

The First Report of *Echinococcus multilocularis* Strain Isolation from Human in Turkey

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SUMMARY: The adult form of the small cestode *Echinococcus multilocularis* (*E. multilocularis*) is found in carnivorous animals, especially in the fox. This cestode, which is observed in the northern hemisphere of the world, is the cause of a generally fatal, progressing disease in humans, known as “alveolar echinococcosis” (AE). The metacestodes of *E. 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 antigens required for diagnosis. It is with this aim that in this study for the first time in Turkey, *E. multilocularis* metacestodes were developed in *Meriones unguiculatus* and an indigenous strain was isolated using the surgical material from a patient diagnosed with alveolar echinococcosis.

Key Words: Alveolar echinococcosis, strain isolation

Türkiye’de insandan ilk izole edilen *Echinococcus multilocularis* suşu

ÖZET : Küçük bir cestod olan *Echinococcus multilocularis*’in erişkini tilkiler başta olmak üzere etçillerde bulunmaktadır. Dünyanın kuzey yarım küresinde görülen bu cestod insanlarda genellikle ölümcül seyreden bir hastalık olan alveolar echinococcosisin etkenidir. *E. multilocularis* metacestodları *Meriones unguiculatus* türü kemirgenlerde deneysel olarak geliştirilebilmekte, elde edilen metacestodlardan başta tanı için gerekli antijenler olmak üzere çok amaçlı yararlanılabilmektedir. Bu amaçla, çalışmada alveolar echinococcosis teşhisi alan bir hastanın operasyon materyalinden Türkiye’de ilk kez *Meriones unguiculatus* üzerinde *Echinococcus multilocularis* metacestodları geliştirilerek yerli bir suş izole edilmiştir.

Anahtar sözcükler: Alveolar echinococcosis, suş izolasyonu

INTRODUCTION

Alveolar Echinococcosis (AE) or honeycomb-like echinococci cyst is the name given to the disease caused by the larvae form of *Echinococcus multilocularis* (*E. Multilocularis*). *E. multilocularis* is seen in the northern hemisphere and it is known to be one of the zoonoses which causes a life threatening disease in humans (8). Although, in nature, the life cycle of *E. multilocularis* takes place among the small rodents and carnivorous animals, humans coincidentally appear in this cycle. Due to the fact that *E. multilocularis* grows very slowly in humans, it may take a long time, such as 10 to 15 years, before the clinical signs appear. When diagnosis is made at the end of this period, it is usually too late. If no

diagnosis is made or mistreated, then 94 % of these cases would might die (9). The metacestodes, which are the larvae form of the parasite, grow towards the periphery with the proliferation of the liver vesicles (2). The disease sustains its importance in Turkey, as it does in countries of the northern hemisphere (1, 6, 8).

Although it has been stated that, the metacestodes of *E. multilocularis* can be developed in some experimental animals such as *Meriones unguiculatus* (*M. unguiculatus*) (7) and mice (3), it has drawn attention that in Turkey no study has been carried out concerning this matter and that no strain isolation belonging to this parasite is present.

In this study, we have aimed to isolate and to grow the *E. multilocularis* strain in experimental animals and by doing so, to obtain antigen for tests which use serological diagnosis and to obtain material for other molecular and biological studies.

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MATERIALS AND METHODS

In this study 4 healthy *M. unguiculatus* type, 8-12 weeks old (on average 60-70 gr.) rodents, known to be sensitive to *E. multilocularis*, from the animal laboratories of the Parasitology Department of Ege University Medical Faculty, were used as experimental animals.

The material required to infect the experimental animals was obtained from the operational material of an operated case in the General Surgery Department of Ege University Medical Faculty, where alveolar echinococcosis in the liver was determined using radiological and serological methods.

Liver tissue infected with AE: The specimens were prepared when the material obtained after the operation was examined microscopically. The diagnosis of alveolar echinococcosis was verified on the observation of germinative membrane and protoscoleces after the specimens were stained with PAS stain.

Tissues containing *E. multilocularis* metacestodes were later cut in to approximately 1cm³ pieces using the aid of a scalpel and placed in petri dishes and washed with physiological serum.

These pieces were placed in to 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 which were put to sleep with ether anaesthetic in a laminar flow cabinet, under laboratory conditions. The animals were later put into their cages and left alone after they had a waken.

The animals were manually examined every 15 days to establish whether or not a mass had developed in the abdomen and were opened in the laminar flow cabinet in the 4th month.

RESULTS

The abdominal examination of the infected animals after the second month revealed the initiation of a mass development. It was observed that from the fourth month the mass had filled in the peritoneal space and that the abdomen had enlarged. After this period those animals, whose movements were noticed to be slower, were dissected. In the macroscopic examination of the dissected animals a dirty white colored hard mass was noticed to have filled in the peritoneal space (Figure 1). The specimens prepared from this mass were examined under the microscope (Figure 2). Protoscoleces and germinative membranes were observed in this examination. The mass was later kept for antigen preparation.

