DOI: https://doi.org/10.2298/SARH230112060L
UDC: 616.993(497.11)

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

The first human case of multilocular echinococcosis recognized in Serbia

Dušan Lalošević[1,2], Mirjana Živojinov[2,3], Valentina Isaković[4], Dejan Ivanov[2,4], Vladan Trivunović[3], Maja Ružić[2,5]

[1]Pasteur Institute, Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia;
[3]University Clinical Center of Vojvodina, Center for Pathology and Histology, Novi Sad, Serbia;
[4]University Clinical Center of Vojvodina, Clinic for Abdominal Surgery, Novi Sad, Serbia;
[5]University Clinical Center of Vojvodina, Clinic for Infectious Diseases, Novi Sad, Serbia

SUMMARY

Introduction *Echinococcus multilocularis* causes multilocular or alveolar echinococcosis, which differs from infection caused by *Echinococcus granulosus* in clinical presentation in humans. The most common definitive hosts for *E. multilocularis* are foxes and jackals, while domestic mammals like dogs and cats are rare. Humans are rare and accidental intermediate hosts. Cystic echinococcosis in humans is endemic in Serbia, while more severe alveolar echinococcosis has not yet been recorded.

Case outline We present a case of a 67-year-old female from a small village in the Sremska Mitrovica municipality. The onset of symptoms was several years ago, with liver pain which progressed over time. Differential diagnoses included benign liver tumors like haemangioma, cystic echinococcosis and abscess formed in the cystic echinococcal lesion. Left lateral hepatectomy was performed, and S II/III liver segments were removed. Pathological examination showed numerous small empty vesicle spaces with chitin membrane without protoscolices, surrounded by massive fibrosis and infiltrative growth into the liver parenchyma, all indicative marks of multilocular echinococcosis. Surgical margins were found positive for echinococcal vesicles showing that echinococcal tissue was not completely removed. Thus albendazole therapy was introduced. Epidemiological interview revealed that the patient lived in an endemic region of multilocular echinococcosis, in a house with two hunting dogs and backyard where contamination of soil with fox feces could occur.

Conclusion This is the first case of human multilocular echinococcosis recorded in Serbia, which should alert the medical community to improve prophylactic and diagnostic procedures and surgical techniques to better manage this zoonotic disease.

Keywords: *Echinococcus multilocularis*; human case; Serbia; Srem region; Mačva region; Vojvodina Province

INTRODUCTION

Echinococcosis is a well-known, often devastating parasitic disease in humans, which also has a great economic impact for livestock production all over the world. More than a milion people are actually infected each year, as reported by the WHO in 2021.

The *Echinococcus* genus involves small taeniid cestodes, with *E. granulosus* and *E. multilocularis* as the two main species. Currently, only *E. granulosus (sensu lato)* is endemic in Serbia, with two variants, *E. granulosus sensu stricto* and *E. canadensis*, causing well-known cystic echinococcosis in humans [1, 2]. Human infection occurs through direct or indirect contact with dogs that carry adult cestodes in their small intestine. Echinococcal cysts in humans, larval stage or metacestode, are primarily localized in the liver but can also be found in other organs, including the brain and bones. Their growing pattern is expansive, but surgical enucleation is possible.

The adult form of *E. multilocularis* lives in the small intestine of carnivores, primarily foxes and jackals. In intermediate hosts (wild rodents such as voles or field mice), this cestode produces numerous small cystic formations, primarily in the liver, representing larval forms filled with protoscolices. In humans, who are not natural hosts, cysts are also primarily localized in the liver but usually remain sterile, not containing protoscolices. Multilocular echinococcosis in humans is dangerous because parasitic cysts grow infiltratively into the liver, resembling malignant tumors, and can metastasize into surrounding tissues and other organs including the brain.

The first report of multilocular echinococcosis in Serbia was in an animal, a beaver from Zasavica, the Special Nature Reserve near the Srem region of the Province of Vojvodina, which is its northern part between the Sava and the Danube rivers [3]. The beaver, being an intermediate host, had a liver lesion, and the dilemma was whether it was a natural local infection or it was imported from Germany, from where the beaver was brought into the Zasavica Reserve. Recently, the Srem region in Serbia was identified as endemic for wild animal multilocular echinococcosis. In this region, 17.9% of foxes and 14.3% of jackals were

Received • Примљено: January 12, 2023
Revised • Ревизија: May 23, 2023
Accepted • Прихваћено: May 23, 2023
Online first: May 30, 2023

Correspondence to:
Dušan LALOŠEVIĆ
Pasteur Institute Novi Sad
Hajduk Veljkova 1
21000 Novi Sad
Serbia
dusan.lalosevic@mf.uns.ac.rs

registered as infected with the intestinal adult form of *E. multilocularis* [4]. Interestingly, a recent paper from Serbia reported a larval form of *E. multilocularis* in the liver of a golden jackal, showing the jackal can be both a definitive and intermediate host [5].

