Detection of the *Echinococcus granulosus* Diagnostic Arc 5 in Sera from Patients with Surgically-Confirmed *E. multilocularis* Infection

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**Summary.** The *Echinococcus granulosus* diagnostic arc 5 was revealed by sera from an Alaskan and a Swiss patient with surgically confirmed *E. multilocularis* infections. The possibility of each patient harboring a concurrent infection with both parasites may be disregarded on the basis of radiologic, scintillographic and surgical data. This conclusion is compatible with ecologic and epidemiologic considerations. These observations suggest that the immunoelectrophoresis test based on arc 5 positivity is not *E. granulosus*-specific as originally described.

**Introduction**

The only immunodiagnostic technique for human *Echinococcus granulosus* infection with which false positive results have not been obtained to date is the immunoelectrophoresis (IEP) test based on the arc 5 positivity criterion (Capron et al., 1967, 1970b; Quilici et al., 1971; Yarzábal et al., 1974, 1975; Varela-Díaz et al., 1975a, b). Evidence accumulated so far is thus in agreement with the original description of arc 5 as being specific to this metacestode. This conclusion was reached by Capron et al. (1967) on the basis of their findings on the comparative antigenic structure of parasites and the testing of human sera.

Antigen sharing between *E. granulosus* cyst fluid and cyst extracts of *E. multilocularis* has been reported (Capron et al., 1968, 1970; Kagan, 1968) and both antigens have been employed in immunological tests for the diagnosis of infections with the heterologous parasite (Bout et al., 1975; Capron et al., 1968; Hess et al., 1974; Kagan et al., 1968). Although serologic cross reactions between sera from persons harboring either metacestode have been demonstrated using each of these antigenic preparations, testing of sera from *E. multilocularis* patients has not resulted in the detection of the *E. granulosus* arc 5 (Bout et al., 1975; Capron et al., 1968, 1970).

The present study was conducted to clarify the potential value of the arc 5 IEP test-positivity criterion in the differential immunodiagnosis of *E. granulosus* infections in areas where these species are sympatric.

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Materials and Methods

The IEP test of Capron et al. (1967) was conducted as modified by Guisantes et al. (1975). The lyophilized sheep hydatid cyst fluid (HCF) antigen employed was processed and characterized by IEP with a sheep antiserum to ovine HCF as described previously (Varela-Díaz et al., 1974). The latter antisera was also employed as a reference reagent in IEP tests of patient's sera to determine reactions of antigenic identity (Varela-Díaz and Coltorti, 1974).

Indirect hemagglutination and indirect fluorescent antibody tests for hydatidosis were performed as described by Hess et al. (1974). Maximal titers observed in non-hydatid sera in IHA and IFA tests were 1:540 and 1:40, respectively. In sera from E. granulosus patients, maximal IHA and IFA titers of 1:393,660 and 1:540, respectively, were recorded under the technical conditions employed.

The latex agglutination test (Williams and Prezioso, 1970) was conducted using lyophilized HCF antigen as described earlier (Varela-Díaz and Coltorti, 1974; Varela-Díaz et al., 1975a). Agglutination at a 1:5 dilution of the serum was considered as a positive test result.

Sera from six persons with parasitologically-confirmed E. multilocularis infection were examined by the IEP test for hydatid disease. Details on each patient's history which were considered pertinent to the present study are as follows:

**Patient A.** Eskimo male born in 1932, resident of Kotzebue, Alaska. First admitted to a hospital at the age of 34 with a history of aching pain over the liver area, the patient had noted a mass in the right upper quadrant and reported to the hospital in Kotzebue. He gave a history of keeping a dog team while at Noatak and on at least one occasion cleaning the dog kennels which were very dusty. He was admitted to the Alaska Native Medical Center, at Anchorage, in 1967. Hepatic scintillography showed multiple defects in both lobes of the liver. On physical exam the liver margin was down well below the costal margin and felt nodular. He was considered non-resectable but was explored at that time to rule out resectability. The liver was found extensively involved with multiple large hard whitish tumor masses in all lobes and probably in all segments of the liver. E. multilocularis infection was diagnosed by histological biopsy at the time. The patient was followed conservatively for a number of years and was able to maintain work as a heavy laborer. There has been a gradual increase in the hepatic mass and on occasions he has developed mild jaundice. In July 1974, X-rays revealed a substernal mass. This was biopsied by mediastinoscopy and proved to be a metastatic E. multilocularis lesion. The serum sample included in the present study was obtained in the same year.

