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Original Scientific Paper

Detection of *Echinococcus* spp. in condemned livers

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ABSTRACT

Echinococcosis, a parasitic zoonosis caused by *Echinococcus* spp. tapeworms, poses a significant public health concern in Europe, but remains under-reported in both humans and animals. Post-mortem examination of slaughtered livestock offers a valuable opportunity for early detection and surveillance of parasitic infections, including echinococcosis, in meat intended for human consumption. In this study, liver samples exhibiting pathological changes were collected from two abattoirs in Serbia. A total of 31 livers—22 from pigs, 7 from lambs, and 2 from bullocks—were analysed microscopically, and molecularly using PCR, to detect *Echinococcus* spp. DNA. The parasite was detected in three pig livers, and three distinct species were identified: *E. granulosus, E. canadensi*s, and *E. multilocularis*. Although the sample size was relatively small, the detection of multiple *Echinococcus* species in pigs suggests a potential role of these animals in the parasite's transmission cycle within Serbia. These findings underscore the ongoing importance of comprehensive meat inspection protocols in abattoirs for zoonotic disease surveillance. They also highlight the need for expanded surveillance efforts and enhanced diagnostic procedures, particularly speciation of the tapeworm, to improve early detection and control of *Echinococcus* infections at the abattoir level.

1. Introduction

Livestock intended for human consumption must undergo slaughter at abattoirs, which consequently serve as valuable places for collecting data on animal health and condition. These data are primarily used to identify meat deemed unfit for human consumption, but they also offer important insights into disease prevalence and potential animal welfare concerns. One such disease, ranked among the top ten foodborne diseases in Europe and therefore of considerable public health significance, is echinococcosis, caused by tapeworms of the genus *Echinococcus* (*Bouwknegt et al*., 2018).

Echinococcus spp. are cestode parasites belonging to the family Taeniidae. Like all taeniid parasites, they possess complex life cycles involving a carnivorous definitive host (typically members of the families Canidae and Felidae) and a herbivorous or omnivorous intermediate host (commonly domestic livestock), in which the larval stage, known as the metacestode, develops. Humans are considered

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intermediate, dead-end hosts in the life cycle of *Echinococcus* spp. In the intestines of Canidae and Felidae, the tapeworm matures into its adult stage and undergoes sexual reproduction, releasing eggs that are subsequently shed in the faeces. Intermediate and dead-end hosts ingest the eggs via food or water, and the hatched larvae reach the various organs where they encyst into the larval stage known as the metacestode. Within these cysts, the parasite reproduces asexually, leading to progressive cyst enlargement, which is the primary cause of symptoms in cystic echinococcosis. The transmission cycle is perpetuated when a definitive host consumes infected tissue from an intermediate host, either through natural predation or the ingestion of raw offal containing viable metacestodes (*WHO Team for Control of Neglected Tropical Diseases*, 2001).

Although reporting of human and animal cases of echinococcosis is mandatory in most endemic countries, including Serbia, global under-reporting remains a major concern (*Casulli,* 2020; *WHO*, 2024). Based on a comprehensive review of human cases in Europe between 2017-2019, it has been estimated that approximately only 25% of cases are actually reported to The European Surveillance System (TESSy), while an earlier study in Germany estimated that between 2003-2005, about 67% of cases of alveolar echinococcosis were never reported (*Casulli et al*., 2023; *Jorgensen et al*., 2008). According to data from the Institute of Public Health of Serbia (IOPHOS) and the official Annual Epidemiological Reports from the European Centers for Disease Control (ECDC), between 2014 and 2019, the number of echinococcosis cases in Serbia exceeded those reported in neighbouring countries. The countries with the highest burden of echinococcosis in Europe are Bulgaria and Germany. In Serbia, 21 new cases were reported in 2023—nearly double the number reported in 2022—suggesting a potential upward trend in the number of reported cases. Since 2022, two confirmed cases of alveolar echinococcosis have been reported in the Vojvodina province, raising significant concern (*Lalošević et al*., 2023; *Milošević et al*., 2024). This may indicate the emergence of an *E. multilocularis* transmission focus in the region—the first documented hotspot in the Balkans. Whether any of the echinococcosis cases reported prior to 2021 were alveolar echinococcosis remains unknown, as the infecting species was not specified.