Since no cases of human multilocular echinococcosis have previously been reported in Serbia, clinical recognition of this disease is a most important issue. We here report the first case of human multilocular echinococcosis in Serbia.

CASE REPORT

We present a case of a 67-year-old female patient with upper abdominal symptoms that started a few years earlier, with weakness and gastritis, and a hiatal hernia. Three years prior to operation, the patient had an episode of collapse, and a subsequent medical examination revealed a liver lesion, which progressively worsened with liver pain. Benign liver tumors such as hemangioma, cystic echinococcosis, and abscess in a cystic echinococcal lesion were included in the differential diagnosis. Blood tests showed mild anemia, erythrocyte sedimentation of 16–20 mm, and no eosinophilia. Immunological markers characteristic of liver tumours such as carcinoembryonic antigen, alpha-fetoprotein and carbohydrate antigen 19-9 were whitin normal limits, as was serology for echinococcosis. Before the operation, pulmonary and cardiac functions were normal. Liver segments S II /III were removed by left lateral hepatectomy.

The specimen received for pathological analysis was a resected liver fragment, 11 × 6.5 × 5.2 cm in size (Figure 1). Capsular surface was thin, slightly uneven and glistening. The surgical margin was coloured green. On a cross-section, one larger (5.3 × 5 × 3.3 cm) and a few smaller cystic cavities were present in the centre of the liver fragment. Cavities contained yellowish, thick and pasty material, and their inner surface was thickened and rugged. The remainder of the liver parenchyma was brown and homogeneous. Pathological examination clearly showed multilocular echinococcosis with numerous small empty vesicle spaces with chitin membrane without protoscolices, surrounded by massive fibrosis and infiltrative growth into the liver parenchyma. Surgical margins were found positive for echinococcal vesicles (Figure 2) showing that echinococcal tissue was not completely removed. Thus albendazole therapy was introduced.

Epidemiological interview revealed that the patient lived in the endemic region for multilocular echinococcosis, in the village Radenković (coordinate: 44° 55′ 16.8″ N, 19° 29′ 21.6″ E) in the municipality of Sremska Mitrovica, Vojvodina Province (Figure 3), in a house with two hunting dogs, and backyard where contamination of soil with fox feces is possible.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

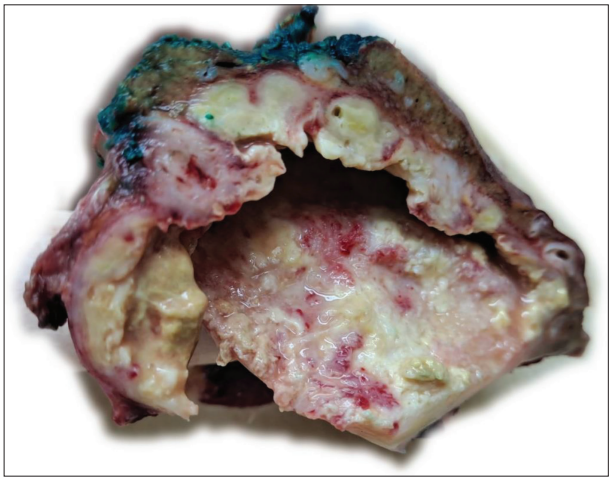

Figure 1. Liver resection with uneven central cavitation, macroscopic view

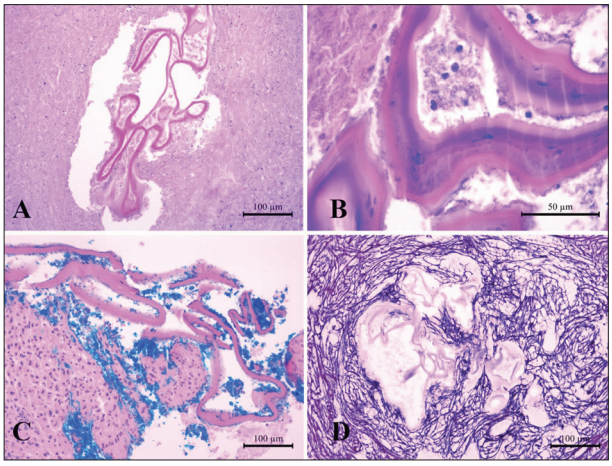

Figure 2. Pathohistology of the liver lesion: A – uneven shapes of echinococcal membranes; B – layered membrane structure; C – membrane on the resection margin, stained with green ink; D – extensive fibrosis around alveolar membranes, reticulin stain (A, B, C – hematoxylin-eosin; D – Gordon and Sweet's silver staining)

