

The First Report in Turkey of *in vivo* Cultivation in *Rattus norvegicus* of *Echinococcus multilocularis* Human Strain

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SUMMARY: The adult form of the small cestode *Echinococcus multilocularis* is found in carnivorous animals, especially in the fox. This cestode, which is found in the northern hemisphere in the world, is the cause of a generally fatal disease in humans, known as alveolar echinococcosis (AE). The metacestodes of *Echinococcus multilocularis* can be experimentally developed in the *Meriones unguiculatus* species of rodents, and it is possible to use these metacestodes for numerous purposes, primarily for supplying the antigens required for serological diagnosis of the disease. It is with this aim that in this study for the first time in Turkey, *Echinococcus multilocularis* metacestodes were developed using different kind of rodent, *Rattus norvegicus*, and an indigenous strain was isolated using the surgical material of a patient diagnosed with alveolar echinococcosis.

Key Words: Alveolar echinococcosis, strain isolation, rat

İnsanda Bulunan *Echinococcus multilocularis* Suşunun *Rattus norvegicus*'ta *in vivo* Kültivasyonu ile Türkiye'de İlk Olarak İzole Edilmesi

ÖZET: *Echinococcus multilocularis*'in erişkin formu, tilkiler başta olmak üzere etçillerde bulunan küçük bir sestoddur. Dünyanın Kuzey Yarımküresinde görülen bu sestod insanlarda genellikle ölümcül seyreden bir hastalık olan alveolar echinococcosisin etkenidir. *Echinococcus multilocularis* metacestodları *Rattus norvegicus* (sıçan) türü kemirgenlerde deneysel olarak geliştirilebilmekte, elde edilen metacestodlardan başta tanı için gerekli antijenler olmak üzere çok amaçlı yararlanılabilmektedir. Bu amaçla, *Echinococcus multilocularis* ile enfekte insandan alınan materyal, farklı bir kemirgen türü olan *Rattus norvegicus* (sıçan)'a inokule edilerek *Echinococcus multilocularis* suşu izole edilmiştir.

Anahtar Sözcükler: *Echinococcus multilocularis*, suş izolasyonu, sıçan

INTRODUCTION

Human alveolar echinococcosis (AE), caused by the metacestode of the *Echinococcus multilocularis* (*E. multilocularis*), is considered to be the most potentially lethal parasitic zoonosis in the nontropical areas in the Northern Hemisphere (2, 15).

The metacestode primarily proliferates in the liver. The clinical signs and symptoms of hepatic alveolar echinococcosis resemble those of hepatic carcinoma or other liver diseases in appearance and behavior (17).

E. multilocularis actively penetrates the epithelial border of the intestinal villi and enters venous and lymphatic vessels to finally reach the liver, where maturation to the asexually pro-

liferating metacestodes takes place. The growth of these larvae cause massive lesions in the liver and occasionally in secondarily infected organs such as the lung and brain, with often fatal consequences for the patient (3).

The usual intermediate hosts are rodents, such as *Microtus*, *Arvicola*, etc. By ingesting the food contaminated with feces of the definitive hosts, in that eggs of the parasite are present, humans become infected (12). As a result of slow growth, the onset of symptomatology is considerably delayed and death of the patient usually occurs 10 to 15 years after diagnosis (1). The proliferation of metacestodes, the larval stage, of *E. multilocularis*, takes place by exogenous budding of daughter vesicles and progressive invasion occurs in the surrounding tissue in the liver (4). It was reported that, the infection was fatal in 94% of the patients during a 10 year follow-up period after the diagnosis (16). The infection is important in Turkey, as well as in all over the world (14, 15).

Metacestodes have been maintained traditionally in the laboratory by serial transplantation passages into susceptible animals such as mice or gerbils for the experimental studies, though there may be different kinds of problems during the maintenance of the strain in the laboratory.

In this study, we have aimed to isolate *E. multilocularis* from human, and to grow the strain in different kind of rodent, *Rattus norvegicus*. By doing so, an indigenous strain may also be used for obtaining antigens and materials for serological tests as well as molecular and biological studies.

MATERIAL AND METHOD

All the experiments in this study were carried out after ethical approval of the Local Ethics Committee of the Experimental Studies at Ege University, Medical Faculty, and the local Ethics Committee of the Clinical laboratory Studies at Dokuz Eylul University, Medical Faculty in compliance with the relevant national laws relating to the conduct of animal experimentation.

In this study, 4 *Rattus norvegicus* (6 months old, 200-250 g weighed) taken from the animal laboratories of the Parasitology Department of Medical Faculty in Ege University, were used as experimental animals.

Liver tissue infected with AE: The material required to infect the experimental animals was obtained from the operational material from a case, operated in the General Surgery Department of Atatürk University Medical Faculty, in that alveolar echinococcosis in the liver was suspected radiologically, and confirmed serologically and pathologically.

After preparation of the specimens, microscopically examination was performed. The diagnosis of alveolar echinococcosis was verified on the observation of germinative membrane and protoscolexes on PAS stained preparations.