From an economic perspective, measured by disability-adjusted life years (DALYs), echinococcosis was estimated to account for 569 DALYs per 1000 capita globally in 2021, with just under three thousand reported cases from 30 countries across the Americas and Europe (WHO, Global Health Estimates; *WHO*, 2024). However, considering the significant under-reporting, the actual burden in terms of DALYs is likely substantially higher. The fatality rates of cystic and alveolar echinococcosis approach 100% if left untreated over time. Based on long-term clinical data from several European countries, the cure rate for cystic echinococcosis is estimated at approximately 75%, with a postoperative fatality rate of around 2%. In contrast, the cure rate for alveolar echinococcosis averages 63%, with postoperative survival rates ranging from 50% to 97% (*Budke et al*., 2006; *Salm et al*., 2019). These cure rates are also influenced by relapse rates, which often result from incomplete removal of the metacestodes. Echinococcosis in animals is almost exclusively diagnosed following slaughter. Metacestodes can be found in various organs, with the liver being the most commonly affected. Unlike cystic echinococcosis, morphological changes caused by *E. multilocularis* are more challenging to attribute to parasitic infection due to its infiltrative growth pattern, which results in lesions and extensive tissue damage. Additionally, *E. multilocularis* tends to metastasise to other organs, making alveolar echinococcosis difficult to detect through visual inspection at the slaughter line.

The aim of this study was to detect *Echinococcus* spp. DNA in condemned livers at slaughterhouses/abattoirs and identify the species of the tapeworm in order to gain insight into the occurrence of infection in livestock.

2. Materials and methods

For the purposes of this study, a total of 31 liver samples collected from two major abattoirs were analysed. The analysed samples were 22 pig livers, 7 lamb livers, and 2 bullock livers. All liver samples were pre-selected and removed from the slaughter line due to discernible pathological morphological changes (clearly identifiable cysts, cyst-like formations, discoloration, and scar tissue). To determine the presence of *Echinococcus* spp., cyst contents (liquid or solid) and/or affected tissue areas were sampled by direct isolation using pipette tips and/or tissue excision, then examined microscopically. DNA was rapidly extracted by boiling the samples in 0.02 M NaOH for 15 minutes, followed by amplification of the *cox1* gene using primers capable of

differentiating *E. granulosus*, *E. multilocularis*, and *E. canadensis* via conventional PCR.

3. Results

A detailed overview of the results by groups of analysed animals is presented in Table 1 for pig, Table 2 for lamb, and Table 3 for bovine liver samples. The analyses revealed that *Echinococcus* spp. DNA was present in a total of three livers, all of which originated from pigs (Table 1).

The first positive finding (Pig 1) came from a liver sample that contained several large, fluid-filled cysts. Tapeworm larvae were microscopically confirmed within the cyst content and were subsequently identified by PCR as *E. granulosus*. The second positive finding (Pig 12) was derived from liver tissue, although small fluid-filled cysts were also present. PCR analysis confirmed the presence of *E. canadensis* in the tissue. The third positive finding (Pig 31) was from a liver sample that contained small, fluid-filled cysts, and analysis of the cyst contents identified *E. multilocularis*.

All positive findings originated from livers with fluid-filled cysts; however, not all cyst-like morphological changes in the livers were caused by the presence of *Echinococcus* spp.

