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| Author(s)        | KAMIYA, Masao; OOI, Hong Kean; OKU, Yuzaburo et al.                                       |
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## ISOLATION OF *ECHINOCOCCUS MULTILOCCULARIS* FROM THE LIVER OF SWINE IN HOKKAIDO, JAPAN

Masao KAMIYA<sup>1</sup>, Hong Kean Ooi<sup>1,2</sup>, Yuzaburo OKU<sup>1</sup>,  
Munehiro OKAMOTO<sup>1</sup>, Masashi OHBAYASHI<sup>1</sup> and Naoki SEKI<sup>3</sup>

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Cysts from the liver of swine, naturally infected with *Echinococcus* in Hokkaido, were squashed finely, then injected into the peritoneal cavities of the Mongolian gerbils. Six months later, although no protoscoleces were observed in the cysts from the swine, well-developed cysts bearing mature protoscoleces of *E. multilocularis* were obtained from the injected gerbils. This observation showed that although *E. multilocularis* cysts in the liver of swine are sterile (i. e., lacking protoscoleces), they are viable and retain their ability to develop protoscoleces in the favourable rodent host.

Key words: *Echinococcus multilocularis*, sterile hydatid cyst, swine, isolation, Mongolian gerbil, Japan

### INTRODUCTION

Following the report by SAKUI et al. (1984), that the hepatic lesions in swine examined at the Higashimokoto Meat Inspection Laboratory, Hokkaido, Japan, between December 1982 and July 1983, were histologically identified as those of *Echinococcus multilocularis*, swine multilocular echinococcosis had been increasingly reported in various areas in Hokkaido (KAWAMOTO et al., 1985; SENUMA et al., 1986). In November 1986, 71 districts or one-third of the whole area of Hokkaido were declared to be endemic for *E. multilocularis* by the Hokkaido prefectural government.

Since the multilocular echinococcal cysts recovered from the liver of swine contained neither protoscoleces nor brood capsules, the swine have been thought to be a dead-end host of the parasite and play no role in the transmission of the cestode. Swine echinococcosis can easily be detected because of the existence of a meat inspection system in Japan in which the individual animal is examined by veterinarians. This, along with the detection of *E. multilocularis* in humans, voles, and carnivores such as foxes and dogs, can be used as an epidemiological indicator to pinpoint the

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<sup>1</sup>) Department of Parasitology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

<sup>2</sup>) Hokkaido Veterinary Center, Sapporo.

<sup>3</sup>) Department of Public Health, Hokkaido Prefectural Government

spread of the parasite in Hokkaido.

However, the biological characteristics of *E. multilocularis* in the swine have not yet been elucidated. Thus, it is desirable to isolate the parasite from the swine for further studies in the laboratory. We report herein the isolation of *E. multilocularis* from the hepatic lesion of swine and its successful passage in the laboratory by intraperitoneal injection of the parasite tissue into the Mongolian gerbil.

#### MATERIALS AND METHODS

Hepatic lesions were observed in a 6-month-old Landrace F<sub>1</sub> female slaughtered in March 1986 at the Kitami Meat Inspection Center, Hokkaido, Japan. Thirty-three lesions, i.e., 11 on the left lobe, 6 on the right lobe and 16 on the quadrate lobe were detected in the respective parts of the liver. These lesions were actually cysts ranging from 5 to 10 mm in diameter. The liver was stored with ice cubes and then sent to Sapporo. On the following day, a portion of the cysts was fixed in 10% formalin for histological examination and the rest was finely cut with scissors, squashed through a wire gauze into phosphate buffered saline and the resulting parasite tissue suspension was made up to a 15 ml solution. Six female laboratory-reared Mongolian gerbils, *Meriones unguiculatus*, aged 10 to 12 weeks old, were each inoculated intraperitoneally with 1 ml of the parasite tissue solution. No proto-scolecemes were observed in the parasite tissue solution.

All the six gerbils were killed 6 months after the intraperitoneal inoculation. A part of the echinococcal cysts recovered from the gerbils was fixed in 10% formalin for histological examination and the rest of the cysts was used for the second passage of the parasite in the gerbils. Formalin-fixed cysts were dehydrated in an alcohol series, embedded in paraffin, sectioned at 4  $\mu$ m and then stained with hematoxylin-eosin (HE) or with periodic acid-Schiff (PAS).