DISCUSSION

Alveolar echinococcosis sustains its importance as a public health problem in Turkey (6, 8). It is observed that although it is seen widely in all regions of our country the number of cases notified are limited due to the difficulties in the diagnosis. It has been stated that the number of alveolar echinococcosis cases are on the increase due to the development of diagnostic techniques and the widely spread health system in recent years (10).



Figure 1. The peritoneal appearance of a dissected *Meriones unguiculatus* with AE

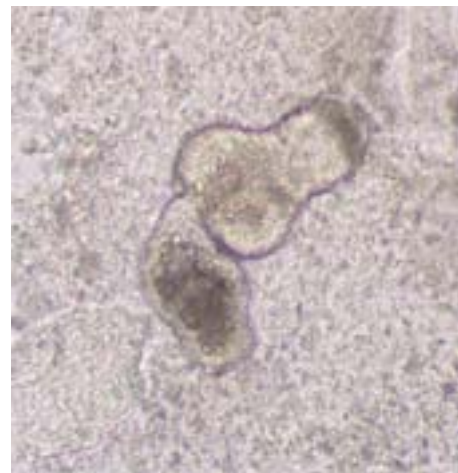


Figure 2. The protoscoleces of *E. multilocularis* prepared from the specimens of the mass.

The most frequently used experimental animal in the isolation of *E. multilocularis* metacestodes and in the making of invivo cultures, is *M. unguiculatus* (7). It has been stated that other types of gerbils (*Cricetulus migratorius* and *Meriones meridianus*) are also being used (5). It has been observed that *E. multilocularis* metacestodes have been developed in some species of mice (C57 BL/6 C57BL6-a/a, C57BL6-Ay/a, C3H/Hej) and rats (3) and it has been observed that some species are resistant to this infection (4).

In this study we have observed that metacestode pieces, which were obtained from a patient with alveolar echinococcosis, have developed by adapting to *M. Unguiculatus* and as a result the *Echinococcus multilocularis* strain has been isolated for the first time in Turkey.

We hold the view that with this isolation, the antigens required for the serological diagnosis of alveolar echinococcosis can be obtained from the indigenous strain.

REFERENCES

1. **Bresson-Hadni S, Laplante JJ**, 1994. Lenys D: Sero-epidemiologic screening of *Echinococcus multilocularis* infection in a European area endemic for alveolar echinococcosis. *Am J Trop Med Hyg*, 51(6): 837-46.
2. **Eckert J, Ammann R**, 1995. Clinical Diagnosis and Treatment of Echinococcosis in Humans In RCA. Thompson and AJ Lymbery (ed). *Echinococcus and Hydatid Disease*. CAB International Wallingford, England. p.411-463.
3. **Emery I, Liance M, Deriaud E, Vuitton D, Houin R, Leclerc C**, 1996. Characterization of T-cell immune response to *Echinococcus multilocularis*-infected C57BL/6J mice. *Parasite Immunol*, 18:463-468
4. **Hildreth MB, Granholm**, 2003. NH. Effect of mouse strain variations and cortisone treatment on the establishment and growth of primary *Echinococcus multilocularis* hydatid cysts. *J Parasitol*, 89(3):493-5.
5. **Osman I, Jiao W, Liao L, Chai J**, 1998. Comparative observation on experimental infection with *Echinococcus multilocularis* in *Cricetulus Migratorius* and *Meriones meridianus*, *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*.16(2):130-2.
6. **Öztek İ**, 1995. Ekinokokkus Alveolaris parazitliği, *T Ekopatol Derg*, 1(3-4): 140-143.
7. **Persat F, Mojon M, Petavy AF**, 1988. Lipids of *Echinococcus multilocularis* metacestodes. *Comp Biochem Physiol*, 91B:133-136.
8. **Rausch R L**, 1995. Life cycle patterns and geografic distribution of *Echinococcus* species, In RCA Thompson and AJ Lymbery (ed.) *Echinococcus and Hydatid disease*. CAB International, Wallingford, England. p.89-134.
9. **Schicker HJ**, 1976. Die Echinokokkose des Menschen. Stand von Diagnose, Therapie und Prognose bei Echinokokkonerkrankungen in Baden-Württemberg in den Jahren p.1960-1972.
10. **Unat EK**, 1991. Ekinokokların ve Enfeksiyonların Tarihçesi. *İnsanlarda ve hayvanlarda Kist Hidatik (Echinococcosis)*. Türkiye Parazitoloji Derneği yayını No:10, p.1-12.