It should also be noted that the patient's mother (ca. 65 years old) was also found to be infected and the lesion was successfully removed by surgery.

**Patient B.** Swiss female born in 1932; resident of Zürich (CH). She was first admitted to a hospital in 1970 with a diagnosis of cholelithiasis. The patient was again hospitalized in September 1972 showing respiratory difficulties, chest pains and 40°C fever. On physical exam hepatomegaly, mainly of the left lobe, was observed and X-rays revealed irregular shadows in the liver due to calcifications. The serum sample was collected at this time.

Laparotomy was performed and a partial liver resection carried out. E. multilocularis infection of the left liver lobe was diagnosed by histological biopsy.

**Patient C.** Swiss male born in 1941; resident of the Schaffhausen area (CH). Hospitalized in 1972 with intrahepatic cholestasis which followed chlorpromazin treatment. He was again hospitalized in 1973 in poor general condition which included emaciation, icterus and hepatomegaly. Scintillographic studies revealed alterations in the left and right hepatic lobes; angiographic studies detected “spotted” areas of pathological processes particularly in the right lobe. Severe cholestasis was diagnosed by laparoscopy and E. multilocularis infection was confirmed by histological biopsy. The distribution of the lesions did not render surgical treatment feasible. The serum sample was collected in August 1973.

**Patient D.** Swiss male born in 1941; resident of Wald (CH). Hospitalized in 1975 with retentive icterus. Hepatic scintillography revealed a large dorso-lateral pathological process in the right liver lobe. An enlarged left hepatic lobe and a large “tumor” of the right liver lobe, with a whitish and localized hard “membrane” were detected by laparotomy. Resection of the right liver lobe was carried out and E. multilocularis infection was diagnosed by histological biopsy. Some of the small vesicles were found to contain protoscolices. The serum sample was collected at this time.

**Patient E.** Swiss male born in 1934; resident of Trin (CH). History of alcoholic abuse; hospitalized in 1970 and 1973 with icterus and fatty liver. General condition deteriorated in 1974 with anorexia, marked loss in body weight, hepatomegaly and increased icterus. Laparoscopy in 1975 revealed hepatomegaly with massive cholestasis, knotty tumour-like masses in left liver lobe and parts of pancreas; hepatogenous splenomegaly. The serum sample was collected at this time. E. multilocularis infection was diagnosed by histological liver biopsy. Surgery was not performed.

**Patient F.** Swiss male born in 1918, resident of Sulz-Rickenbach (CH). First complained of abdominal pain in February 1975 at which time a subfebrile non-icteric state was evident. Hospitalized in May 1975 with a diagnosis of cholecystitis. Serum samples were collected at this time.
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Fig. 1. Immunoelectrophoresis test results. Sheep hydatid cyst fluid antigen (*SHF*); reference sheep antiserum to ovine hydatid fluid (*SASHF*). Slide shows a reaction of antigenic identity between the arc 5 developed by the reference SASHF and that developed by the serum from an Alaskan patient with surgically confirmed *Echinococcus multilocularis* infection.

1975 in poor general condition with hepatomegaly and loss of body weight. Hepatic scintillography revealed a large pathological process in the right liver lobe. During laparotomy, a "tumor" with small vesicles was found in the right liver lobe. The latter was resected and an area of central necrosis, surrounded by tumor-like knotty masses, was observed macroscopically, *E. multilocularis* infection was diagnosed by histological biopsy of this material.

The serum sample included in the present study was collected four months after surgery.

Results

Data on serologic test results obtained in sera from six patients with confirmed *E. multilocularis* infections are shown in Table 1.