#### RESULTS

Intact cysts consisting of PAS-positive laminated cyst wall were observed in the section of the hepatic lesion of the swine (Fig. 1). Germinal cells were also seen on the inner surface of the cyst wall. However, regressive foci of the cyst were also observed (Fig. 2). Nevertheless, neither brood capsules, protoscolecemes nor calcareous corpuscles were observed, and strong cellular response of the host around the cyst was evident in the form of granuloma.

Two of the 6 parasite tissue-inoculated gerbils were found to be infected with *E. multilocularis*. The *E. multilocularis* cyst from the abdominal cavity of the gerbil macroscopically resembles the roes of codfish (Fig. 3). Cysts with a maximum diameter of 2 cm and weighing a total of 11 g, were recovered from the serous surface of the soft organs in the thoracic and abdominal cavities of one of the two infected gerbils. In the other infected gerbil, cysts with a maximum diameter of 3 cm

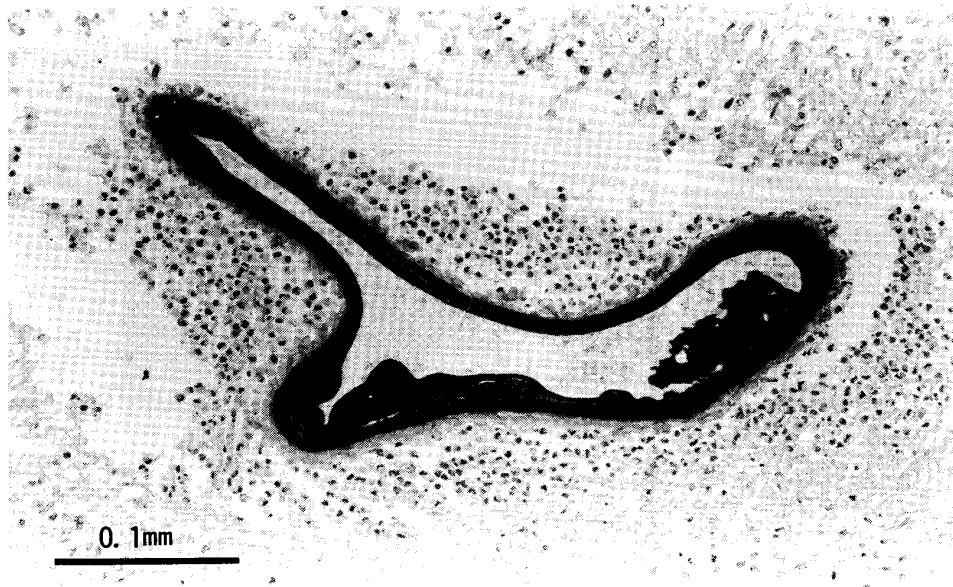


FIGURE 1 Sterile cyst of *E. multilocularis* in hepatic lesion of swine. PAS stain.