Table 1. Analysis of pig livers

Species - sample number(s)	Liver morphology/damage	Presence of cyst fluid	Sample examined	Result
Pig - 1	Large, fluid filled cysts (3)	Yes	Cyst fluid	*E. granulosus*
Pig - 2,5	Flat, button-shaped scar or abscess	No	Tissue sections (2)	Negative
Pig - 3,9	Small cyst or abscess, hard	No fluid but grainy, thick white content	Tissue section and content	Negative
Pig - 4,12	Small, fluid filled cyst	Yes	Cyst fluid and tissue section	*E. canadensis*
Pig - 8	Flat, button-shaped scar or abscess	No	Tissue section	Negative
Pig - 10	Flat, button shaped scar or abscess	No	Tissue section	Negative
Pig - 13	Flat, scar	No	Tissue section	Negative
Pig - 14	Flat, scar	No	Tissue section	Negative
Pig - 15; 20	Flat, button shaped scar or abscess	No	Tissue sections (2)	Negative
Pig - 16	Flat, scar	No	Tissue section	Negative
Pig - 17	Flat, scar	No	Tissue section	Negative
Pig - 18; 19	Flat, button shaped scar or abscess	No	Tissue sections (2)	Negative
Pig - 21	Discoloration, no scarring, no cyst or abscess	No	Tissue section	Negative
Pig - 27	Flat, scar	No	Tissue section	Negative
Pig - 28	Flat, scar	No	Tissue section	Negative
Pig - 29	Flat, scar	No	Tissue section	Negative
Pig - 30	Flat, scar	No	Tissue section	Negative
Pig - 31	Small, fluid filled cyst	Yes	Cyst fluid	*E. multilocularis*
Pig - 32	Small, fluid filled cyst	Yes	Cyst fluid	Negative
Pig - 33	Flat, scar	No	Tissue section	Negative
Pig - 34	Flat, scar	No	Tissue section	Negative
Pig - 35	Flat, scar	No	Tissue section	Negative

Table 2. Analysis of lamb livers

Species - sample number(s)	Liver morphology/damage	Presence of cyst fluid	Sample examined	Result
Lamb - 22	Flat, scar	No	Tissue section	Negative
Lamb - 23	Flat, scar	No	Tissue section	Negative
Lamb - 24	Flat, scar	No	Tissue section	Negative
Lamb - 25	Flat, scar	No	Tissue section	Negative
Lamb - 26	Flat, scar	No	Tissue section	Negative
Lamb - 36	Small, fluid filled cyst	Yes	Cyst fluid	Negative
Lamb - 37	Small, fluid filled cyst	Yes	Cyst fluid	Negative

Table 3. Analysis of bovine livers

Species - sample number(s)	Liver morphology/damage	Presence of cyst fluid	Sample examined	Result
Bullock - 7	Flat, scar	No	Tissue section	Negative
Bullock - 6; 11	Small cyst or abscess, hard	No fluid but thick yellow content	Tissue section and content	Negative

4. Discussion

The abattoir serves as a critical control point for the surveillance and management of zoonotic agents responsible for foodborne infections and intoxications. In addition to pathogen detection, meat inspection provides valuable information on pathological lesions associated with animal health and production diseases. The relevant pathological findings identified in the analysed slaughter by-products underscore the importance of animal health control at the slaughterhouse level and demonstrate its role as a key tool for the surveillance and management of animal diseases at the primary production stage. For example, in the United States, approximately 10–20% of feedlot cattle have presented with liver abscesses at slaughter (*Amachawadi and Nagaraja*, 2016). Similarly, a large-scale study in the Czech Republic reported liver damage in 34% of cows, 17.51% of sows, and 12.97% of ewes (*Valkova et al*., 2023). While liver abscesses were primarily of bacterial or, more rarely, viral origin, parasitic lesions were comparatively rare, accounting for only 1.31% of liver damage in heifers, 7.51% in ewes, and 3.68% in finishing pigs (*Valkova et al*., 2023). These findings along with previous reports clearly show that infections caused by environmentally transmitted parasites are rather uncommon in intensively farmed animals as opposed to extensively farmed and free-range animals (*Betić et al*., 2022; *Kijlstra et al*., 2004). As slaughterhouses and abattoirs in Serbia mostly process animals from intensive farming systems, echinococcosis is expected to be a minor finding during meat inspection. However, as shown here and by *Valkova et al*. (2023), echinococcosis diagnostics for pigs, particularly finishing pigs, may need to be prioritised, as 3/22 were found to be positive. Overall, the results of our study show that the majority of liver changes observed at slaughter are scars, small abscesses and discolorations. Hard cysts with grainy, thick, white contents are likely associated with earlier bacterial infections, while button-shaped scars are, according to literature, most commonly linked to prior *Ascaris* spp. infections. Regarding the detection of *Echinococcus* based solely on liver morphology and visible lesions, a definitive diagnosis cannot be made with absolute certainty or reliability. Smaller fluid-filled cysts may be associated with certain *Echinococcus* species, such as *E. granulosus* and *E. canadensis*, which only rarely infect humans. Unlike *E. granulosus*, which typically forms a single, large, fluid-filled cyst, *E. multilocularis* produces numerous small, infiltrative cysts (locules) that spread throughout the host's organs, mimicking the growth pattern of a malignant tumour (*Macpherson et al*., 2003). Consequently, the cavitations and tissue damage caused by *E. multilocularis* are often difficult to detect visually, particularly in younger animals, which are commonly processed at slaughterhouses/abattoirs, as cyst growth and damage may not have developed sufficiently.