Figure 3. Village Radenković in Sremska Mitrovica municipality near Zasavica Reserve, where a beaver infected with *E. multilocularis* was found ten years earlier [from Wikipedia, modified (https://upload.wikimedia.org/wikipedia/commons/archive/8/85/20110531171606%21Northern_macva03_map.png, 07.01.2023]

DISCUSSION

Rudolf Virchow first recognized multilocular echinococcosis in 1855 ("Die multiloculäre, ulcerirende Echinokokkengeschwulst der Leber") as a distinct form

of the disease characterized by many small alveolar spaces surrounded by echinococcal membranes, with rare or no protoscolices at all [6]. Virchow described an infiltrative lesion growth in a liver, with central cavitation filled with necrotic fluid, similar to what we found in our patient.

Pathohistological diagnosis of multilocular echinococcosis based on surgical material is completely sufficient, as described in classical textbooks (Binford, Conor. AFIP, 1976; Saltykow, 1951). In liver needle biopsy of a suspected lesion, the histomorphological diagnosis of echinococcosis is easy if chitin membranes are found. The presence of protoscolices or at least chitin hooklets suggests hydatid or cystic echinococcosis because alveolar or multilocular echinococcus does not produce protoscolices in humans. Hence the absence of protoscolices or hooklets in a material is considered an important morphological diagnostic criterion of multilocular or alveolar echinococcosis. The distinction between multilocular echinococcosis and multiple cystic echinococcosis, following the rupture of a primary liver cyst and dissemination of protoscolices with their cystic metamorphosis, may represent a diagnostic challenge. We described a case in a 12-year-old girl with disseminated peritoneal cystic echinococcosis localized in the small pelvis and ovary [1]. When only biopsy material is taken from a patient, the monoclonal antibody Em2G11, highly specific for *E. multilocularis*, may be helpful [7].

The increase in the fox and jackal populations as a result of the eradication of rabies in Serbia [8] caused the spread of their parasites, not only *E. multilocularis* [9] but *Capillaria aerophila*, agent of pulmonary capillariasis, too [10]. Multilocular echinococcosis has recently been reported in the neighbouring countries of Serbia, including Croatia [11] and Bosnia and Hercegovina [12]. The first human case in Croatia had mushroom-picking in the forest as a risk factor, and interestingly, the patient in question also came from the Srem region. The part of Sremska Mitrovica municipality, where the village Radenković is located, is south of the river Sava, which is near the place where the first case of *E. multilocularis* was found in a beaver 10 years ago [3]. This part geographically belongs to the Mačva region, but administratively to the Vojvodina Province. Taken together, these findings show an expansion of the area of multilocular echinococcosis to the south.

In Serbia, where cystic echinococcosis is endemic, multilocular echinococcosis is becoming a new diagnostic and therapeutic challenge. To start with, careful use of terminology is important, because multiple cystic echinococcosis is not multilocular echinococcosis [13, 14]. The range of diagnostic tools for multilocular echinococcosis has increased lately, especially with the introduction of molecular methods [15, 16, 17], and those need to be introduced in routine medical practice in Serbia.

E. multilocularis, a zoonotic agent whose eggs are very resistant to low temperatures and disinfectants, is in expansion in Europe, including in countries neighboring Serbia such as Hungary [18] and Croatia [11], as well as across the world, notably in some Asian countries [19, 20]. The treatment of choice is obviously surgery, but therapeutic options in complicated inoperable cystic and multilocular echinococcosis are quite limited, and long-term (up to lifelong) albendazole is the first line treatment [21].

ACKNOWLEDGEMENT

The paper was reported at the 1st Forum of the Academy of Medical Sciences of the Serbian Medical Society held on March 24, 2023 in Belgrade (Serbia), and published as an abstract (Srp Arh Celok Lek. 2023 Jan–Feb;151(1–2):120–34).

Conflict of interest: None declared.

