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Echinococcus granulosus antigens: Immunoelectrophoretic and Western blot analysis of hydatid cyst fluids

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For the serodiagnosis of echinococcosis (hydatidosis) in humans many different test systems and a variety of mostly crude, undefined antigens are presently used (Rickard and Lightowlers 1986; Schantz and Gottstein, 1986). In order to obtain uniform and comparable results, standardisation of techniques and characterisation of antigens are required. As fluids from hydatid cysts originating from cattle, camels and other intermediate hosts are employed as serodiagnostic antigens in various countries, a comparative immunoelectrophoretic analysis was carried out, supplemented by SDS-PAGE and Western blot analysis.

Hydatid cysts were collected at abattoirs from the following host animals: cattle and horse in Zürich, Switzerland; camel in Cairo, Egypt; sheep and cattle in Australia. Human hydatid fluid was obtained from a cyst excised from a Swiss patient. In Switzerland *Echinococcus granulosus* occuring in cattle and dogs has recently been identified as a distinct "cattle strain" with certain morphological and biological characteristics (Thompson et al. 1984). They include the nearly exclusive localisation of hydatid cysts in the lungs of cattle, a high fertility rate of these cysts of about 95% (Eckert 1981) and a pronounced antigenicity of the cyst fluid (Hess et al. 1974).

In camels, organ localisation of *E. granulosus* is similar to that in cattle, as the majority of the hydatid cysts are found in the lungs. According to various authors 68% to 86% of camels infected with larval *E. granulosus* harboured cysts in the lungs whereas cysts in the liver were much less frequent (20%–32%) (Hamdy et al. 1980; Abdel-Gawad et al. 1981; Saad et al. 1983). According to our own observations, about 50% of the pulmonary cysts in camels are fertile. The main localisation of hydatid cysts in horses slaughtered in Switzerland is the liver. The same applies to sheep and cattle in Australia although multiple infections of the liver and lung are also common. Differences in organ localisation may be an expression of intraspecific variation of *E. granulosus* (Thompson 1986).

After transportation to the laboratory the cysts were opened, the fluid was aspirated, cleared of protoscolices and other particles by sedimentation and frozen at −20° C until use. The following batches of hydatid fluid [HF] (each pooled from several cysts) were examined: those from fertile cattle lung cysts ($BoHF_f$) and those from fertile ($CaHF_f$) or sterile ($CaHF_s$) camel lung cysts. For comparison, the following hydatid fluids were additionally included in immunoelectrophoresis: those from fertile horse liver cysts ($HoHF_f$, Swiss origin), from fertile liver cysts of sheep ($ShHF_f$, Australian origin) and from a fertile human liver cyst ($HuHF_f$, Swiss origin). In addition, bovine fertile HF from Australia and camel fertile HF from CDC, Atlanta, USA were included in SDS-PAGE and Western blot investigations. The fluids were concentrated and dialysed against phosphate buffered saline at pH 7.2 with an Amicon ultrafiltration cell using a YM-10 membrane. The material was then lyophilized and reconstituted with distilled water to give a final protein concentration of 50 mg/ml.

Antiserum against *E. granulosus* was obtained by pooling 10 sera of Swiss human patients with clinically and serologically confirmed cystic echinococcosis. An antiserum against camel serum proteins was prepared by hyperimmunization of a rab-