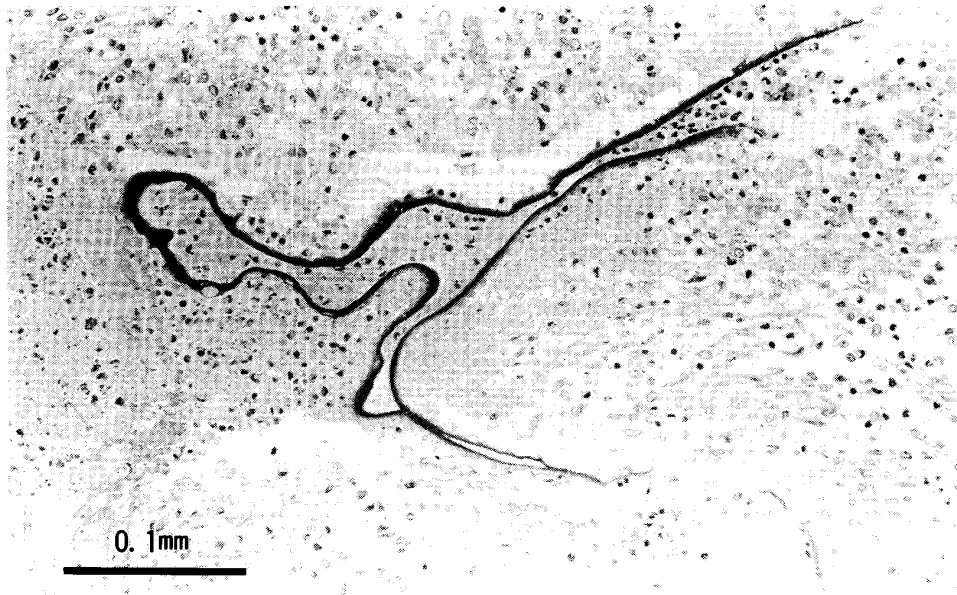


FIGURE. 2 Regressive cyst of *E. multilocularis* in hepatic lesion of swine. Note the disruption of cuticular layer and the infiltration of leucocytes into the cyst. PAS stain.

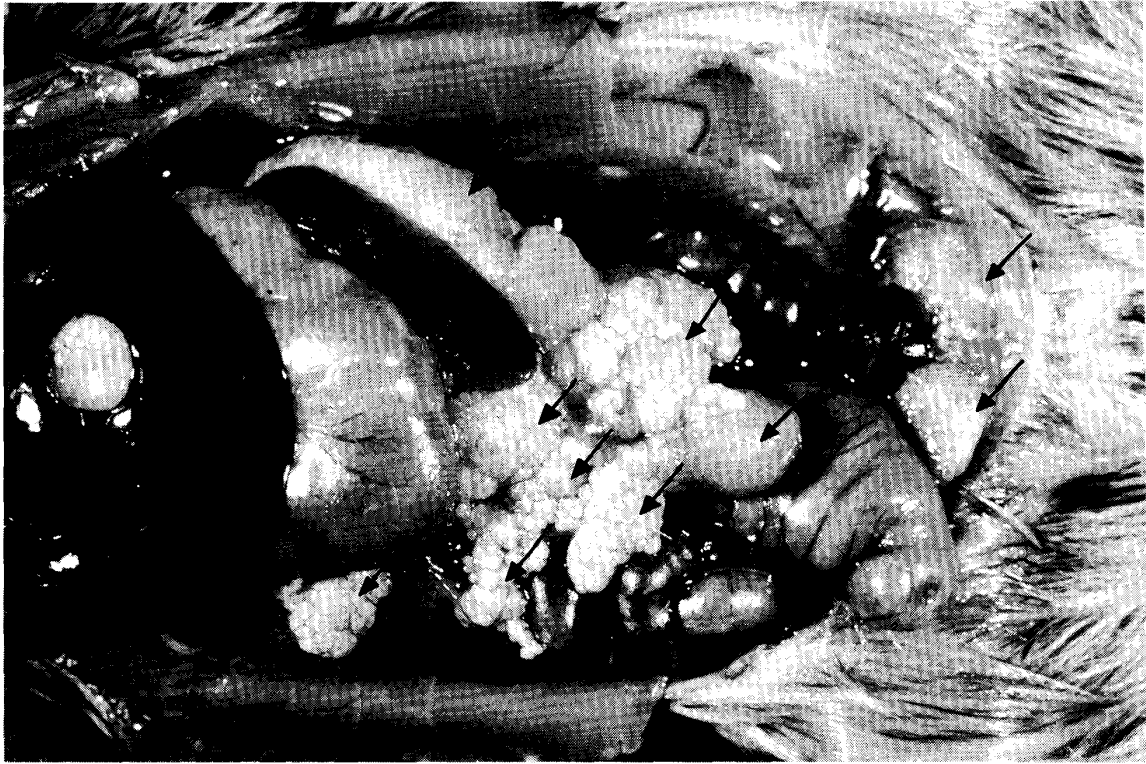


FIGURE 3 *E. multilocularis* cyst (→) in abdominal cavity of Mongolian gerbil, 6 months after intraperitoneal inoculation of the swine hepatic lesion.

and weighing a total of 6 g were recovered only from the organs of the abdominal cavity.

