; New England Nuclear, Boston, Mass., USA) fluorography on Kodak XAR-2 films (48 h exposure at −80° C).

Surprisingly, this investigation revealed a striking heterogeneity between the banding patterns of the different isolates (Fig. 1c). Two of the isolates (CH6 and B) obviously demonstrated the absence of antigenic proteins showing potential cross-reactivity with serum antibodies from human *E. granulosus* patients. On the other hand, the CH1 isolate showed the most complex banding pattern over the entire pH range used, and the CH10 and CH11 isolates had relatively few cross-reactive components. All IEF procedures were qualitatively confirmed by at least duplicate runs (data not shown).

From these results, we conclude that there is a polymorphism in antigen synthesis between different *E. multilocularis* isolates, shown in the present study by the lack of or variations in the patterns related to antigenic components potentially cross-reacting with sera from human patients infected with *E. granulosus*. We do not intend to draw any conclusions with regard to the biological mechanism underlying this variance, as multiple and complex effects such as host or other extrinsic factors may be involved. Rather, the practical significance of the reported variance becomes evident when crude antigen extracts or antigen fractions derived from different metacestode isolates are used for immunodiagnosis: different degrees of cross-reactivity prevent the standardization of tests and complicate the interpretation and comparability of test results, especially with respect to specificity criteria.

From a biological point of view, our observations confirm the occurrence of antigenic variance between *E. multilocularis* isolates previously described by Liance et al. (1984). The evidence for intraspecific variance in *E. granulosus* (Thompson and Lymbery 1988) rather suggests a putative similarity for *E. multilocularis*. Besides the extrinsic criteria that have been discussed for many years, intrinsic criteria such as those involving molecular biology have more recently been applied to demonstrate genetically restriction-fragment-length polymorphisms (RFLPs) among *E. multilocularis* isolates (Vogel et al. 1990). Future work combining multiple criteria and parameters such as morphology, biochemistry and pathology, among others (Thompson and Lymbery 1988), should prospectively help to determine taxonomically the extent of variation among *E. multilocularis* isolates or, eventually, among *E. multilocularis* strains or subspecies.

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