REFERENCES

1. Lalošević D, Fenyvesi A, Lalošević V, Popović Ž, Mesaroš A. Dilemmas in diagnostics of disseminated echinococcosis. Med Pregl. 2008;61(11–12):607–14. [DOI: 10.2298/mpns0812607l] [PMID: 19368281]
2. Bobić B, Nikolić A, Katić Radivojević S, Klun I, Djurković-Djaković O. Echinococcosis in Serbia: An issue for the 21st century? Foodborne Pathog Dis. 2012;9(11):967–73. [DOI: 10.1089/fpd.2012.1227] [PMID: 23113724]
3. Ćirović D, Pavlović I, Kulišić Z, Ivetić V, Penezić A, Ćosić N. *Echinococcus multilocularis* in the European beaver (*Castor fiber* L.) from Serbia: first report. Vet Rec. 2012;171(4):100. [DOI: 10.1136/vr.100879] [PMID: 22791524]
4. Lalošević D, Lalošević V, Simin V, Miljević M, Čabrilo B, Čabrilo OB. Spreading of multilocular echinococcosis in southern Europe: the first record in foxes and jackals in Serbia, Vojvodina Province. Eur J Wildl Res. 2016;62:793–6. [DOI: 10.1007/s10344-016-1050-9]
5. Marinković D, Gavrilović P, Vidanović D, Ćirović D, Kuručki M, Vasković N, et al. First report of alveolar hydatid disease (*Echinococcus multilocularis*) in a golden jackal (*Canis aureus*). Acta Parasit. 2022;67(3):1401–6. [DOI: 10.1007/s11686-022-00556-y] [PMID: 35536425]
6. Tappe D, Frosch M. Rudolf Virchow and the recognition of alveolar echinococcosis, 1850s. Emerg Infect Dis. 2007;13(5):732–5. [DOI: 10.3201/eid1305.070216] [PMID: 17553252]
7. Barth T, Casulli A. Morphological characteristics of alveolar and cystic echinococcosis lesions in human liver and bone. Pathogens. 2021;10(10):1326. [DOI: 10.3390/pathogens10101326] [PMID: 34684275]
8. Stankov S, Lalošević D, Fooks AR. History of Rabies Incidence and Rabies Control in Serbia in Support of the Zero by 2030 Campaign to Eliminate Dog-Mediated Human Rabies. Viruses. 2022;14(1):75. [DOI: 10.3390/v14010075] [PMID: 35062279]
9. Miljević M, Lalošević D, Simin V, Blagojević J, Čabrilo B, Čabrilo OB. Intestinal helminth infections in the golden jackal (Canis aureus L.) from Vojvodina: Hotspot area of multilocular echinococcosis in Serbia. Acta Vet Hung. 2021;69(3):274–81. [DOI: 10.1556/004.2021.00030] [PMID: 34506299]
10. Lalosević D, Lalosević V, Klem I, Stanojev-Jovanović D, Pozio E. Pulmonary capillariasis miming bronchial carcinoma. Am J Trop Med Hyg. 2008;78(1):14–6. [DOI: 10.4269/ajtmh.2008.78.14] [PMID: 18187778]
11. Dušek D, Vince A, Kurelac I, Papić N, Višković K, Deplazes P, et al. Human alveolar echinococcosis, Croatia. Emerg Infect Dis. 2020;26(2):364–6. [DOI: 10.3201/eid2602.181826] [PMID: 31961317]
12. Omeragić J, Goletić T, Softić A, Goletić Š, Kapo N, Soldo DK, et al. First detection of Echinococcus multilocularis in Bosnia and Herzegovina. Int J Parasitol Parasites Wildl. 2022;19:269–72. [DOI: 10.1016/j.ijppaw.2022.11.005] [PMID: 36406035]