The cysts contained about 120,000 protoscoleces per gram and all the protoscoleces, with abundant calcareous corpuscles, were fully developed (Fig. 4). Histological examination of the cyst showed that host cellular reactions were mild except for the presence of slight lymphocytic and polymorphonuclear cellular infiltration around the vesicles. The vesicles were composed of fine reticular tissue in which brood capsules with fully developed protoscoleces were embedded (Fig. 5). From these histological findings, the cysts were identified as those of *E. multilocularis*.

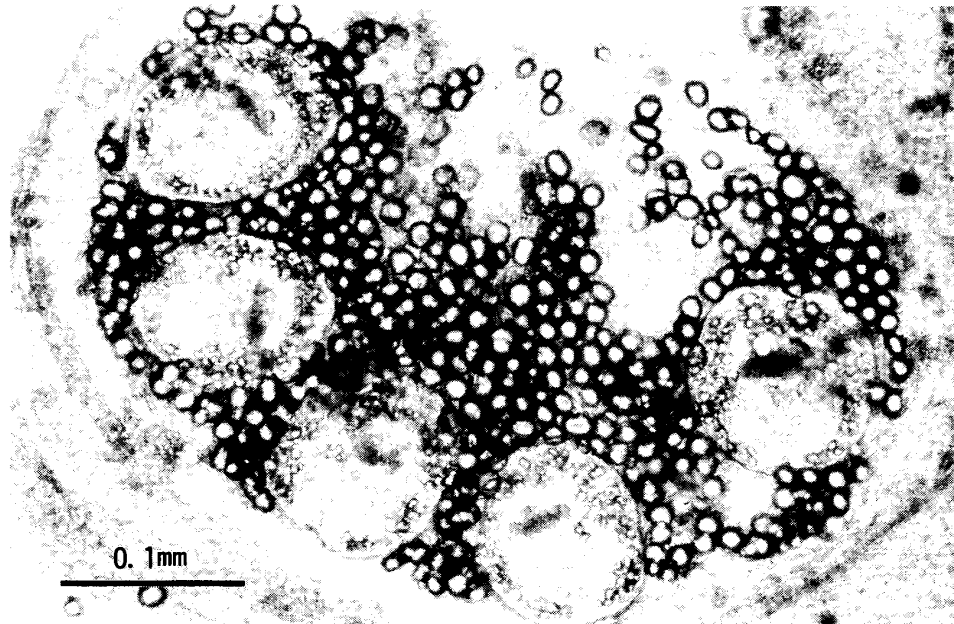


FIGURE 4 *E. multilocularis* cyst from Mongolian gerbil containing fully mature protoscoleces with abundant calcareous corpuscles. Unfixed specimen.

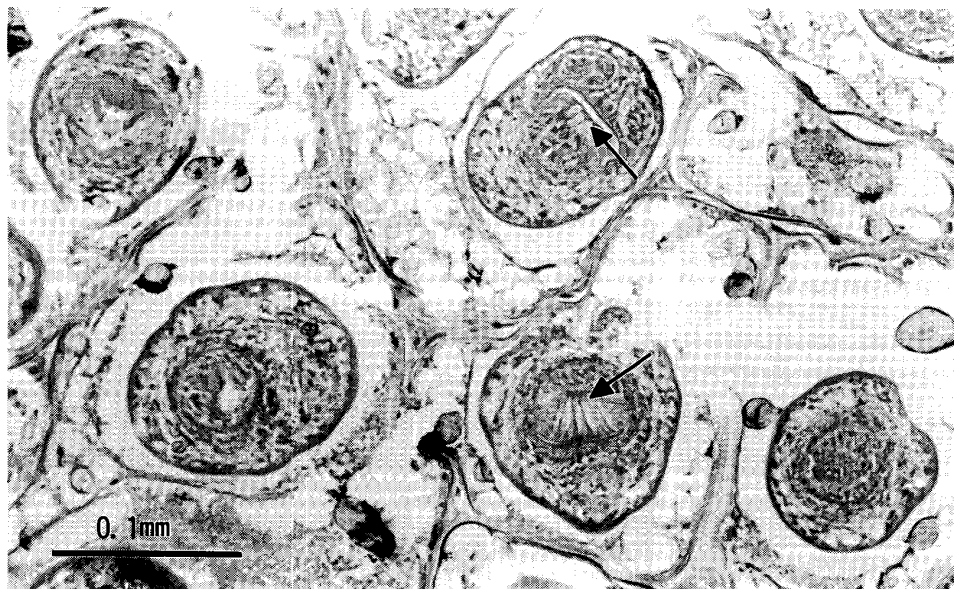


FIGURE 5 Section of *E. multilocularis* cyst from Mongolian gerbil showing fully mature protoscoleces within small vesicles. Note the development of hooks (→) in the protoscoleces. HE stain.

