Dear Dr. Daly,

Enclosed please find a final report on PSTC Project No. 6.132 "Development and Characterization of monoclonal antibodies to hydatid disease antigens", which has been completed December 1989.

Thank you.

Yours sincerely,

Sami K. Abdel-Hafez, Ph.D.
Professor

c.c. Dean of Research and Graduate Studies, Yarmouk University.

Project Title: Development and Characterization of Monoclonal Antibodies to Hydatid Disease Antigens.

Principal Investigator: Professor Sami K. Abdel-Hafiez.
Co-investigator: Dr. Fadwa M. Al-Yaman.

I. Background:

The project started December 1986 and was completed December 1989. The following were the objectives of the project:

1. Prepare hydatid antigenic material from human and domestic animal sources as well as from in vitro cultured stages (excretory/secretory antigens).

2. Produce monoclonal antibodies (MCA) against some of these antigens.

3. Screen the MCA produced against a battery of antigens, and select 'relevant' ones for potential of utilization in serodiagnosis and isolation of appropriate antigens.

4. Use of selected MCA in seroepidemiological situations and for the detection of E/S antigens in sera of humans and domestic animals infected with the hydatid cysts.

II. Work Carried Out:

To fulfil these objectives we have carried out the following research work:

1. Preparation of antigenic material from hydatid cyst fluid, protoscoleces, and protoscolacces tegument as well as in vitro cultured stages at various phases of cultures.
2. Establishment of *in vitro* culture of *Echinococcus* up to the adult sexual stage. These cultures were initiated from sheep, donkey and human hydatid protoscoleces. Differences in the behaviour and success of *in vitro* cultures from the different sources were noted. These studies helped in the strain identification of *E. granulosus* in Jordan and in the preparation of *in vitro* derived antigens.

3. Excretory/secretory antigens were prepared from protoscoleces of sheep hydatid cysts which were *in vitro* cultured up to 35 days. Characterization of these antigens was carried out using SDS-PAGE and immunoblot techniques and was found to contain many protein and glycoprotein fractions.

4. Monoclonal antibody production against protoscoleces tegumental antigenic extract was carried out using immunized Balb/c mice as spleen cell donors. Three cell lines were expanded and characterized. The specificity of these MCA's was studied using a battery of antigenic preparations from different parasites. Under non-reducing conditions immunoblot analysis showed that one of the prepared MCA's identified a 31 kDa single band while the other two MCA identified 2 or 3 bands simultaneously. Under reducing conditions, the 1st MCA identified a <14.4 kDa single band while the other 2 MCA identified many small Mr bands. The respective antigens for each of these 3 MCA's were affinity purified but were not found to be *E. granulosus* specific. Affinity purified antigens with these MCA were tested for possible use in serodiagnosis. The greatest sensitivity (81.3% of positive cases) was obtained using affinity purified antigens by 5B4G7 MCA. The epitopes recognized by one of these 3 MCA were of protein nature (in 2 MCA) and glycoprotein nature (1 MCA).

5. Monoclonal antibody production against a purified antigen from crude sheep hydatid fluid. The antigen used was a 50-100 kDa fraction. Immunized Balb/c mice were used as spleen cell donors. Five MCA's whose isotypes were IgG1(2), IgG2b(1) and IgM(2) were characterized. Two of these MCA's were *E. granulosus* specific, one was taenia specific and two were crossreactive with *Fasciola hepatica*, and *Dictyocaulus floridus* antigens. The epitopes recognized by these MCA's appeared to be non-carbohydrate in nature.
III. Publications Supported by Grant USAID 6.132:


IV. Publications Submitted or in Preparation:


V. **Titles of Thesis Work Supported by the Grant:**

These include thesis work which utilized equipment and facilities made possible through this grant.

<table>
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<tr>
<th>Title</th>
<th>Graduate Student</th>
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<td>3 Isolation and characterization of excretory/secretory antigens from various <em>in vitro</em> developmental stages of <em>Echinococcus granulosus</em>.</td>
<td>Ayman S. Hussein</td>
<td>Sept. 1990</td>
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<td>5 Studies on the <em>in vitro</em> culturing of <em>Echinococcus granulosus</em> from sheep and donkey cysts in Jordan.</td>
<td>Nawal S. Hijjawi</td>
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<td>7 Histopathological, cellular and immunological changes in the spleen of sheep and mice infected with hydatid cysts of <em>Echinococcus granulosus</em>.</td>
<td>Siddieg A. Rahoud</td>
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