A precipitation band which was indistinguishable from the *E. granulosus* diagnostic arc 5 in electrophoretic mobility and morphological characteristics was detected in sera from patients A and B. In each case, an antigenic identity was demonstrated (Fig. 1) between this band and the arc 5 developed by the reference sheep antiserum to ovine HCF.

Discussion

In the present study sera from two patients with surgically confirmed *E. multilocularis* infection revealed a precipitation band in IEP tests which was indistinguishable from...
able from the *E. granulosus*-diagnostic arc 5 and produced a reaction of antigenic identity with the arc 5 developed by a reference sheep antiserum to ovine HCF. These observations suggest that the antigens responsible for arc 5 formation are shared by both species of *Echinococcus* and thus seem to be genus-specific, rather than species-specific for *E. granulosus* as originally described (Capron et al., 1967).

It is well known that not all hydatidosis patients are positive to the immunodiagnostic techniques applied to date for this purpose. In the case of *E. granulosus* infections, test sensitivity has been associated with the technique and positivity criterion employed (Varela-Diaz et al., 1975a, b) and with the physical condition of cysts (Capron et al., 1970a; Yarzabal et al., 1974; Coltorti and Varela-Diaz, 1977). Factors which may be associated with the induction of an antibody response in alveolar hydatidosis patients, however, have not been similarly studied. Since the central portion of the *E. multilocularis* metacestode progressively degenerates and the peripheral, proliferative layer invades the host tissues (Rausch, 1958), variations in the quality and/or quantity of antigens released during this growth process may be related to the detectability of antibody responses to this metacestode.

In any case, the observation of arc 5 in only two of six *E. multilocularis* sera in the present study and its absence from the sera of previously reported alveolar hydatidosis cases (Bout et al., 1975; Capron et al., 1968, 1970a) may be accounted for, at least partly, in terms of an insufficient degree of stimulation of the host immune system by arc 5 antigens in this metacestode. As such, these findings may be suggestive of a lower diagnostic sensitivity of the arc 5 IEP test in *E. multilocularis* patients than that observed in persons harboring cysts of *E. granulosus*.

Some cases of persons with concurrent *E. granulosus* and *E. multilocularis* infections have been reported (Nadjgieriev and Kochegarov, 1962; Posselt, 1928, 1931; Pampiglione et al., 1968; Semenov, 1954). Such mixed infections, however, have not been recorded in Alaska (Rausch, 1967; Wilson et al., 1968), Switzerland (Schmid, 1958; Drolshammer et al., 1973) or Southern Germany (Schicker, 1976). The possibility that both arc 5-positive alveolar hydatidosis patients in the present study may have been harboring a concurrent *E. granulosus* infection was thus contemplated.

Patient B has been a resident of Zürich and both *Echinococcus* species are known to be responsible for human infection in this Canton (Drolshammer et al., 1973). No evidence to this effect, however, was available on the basis of radiological, scintillographic and surgical data on this patient.

Patient A has been a life-long resident of the region of Kotzebue, situated on the Baldwin Peninsula on the western coast of Alaska. The area is in the zone of tundra, where *E. multilocularis* is enzootic (Rausch, 1958, 1960, 1967; Fay, 1973). Of the 19 alveolar hydatidosis cases for which information is available to one of the writers (R.L.R.) five originated in Kotzebue, thus making it second only to St. Lawrence Island in the number of known cases in Alaska (Rausch, unpublished data).

In contrast, most of the surgically-confirmed cases of *E. granulosus* infection in Alaska have originated in the forested areas of the interior (Rausch, 1967; Wilson et al., 1968). There is a possibility, however, that this metacestode also occurs in Kotzebue. In a survey of helminths of dogs in western-southwestern Alaska, including Kotzebue to the north, this parasite was found in only two of 97 animals examined at autopsy (Rausch, unpublished data). In the case of Patient A, however, no evidence of a concurrent *E. granulosus* infection was obtained from radiologic, scintillographic and surgical data (J.F. Wilson, personal communication).
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