## DISCUSSION

The first human case of alveolar hydatid disease, which is caused by *E. multilocularis*, in Hokkaido was reported from the island of Rebun in 1937 (reviewed by YAMASHITA, 1978). This was followed by a large scale eradication campaign of the definitive hosts such as foxes and dogs in the island to control the parasite. Since no new human cases of *E. multilocularis* infection have been reported after the early '70's on the Rebun Island, the campaign was declared to be successful in controlling the parasite.

However, in 1965, new human cases of echinococcosis were detected in the so-called "Konsen" area in eastern Hokkaido and the endemic area was limited to 10 administrative districts. After 1983, *E. multilocularis* infections were found in ungulates such as swine (SAKUI et al., 1984) and horse (MIYAUCHI et al., 1984) in the hitherto "non-endemic" area. This led to a follow-up survey of the parasite in swine as well as in other hosts and the result showed that *E. multilocularis* is prevalent in one-third of the total administrative districts of Hokkaido (Fig. 6).

Thus, *E. multilocularis* infection in swine has been effectively demonstrated to be a reliable indicator for pinpointing the distribution of the parasite in the supposedly "non-endemic" area in Hokkaido.

Besides the reports by the Soviet researchers, ungulates were thought to be refractory to *E. multilocularis* infection. LUKASHENKO(1968, 1971) reported experimental *E. multilocularis* infection in 27 swine, 22 lambs and 15 calves. He noted that *E. multilocularis* lesions in these ungulates were limited to granuloma formation and the parasite tissues remained at the initial stage of the infection. Strong host cellular reactions were observed and the cysts were sterile. He concluded that ungulates play no role in the life cycle of the cestode in nature. In addition, OHBAYASHI et al. (1971), reported only regressive foci in a horse and goats experimentally infected with *E. multilocularis*.

One of the explanations for the lack of reports of spontaneous *E. multilocularis* in ungulates in Europe and North America is that the parasite is mainly being maintained sylvatically among the wildlife. The areas inhabited by these wildlife do not overlap much with the pastoral area and thus *E. multilocularis* infected animals do not have substantial contact with the livestock. Moreover, as pointed out by SCHWABE (1986), this paucity in literature might be due to the truncated life-spans of most domesticated food animals. In Hokkaido, however, the red foxes come foraging around the piggeries and this has been implicated as the reason for the increase in the incidence of *E. multilocularis* in swine in the past few years. Notwithstanding, we still need to clarify whether the high prevalence of *E. multilocularis* in swine is actually due to the increase in the number of infected foxes that come to the area around the pig pens foraging for food or whether the strain of *E. multilocularis* observed in the swine liver