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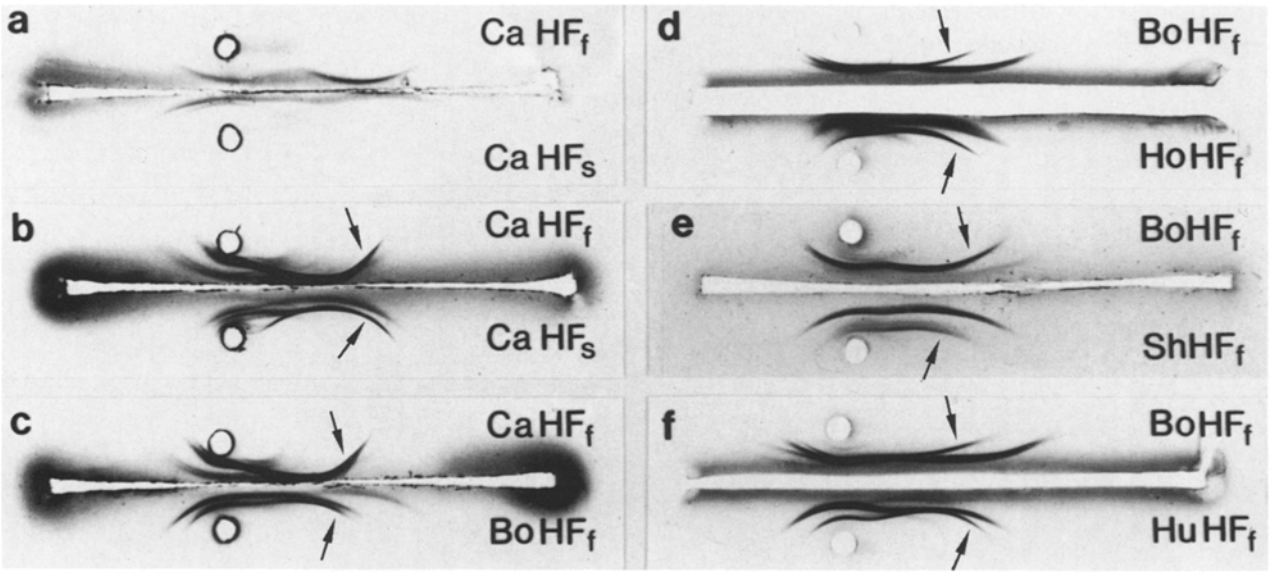

Fig. 1a–f. Results of immunoelectrophoresis. Hydatid fluid (*HF*) antigens (*s*, sterile; *f*, fertile) were placed in the circular-shaped wells. *Ca*, camel; *Bo*, bovine; *Sh*, sheep; *Ho*, horse; *Hu*, human origin. In the lateral troughs the following sera were placed: **a**, rabbit anti-camel-serum; **b–f**, human anti-*Echinococcus granulosus* (pool from 10 pre-operative hydatid cases). *Arrow*, arc 5 precipitation line

bit with 1 mg native camel serum per injection in Freund's complete adjuvant. Immunoelectrophoresis was carried out according to Varela-Diaz and Coltorti (1976).

The results of immunoelectrophoresis are demonstrated in Fig. 1 and Table 1. The reaction of camel hydatid antigens ($CaHF_f$ and $CaHF_s$) with anti-camel serum (from rabbit) resulted in 3 and 2 weak arcs respectively, indicating the minor importance of host components in this immune reaction (Fig. 1a).

When antigens from hydatid fluids of various origins were tested against anti-*E. granulosus* serum (from human patients) the following results were obtained: all antigens, irrespective of origin, produced arc 5 precipitation; within the same animal species the patterns of precipitated arcs were reproducible in different experiments (Fig. 1b, c; Fig. 1d, e and f). In precipitation patterns of hydatid antigens from the various hosts, minor differences could be recognized which were quite distinct between antigens of camel and cattle origin (Fig. 1c, Table 1).

SDS-PAGE, silver-staining and Western blot were performed exactly as previously described (Gottstein et al. 1986a). Figure 2 shows on the left hand side the silver-stained SDS-PAGE analysis of HF isolated from various hosts. A complex pattern of separated polypeptides over the complete range of relative molecular masses (Mr) demonstrated some obvious relationships between HF of the same host species origin. This has to be partially related to host or nonantigenic parasite components, due to the observation of a comparatively reduced number of antigenic bands demonstrated in Western blot (Fig. 2, right hand side). In this analysis, camel HF (a, b and c) exhibited the largest number of antigenic polypeptides, indicating a relatively high proportion of antigenic components in the whole HF protein compound. Camel HF from Cairo (a, b) and CDC/Atlanta (c) exhibited a similar antigenic polypeptide pattern. The patterns of camel HF (a–c) differed slightly from those of sheep HF (d) but could clearly be sepa-

