Hydatid disease is caused by the larval stage of Echinococcus granulosus, which can establish itself in a wide range of intermediate hosts including cattle, sheep, pigs, horses and humans. These hosts become infected by ingestion of eggs in contaminated food or water (Breijo et al., 1998).

Protoscolecies have a dual potential for development; on one hand, they are the infective stage of the adult parasite in the definitive host, on the other hand, they are capable of reversibly developing into cysts in the intermediate host when released by accidental rupture of the mother cyst (secondary hydatidosis) (Lightowers, 1990).

The first attempts of in vitro cultivation of E. granulosus protoscolecies were made by Dévé (1901, 1902). However these assays were unsuccessful because protoscolecies died after 28 days in in vitro conditions. The first successful in vitro cultivation in a cystic direction was reported by Smyth (1962). Since then, a significant progress was made using a variety of media and conditions (Benex, 1968; Brudnjak et al., 1970; Heath & Osborn, 1976; Rogan & Richards, 1986; Casado et al., 1986; Rodriguez-Caabeiro & Casado, 1988; Casado & Rodriguez-Caabeiro, 1988a & b).

Rodriguez-Caabeiro & Casado (1988) worked with hydatid cysts of sheep origin. They observed microcysts formation after 15 days. Ponce Gordo & Cuesta Bandera (1997) worked with cysts of sheep, cattle, horse, pig and human origin. Working with this material, they found differences not only in the time of microcysts formation, but also in the development process. They obtained microcysts from protoscolecies of sheep, pig, horse and human origin. The development to microcysts could not be achieved with material of cattle origin. Their initial development was normal but in no case microcysts finally formed.

The in vitro cultivation of E. granulosus protoscolecies allows to obtain information applicable to the hydatid disease control programmes and in biochemical, immunological and treatment studies (Casado et al., 1986).

The aim of this work was the achievement of microcysts formation from protoscolecies of E. granulosus of cattle origin using the in vitro vesicular culture technique.
Fig. 1. - Protoscoleces obtained of lung bovine cysts. The typical shape of protoscoleces is observed, hooks (H) and calcareus corpuscles (CC). Dead stained protoscolece (white arrow). Methylene blue exclusion test 1:10.000.

Fig. 2. - Evaginated protoscolece.

Fig. 3. - Vesiculated protoscolece. An evaginated protoescolece showing a posterior bladder is observed (arrow).

Fig. 4. - Vesiculating protoscolece (three days of culture). Note the increment in the size.

Fig. 5. - Microcyst showing laminated layer (20 days of culture) (arrow).

Fig. 6. - Microcyst completely developed (48 days of culture).
MATERIALS AND METHODS

Hydatid cysts from naturally infected bovine lungs and livers were obtained from two abattoirs located in the southeast of Buenos Aires province, Argentina. Protoscoleces were removed from cysts by asptic techniques and washed several times with phosphate-buffered saline (PBS) pH 7.2. Viability was assessed by the methylene blue exclusion test (Casado et al., 1986) or by muscular movements, morphological perfection of the whole body, and motility of flame cells. Groups of 1,500 viable and free protoscoleces were transferred to Leighton tubes, one to five replicates per sample, depending on quantity of available protoscoleces. The culture medium was 199 (Gibco) containing 100 IU penicillin, 100 µg/ml streptomycin, 4 mg/ml glucose and 20 % (v/v) foetal calf serum. Cultures were maintained at 37°C and the medium was changed every three-four days. A total of 10 samples (seven from lung and three from liver) was cultured. Development was followed microscopically every day. Development time until cysts was determined in days. The data recorded for each sample correspond to the moment when a stage was reached by the most advanced individuals of the culture.

RESULTS

At the beginning of the culture protoscoleces were invaginated (Fig. 1). After 48-72 hours of incubation some evaginated protoscoleces could be observed (Fig. 2). The amount of them is variable in the different cultures, between 5 and 20 %, but it never reached 100. Vesiculated protoscoleces and protoscoleces with posterior bladders (Figs 3 & 4) appeared during the first week of incubation. Moreover, a considerable increment in the size of them could be observed. After 14 days of culture, a laminated layer appeared like a fine membrane in one of the extremes of the protoscoleces. Seventy percent of all samples reached the microcyst formation (57.1 % of lung and 100 % of liver samples). On day 20, some microcysts with a complete laminated layer were observed (Fig. 5). Some samples showed microcyst formation between 24-38 days. By day 48, microcysts completely developed could be observed (Fig. 6). In all cultures, the total number of microcyst formed was approximately 1.6 % of the number of initial cultured protoscoleces. Some cultures could be maintained until day 95.

DISCUSSION

This is the first study where microcysts formation was obtained using protoscoleces of E. granulosus of cattle origin. As Smyth described (1962), we found two patterns of cystic development in vitro. In the first, some protoscoleces became swollen or vesicular within a few days of culture and grew into thin walled cysts. In the non-vesicular pattern of development, the protoscoleces each develop a posterior bladder which increased substantially in size; eventually a spherical cyst formed and this became enveloped by a laminated layer. Such cysts were morphologically indistinguishable from cysts that developed from vesicular protoscoleces. This also coincides with the works done by Casado & Rodríguez-Caabeiro (1988a) and Ponce Gordo & Cuesta Bandera (1997).

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