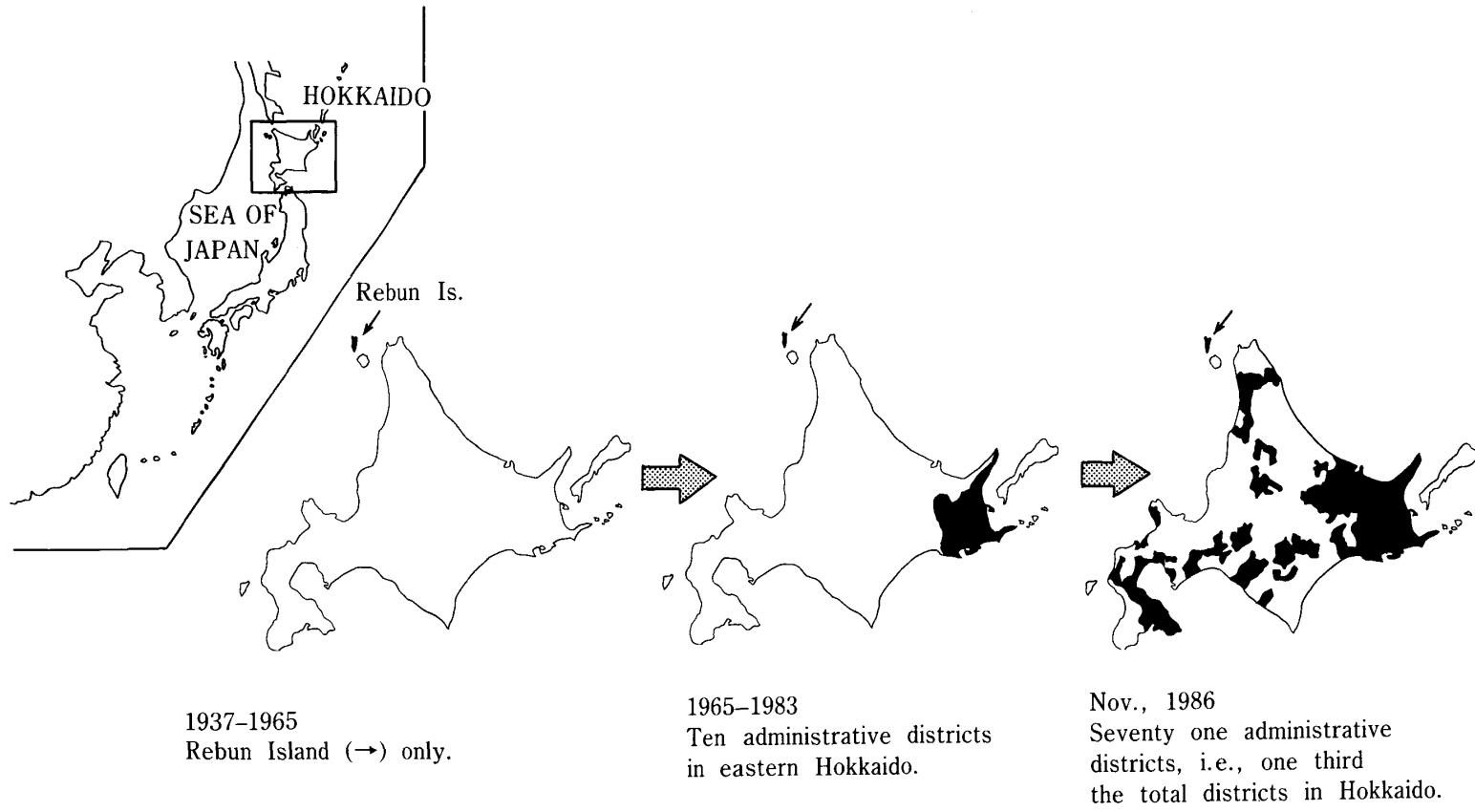


FIGURE 6 Distribution (■) of *E. multilocularis* in Hokkaido, Japan, since 1937

*E. multilocularis* isolation from swine

is biologically distinct from the hitherto known *E. multilocularis*.

*E. granulosus* infection has also been reported in imported swine in Hokkaido (UEDA et al., 1958). However, there is no evidence that the life cycle of *E. granulosus* has ever been maintained in Hokkaido. Moreover, cysts of *E. granulosus* and *E. multilocularis* differed markedly in the swine. In the former, the cysts are simple, much larger and fertile, while in the latter they are composed of small vesicles, comparatively smaller in size and regressive in nature.

Previously, we have tried twice without success to isolate the *E. multilocularis* in the cyst from swine by implanting the whole cyst mass into the peritoneal cavity of two groups of six female gerbils aged 12 to 24 weeks old (unpublished data). The success of the present experiment may be due to the use of finely cut and sieved parasite tissue. This method has also been reported to be successful for the isolation of *E. multilocularis* from human cysts (RAUSCH & WILSON, 1973).

The strobilar stage of the present swine isolate of *E. multilocularis*, obtained from experimentally infected definitive host, will have to be further studied in order to elucidate the biological characteristics of the parasite in spontaneous infection.

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