The findings of this study underscore the continued relevance of post-mortem meat inspection at the slaughterhouse level, employing visual assessment, palpation, and incision techniques. This approach remains a valuable tool for the detection of notifiable diseases and zoonoses, identification of production-related conditions, and provision of epidemiological feedback to veterinary practitioners regarding subclinical disease occurrences at the farm level. In particular, palpatory inspection plays a crucial role in identifying internal abnormalities—such as cysts—that may not be visible on the surface of the liver, thereby enhancing diagnostic sensitivity. While conventional meat inspection methods are adequate and successful in detecting *Trichinella* spp., it provides limited information on the presence of other major foodborne parasites, which require laboratory-based molecular diagnostic methods for detection and identification.

5. Conclusion

Although the number of samples examined was limited and *Echinococcus* spp. was not the predominant cause of (morphological) liver changes, the detection of three different tapeworm species remains a cause for concern. While the study shows that most carcasses flagged for conditional use based on liver examination are not infected with *Echinococcus*, the results suggest that pigs can still play a role in the transmission of the parasite in Serbia. These results emphasise the need for more comprehensive studies to obtain reliable data on the prevalence and diversity of *Echinococcus* spp. in pigs. Such studies would provide valuable insights that can support the harmonisation of post-mortem inspection protocols and assist risk managers to develop and implement modernised control strategies in slaughterhouses.

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References

Amachawadi, R. G., & Nagaraja, T. G. (2016). Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. *Journal of Animal Science*, 94(4), 1620–1632. https://doi.org/10.2527/jas.2015-0261.

Betić, N., Karabasil, N., Djurković-Djaković, O., Ćirković, V., Bobić, B., Branković Lazić, I., Djordjević, V., & Klun, I. (2022). Seroprevalence, Direct Detection and Risk Factors for *Toxoplasma gondii* Infection in Pigs in Serbia, and Influence of Biosecurity Measures. *Microorganisms*, 10(5), 1069. https://doi.org/10.3390/microorganisms10051069

Bouwknegt, M., Devleesschauwer, B., Graham, H., Robertson, L.J., van der Giessen, J. W. & Euro-FBP workshop participants (2018). Prioritisation of foodborne parasites in Europe, 2016. *Euro Surveillance*, 23 (9), 17–00161. https://doi.org/10.2807/1560-7917.ES.2018.23.9.17-00161.

Budke, C. M., Deplazes, P., & Torgerson, P. R. (2006). Global Socioeconomic Impact of Cystic Echinococcosis. *Emerging Infectious Diseases*, 12(2), 296–303. https://doi.org/10.3201/eid1202.050499.

Casulli, A. (2020). Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis. *The Lancet Global Health*, 8(4), Article e470 –471. https://doi.org/10.1016/S2214-109X(20)30066-8.