Table 1. Number and type of precipitated arcs in immunoelectrophoresis (see Fig. 1)

Hydatid antigen origin	Human anti-*Echinococcus granulosus*	
	No. of arcs	arc 5
Camel: – $CaHF_f$	4	+
– $CaHF_s$	4	+
Cattle: $BoHF_f$	3	+
Horse: $HoHF_f$	3	+
Sheep: $ShHF_f$	3	+
Human: $HuHF_f$	3	+

Abbreviations of antigens: $CaHF_f$, fertile camel lung cysts; $CaHF_s$, sterile camel lung cysts; $BoHF_f$, fertile cattle lung cysts; $HoHF_f$, fertile horse liver cysts; $ShHF_f$, fertile sheep liver cysts; $HuHF_f$, fertile human liver cyst; +, present

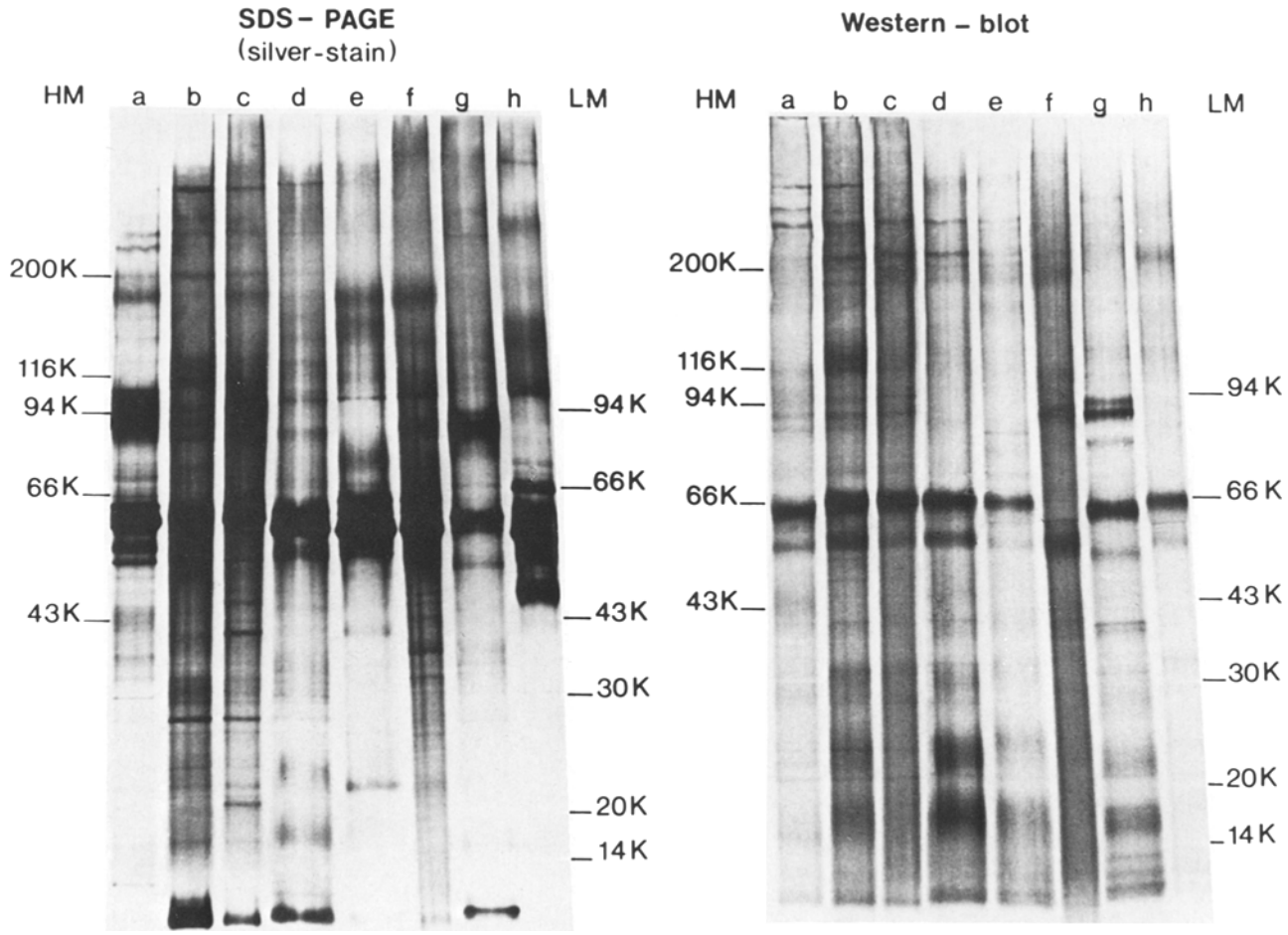

Fig. 2. Results of silver-stained SDS-PAGE and Western blot analysis. The following antigens were separated by SDS-PAGE: *lane a*, camel sterile HF from Cairo; *lane b*, camel fertile HF from Cairo; *lane c*, camel fertile HF from Atlanta; *lane d*, sheep fertile HF from Australia; *lane e*, bovine fertile HF from Australia; *lane f*, bovine fertile HF from Zürich; *lane g*, horse fertile HF from Zürich; *lane h*, human fertile HF from Zürich. For Western blot, the same human anti-*Echinococcus granulosus* serum pool was used as described in the legend of Fig. 1. *HM* and *LM*, high and low relative molecular masses

rated from bovine (e, f), horse (g) and human (h) HF. Between bovine HF from Zürich (f) and bovine HF from Australia (e) differences were discernible. The number of antigenic polypeptides in human HF (h) is much smaller than in camel HF. The reason for this is not known but might be found in the fact that the material was not pooled but isolated from a single cyst. No immunoreactive bands were found in control experiments with a pool of sera from healthy blood donors.

Based on these findings fluids of fertile and sterile hydatid cysts of camel origin were used as a source for an antigen prepared according to Farag et al. (1975) and employed in the enzyme-linked immunosorbent assay for routine serodiagnosis of human echinococcosis. The ELISA was performed according to the procedure