13. Đenadić G, Joković D, Vučković D, Živojinov M, Lalošević D. Review of echinococcosis cases diagnosed in Mačva region, Serbia. MD-Medical Data. 2022;14(3):69–72.
14. Vuitton DA, McManus DP, Rogan MT, Romig T, Gottstein B, Naidich A, et al. International consensus on terminology to be used in the field of echinococcoses. Parasites. 2020;27:41. [DOI: 10.1051/parasite/2020024] [PMID: 32500855]
15. Fan H, Gai W, Zhang L, Ma Y, Wang H, Chen X, et al. Parasite Circulating Cell-free DNA in the Blood of Alveolar Echinococcosis Patients as a Diagnostic and Treatment-Status Indicator. Clin Infect Dis. 2021;73(1):e246–e251. [DOI: 10.1093/cid/ciaa1679] [PMID: 33146713]
16. Knapp J, Lallemand S, Monnien F, Felix S, Valmary-Degano S, Courquet S, et al. Molecular diagnosis of alveolar echinococcosis in patients based on frozen and formalin-fixed paraffin-embedded tissue samples. Parasite. 2022;29:4. [DOI: 10.1051/parasite/2022004] [PMID: 35113014]
17. Umhang G, Bastid V, Avcioglu H, Bagrade G, Bujanić M, Bjelić Čabrilo O, et al. Unravelling the genetic diversity and relatedness of Echinococcus multilocularis isolates in Eurasia using the EmsB microsatellite nuclear marker. Infect Genet Evol. 2021;92:104863. [DOI: 10.1016/j.meegid.2021.104863] [PMID: 33857665]
18. Dezsényi B, Dubóczki Z, Strausz T, Csulak E, Czoma V, Káposztás Z, et al. Emerging human alveolar echinococcosis in Hungary (2003-2018): a retrospective case series analysis from a multi-centre study. BMC Infect Dis. 2021;21(1):168. [DOI: 10.1186/s12879-021-05859-5] [PMID: 33568075]
19. Casulli, A. Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. Lancet Glob Health. 2020;8(4):e470–e471. [DOI: 10.1016/S2214-109X(20)30066-8] [PMID: 32199112]
20. Paternoster G, Boo G, Wang C, Minbaeva G, Usubalieva J, Raimkulov KM, et al. Epidemic cystic and alveolar echinococcosis in Kyrgyzstan: an analysis of national surveillance data. Lancet Glob Health. 2020;8(4):e603–e611. [DOI: 10.1016/S2214-109X(20)30038-3] [PMID: 32199126]
21. Mikić D, Jevtić M, Arsić-Komljenović G, Ristanović E, Stanković N, Sjenicić G, et al. [Impossibility of the treatment of inoperable liver multicystic echinococcosis due to adverse reactions to antihelminitics]. Vojnosanit Pregl. 2009;66(10):833–9. [DOI: 10.2298/vsp0910833m] [PMID: 19938764]

Први препознати случај мултилокуларне ехинококозе код људи у Србији

Душан Лалошевић[1,2], Мирјана Живојинов[2,3], Валентина Исаковић[4], Дејан Иванов[2,4], Владан Тривуновић[3], Маја Ружић[2,5]

[1]Пастеров завод, Нови Сад, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[3]Универзитетски клинички центар Војводине, Центар за патологију и хистологију, Нови Сад, Србија;
[4]Универзитетски клинички центар Војводине, Клиника за абдоминалну хирургију, Нови Сад, Србија;
[5]Универзитетски клинички центар Војводине, Клиника за инфективне болести, Нови Сад, Србија

САЖЕТАК

Увод Инфективни агенс мултилокуларне или алвеоларне ехинококозе, *Echinococcus multilocularis*, врста је различита од *E. Granulosus*, који изазива цистичну ехинококозу, не само по биолошким својствима него и по клиничком значају код људи, који могу бити ретки и случајни прелазни домаћини. Дефинитивни домаћини за *E. multilocularis* обично су лисице и шакали, а ретки су домаћи сисари попут паса и мачака. Цистична ехинококоза код људи у Србији је ендемска, док по клиничком току и исходу много опаснија мултилокуларна ехинококоза није још препозната.

Приказ болесника Наш случај је била жена, 67 година, из малог села у општини Сремска Митровица. Њени симптоми су почели неколико година раније и прогресивно су се погоршавали са болом у јетри. У диференцијалну дијагнозу укључени су бенигни тумори јетре, попут хемангиома, цистичне ехинококозе и апсцеса у цистичној ехинококној лезији. Сегменти јетре С II/III су уклоњени левом латералном хепатектомијом. Патохистолошким прегледом јасно је утврђена мултилокуларна ехинококоза са бројним малим празним везикулама од хитинских мембранозних простора без протосколекса, окружених масивном фиброзом и са инфилтративним типом раста у паренхиму јетре. Ехинококне везикуле су нађене и на хируршким маргинама и закључујемо да није потпуно уклоњено цело ехинококно ткиво, те је препоручена терапија албендазолом. Епидемиолошком анкетом утврђено је да болесница живи у ендемском региону мултилокуларне ехинококозе, у кући са два ловачка пса и двориштем где је могућа контаминација земљишта лисичјим изметом.

Закључак Овај случај је први препознати случај мултилокуларне ехинококозе код људи у Србији. Морамо побољшати профилактичке и дијагностичке процедуре и хируршку технику за ову зоонотску болест.

Кључне речи: *Echinococcus multilocularis*; хумани случај; Србија; Сремски регион; Мачвански регион; Војводина