Casulli, A., Abela-Ridder, B., Petrone, D,, Fabiani, M., Bobić, B., Carmena, D., Šoba, B., Zerem, E., Gargaté, M. J., Kuzmanovska, G., Calomfirescu, C., Rainova, I., Sotiraki, S., Lungu, V., Dezsényi, B., Herrador, Z., Karamon, J., Maksimov, P., Oksanen, A., Millon, L., Sviben, M., Shkjezi, R., Gjoni, V., Akshija, I., Saarma, U., Torgerson, P., Šnábel, V., Antolová, D., Muhovic, D., Besim, H., Chereau, F., Belhassen, García M,, Chappuis, F., Gloor, S., Stoeckle, M., Müllhaupt, B., Manno, V., Santoro, A., & Santolamazza, F. (2023). Unveiling the incidences and trends of the neglected zoonosis cystic echinococcosis in Europe: a systematic review from the MEmE project. *Lancet Infectious Diseases*, 23(3), Article e95-e107. https://doi.org/10.1016/S1473-3099(22)00638-7

Jorgensen, P., an der Heiden, M., Kern, P., Schöneberg, I., Krause, G., & Alpers, K. (2008). Underreporting of human alveolar echinococcosis, Germany. *Emerging Infectious Diseases*, 14(6), 935–937. https://doi.org/10.3201/eid1406.071173.

Kijlstra, A., Eissen, O. A., Cornelissen, J., Munniksma, K., Eijck, I., & Kortbeek, T. (2004). *Toxoplasma gondii* infection in animal-friendly pig production systems. *Investigative Ophthalmology & Visual Science*, 45(9), 3165–3169. http://doi.org/10.1167/iovs.04-0326.

Lalošević, D., Živojinov, M., Isaković, V., Ivanov, D., Trivunović,V., & Ružić, M. (2023). The first human case of multilocular Echinococcosis recognized in Serbia. *Srpski arhiv za celokupno lekarstvo*, 151(7–8), 453–456. https://doi.org/10.2298/SARH230112060L.

Lightowlers, M. W., Lawrence, S. B., Gauci, C. G., Young, J., Ralston, M. J., Maas, D. & Heath, D. D. (1996). Vaccination against hydatidosis using a defined recombinant

antigen. *Parasite Immunology,* 18, 457–462. https://doi.org/10.1111/j.1365-3024.1996.tb01029.x

Macpherson, C. N., Bartholomot, B., & Frider, B. (2003). Application of ultrasound in diagnosis, treatment, epidemiology, public health and control *of Echinococcus granulosus* and *E. multilocularis*. *Parasitology*, 127, Suppl:S21-35. https://doi.org/10.1017/S0031182003003676

Milošević, M., Šarčev, K., Živojinov, M., Milošević, Z., Lalošević, D., Torbica, S., & Ružić, M. (2024). Alveolar Echinococcosis of the Liver with Initially Suspected Intrahepatic Cholangiocarcinoma: Case Report—The Significance of Preoperative Serological Diagnostics. *International Surgery*, 108(2), 89–96. https://doi.org/10.9738/INTSURG-D-24-00008.1

Salm, L. A., Lachenmayer, A., Perrodin, S. F., Candinas, D., & Beldi, G. (2019). Surgical treatment strategies for hepatic alveolar echinococcosis. *Food Waterborne Parasitology*, 15, Article e00050. https://doi.org/10.1016/j.fawpar.2019.e00050

Valkova, L., Voslarova, E., Nincakova, S., Passantino, A., & Vecerek, V. (2023). The Incidence of Liver Damage Found during Postmortem Examination at the Slaughterhouse. *Animals*, 13(5), 839. https://doi.org/10.3390/ani13050839.

WHO Team for Control of Neglected Tropical Diseases (NTD), (2001). WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. In: Eckert, J., Gemmell, M.A., Meslin, F-X., & Pawłowski, Z.S. (Eds), World Organisation for Animal Health (Office International des Epizooties), Paris, France, and World Health Organization, Geneva, Switzerland.

WHO, (2024). Global report on neglected tropical diseases 2024. Geneva: World Health Organization; 2024. Retrieved from https://www.who.int/teams/control-of-neglected-tropical-diseases/global-report-on-neglected-tropical-diseases-2024. Accessed June 7, 2025

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