described in Gottstein et al. (1986a). The sensitivity of the ELISA using $CaHF_f$ was determined to be 85% in hepatic (23 Swiss patients) and 50% in pulmonary (6 Swiss patients) cystic echinococcosis (Gottstein et al. 1986b). $CaHF_f$ and $BoHF_f$ were compared in ELISA using 17 patients with confirmed echinococcosis of the liver. Using a positive / doubtful / negative interpretation of the results, a correlation coefficient of $r = 0.7$ was obtained.

The findings of our study demonstrate that antigens derived from hydatid cysts of camel origin are of similar quality for routine serodiagnosis as antigens of Swiss bovine origin. In immunoelectrophoresis, all antigens derived from the various host species produced arc 5 precipitation. Using the same technique, some differences were observed between hydatid antigens derived from various host species. Western blot analysis in which nonantigenic host and parasite components are excluded

as contaminating factors revealed similarities between hydatid antigens from camels and sheep but antigens of cattle, horse and human origin were quite different. The antigenetic differences between the hydatid fluids of cattle origin from Switzerland (Zürich) and Australia agree with the finding that the Swiss and Australian cattle isolates also differ in morphological and biological features (Thompson et al. 1984). Interpretation of SDS-PAGE analysis was rendered more difficult due to a high number of contaminating nonantigenic components. Other studies (Pezzella et al. 1984) and our preliminary results can contribute to the characterisation of antigens of various sources used in serology but the biological significance of such results in relation to differences of *E. granulosus* strains has to be further evaluated. It appears that for extended studies, the employment of immunoelectrophoresis in combination with Western blot analysis under standardized conditions may be most promising.

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