Tissue blocks of *E. multilocularis* vesicles with a volume of 3 cm³ were cut into pieces very carefully, with a sterile scalpel and then were placed in a Petri dish and washed with physiological serum under the sterile conditions. In vitro cultivation at +37 °C of *E. multilocularis* metacestodes was carried out as described previously by Hemphill and Gottstein (4). Two pieces of tissue were placed in 40 ml of culture medium (RPMI 1640 containing 10% fetal calf serum). The tissue blocks were kept in tightly-closed culture flasks (75 cm³), placed in upright position in an incubator at +37 °C *in-vitro* during 232 days.

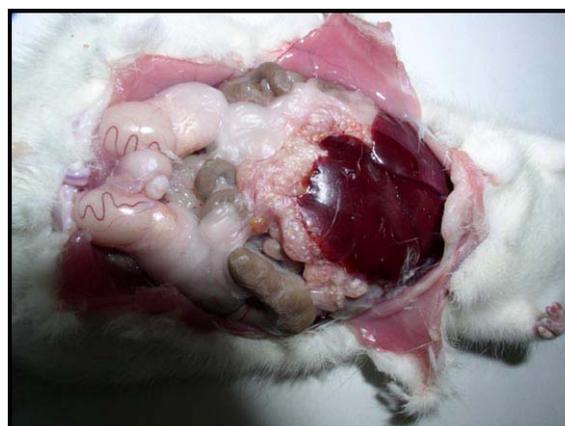
After this waiting period, these pieces were placed into the peritoneal space by making a small insertion in the linea alba on the abdominal wall of the lower part of the abdomen of animals under Ketamine (Ketalar®, Pfizer, Turkey) anesthesia in a laminar flow cabinet in the laboratory. After the recovery, the animals were put in their cages.

All operated animals were examined manually in every 15 days to establish whether or not a mass had developed in the abdomen. Animals were killed by cervical dislocation, and laparotomy was performed in the laminar flow cabinet at the 12th months.

RESULTS

All of the animals have developed an abdominal mass. The abdominal examination of infected *Rattus norvegicus* after 6-7 months revealed the initiation of a mass development. It was observed that at the 9th month, the mass had filled the abdomen. After this period, animals that were hardly moving due to the large abdominal masses were dissected. During the macroscopic examination of the dissected animals, a dirty white-colored hard mass filling the whole abdominal cavity was noticed (Figure 1).

Figure 1: The appearance of abdominal cavity of *Rattus norvegicus* infected with *E. multilocularis* at 12th month



DISCUSSION

Maintenance of the metacestodes in the laboratory by serial transplantation passages is very important for the in vivo cultivation of *E. multilocularis*. In many researches, different animals and methods have been suggested for this purpose. More commonly, researchers have used mice or gerbils infected by intraperitoneal or intrahepatic injection of metacestode-infected tissue, which subsequently resulted in secondary AE (5). In a recent study, it was reported that some of the strains of mice such as, C57BL6-a/a, C57BL6- A³/a, C3H/HeJ were susceptible to *E. multilocularis*, while some of them were relatively resistant to this infection (6). They have reported that, such susceptibility was also consistent with the previous study (6, 13). It is known that the infection can occur in other gerbil species such as *Cricetulus migratorius* and *Meriones meridianus*, and female 6-10-week-old C57BL/6 mice (13). It was also reported that *Rattus norvegicus* has been naturally infected with *E. multilocularis* in Japan (10). We have demonstrated that metacestodes in tissue pieces that were

obtained from a patient with alveolar echinococcosis have successfully been developed in *M. unguiculatus*, and this was the very first report of *E. multilocularis* strain isolation in Turkey and nuclear diagnoses method (7, 8, 9). We hold the view that with this strain, the antigens required for the serological diagnosis of AE can be obtained from the indigenous strain (11).

These animal models have provided important information's about the immunology in AE, host-parasite interactions, and the effects of various potential drugs in the treatment of AE (5). In addition, a metacestode in vitro culture model has been developed for the experimental studies. In future, these in vitro models may be a valuable alternative to animal experimental studies, however today it is believed that, it can never replace the use of laboratory animals entirely (4).

There may be lots of different kinds of problems for maintaining the strain of *E. multilocularis*. For instance, *Meriones unguiculatus* species are monogamic, and their number may decrease dramatically due to their fights in their cages. Therefore, different types of animals may be used for the maintenance of strains, so that such problems can be avoided. Comparing with the previous study, we may suggest that *Rattus norvegicus* is more favorable than *Meriones unguiculatus* in terms of maintenance of *E. multilocularis* strain, because the duration of the passaging from one animal to another is longer in *Rattus norvegicus*, thus, less number of animals are required to maintain the strain (7).

In this study, metacestodes in tissue specimens obtained from the patient with AE infection were implanted in *Rattus norvegicus*. The cyst formation was observed in these animals at 12th month. This is the first in vivo cultivation of *E. multilocularis* in *Rattus norvegicus*, is the first report in Turkey.

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