Case Report

Double Trouble on the Lower Leg—Unique Human Coinfection with *Echinococcus granulosus* and *Echinococcus multilocularis* without Liver Involvement

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Case Report

Double Trouble on the Lower Leg—Unique Human Coinfection with *Echinococcus granulosus* and *Echinococcus multilocularis* Without Liver Involvement

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Abstract: The tapeworms *Echinococcus granulosus* and *Echinococcus multilocularis* cause two different clinical manifestations in humans: cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. Both forms of echinococcosis manifest primarily in the liver, while other organs or tissues are less frequently affected. Simultaneous occurrence of CE and AE is extremely rare and all previously reported patients had affected liver, while simultaneous infection without liver involvement has not yet been described. Hereby, we present an exclusively extrahepatal *E. granulosus* and *E. multilocularis* coinfection localized between the calf muscles of a patient. Due to progressive painful local swelling, an abscess was suspected, but there was no improvement on multiple antibiotic courses. When imaging diagnostics suggested a parasitic origin of the two identified cystic lesions, positive serology on both species indicated a dual infection. Albendazole therapy was started, and extensive surgical excision was performed. Both species were confirmed using PCR and sequencing from intraoperative samples. The current case shows that coinfection without liver involvement can occur even in patients from the low-incidence regions, which should be considered in the differential diagnosis of patients with unusual clinical presentation.

Keywords: *Echinococcus multilocularis*; *Echinococcus granulosus*; alveolar echinococcosis; cystic echinococcosis; coinfection; extrahepatic; muscle

1. Introduction

Human echinococcosis is a zoonotic disease caused by the metacestode stage of tapeworms from *Echinococcus* genus. The disease can be transmitted to humans through the ingestion of embryonated parasite eggs from the soil, contaminated food or drink, or after direct contact with an infected animal [1]. In Europe, echinococcosis can be caused by two species: *Echinococcus granulosus* and *Echinococcus multilocularis*, which cause two different clinical manifestations in humans: cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. In both infections, humans serve as accidental hosts,

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and although the transmission routes are the same, the main hosts and thus the geographical distribution and risk factors for infection differ considerably depending on the involved *Echinococcus* species [2]. The main hosts for *E. granulosus* are canines, of which the domestic dog is the most important, while the main host for *E. multilocularis* is the red fox (*Vulpes vulpes*), although other definitive hosts such as domestic dogs might have a substantial role in the transmission to humans [3,4]. Due to the different biology of the parasitic cysts, the clinical manifestations of infections in humans are characterised by different clinical courses and prognoses. In case of CE, a single cyst or, more rarely, several cysts gradually increase in size by concentric growth, which is usually asymptomatic. But, in rare cases, symptoms of local compression, secondary bacterial infection or even rupture can develop. In contrast, the growth of an AE lesion is slower and more insidious, consisting of a conglomerate of many small cysts, often interspersed with necrotic tissue and calcifications, growing by lateral budding across the tissue, regardless of the anatomical boundaries - similar to a cancerous lesion [5]. In this way, human AE is usually diagnosed in late stages of the disease, and has much worse prognosis with sometimes even lethal outcome.

In general, the liver serves as the primary site for the manifestation of both forms of echinococcosis. In case of CE, the liver is affected in 69-75% of cases, while the lungs represent the second most commonly involved organ, accounting for 17-22% of cases [5]. AE can be even more considered as a primary liver disease, as the liver is affected in almost all, and extrahepatic growth is present in only 3% of cases [6]. In addition, hepatic AE lesions are more prone to continuous spread to the neighboring tissues or organs, which could be found in 34% of cases, e.g. diaphragm, retroperitoneal tissue, abdominal lymph nodes, extrahepatic vessels or ligaments and the peritoneum, and in rare cases metastatic spread to more distant organs can be found [6].

The dual infection with *E. granulosus* and *E. multilocularis* is very rarely observed in humans. The majority of coinfected patients are reported from highly endemic areas in China [7,8]. In all cases reported, the liver was always involved, containing lesions caused by either both, or at least one *Echinococcus* species [7].

Apart from being a rare disease, extrahepatic echinococcosis, whether primary, secondary or as a coinfection, typically has an unusual clinical presentation, which can mislead clinicians, and affect negatively the diagnostic and therapeutic decisions, especially in regions with a low or extremely low incidence of disease. Delayed diagnosis, as well as inappropriate treatment, can thus have a negative impact on the course of the disease.

Dalmatia, the Mediterranean part of Croatia, has historically been an endemic area for CE. There has been a decrease in the CE incidence of over 70% from the mid-1950s until late 1990s [9]. However, patients with CE are occasionally still diagnosed all around the country. On the contrary, the first evidence of infection with *E. multilocularis* was described only recently, in 2015, in red foxes [10]. Shortly thereafter, the first human case of AE was described in 2018 [11]. Since then, the incidence of human AE appears to be increasing, with most cases concentrated in the region of central continental Croatia [12]. However, both human CE and AE are rare diseases in Croatia, and an exclusively extrahepatic focus of either of these infections hasn't been reported in our patients yet.

Hereby we present, to the best of our knowledge, the first case of human concurrent CE and AE coinfection localized solely outside the liver, among muscles of the calf. The surprising localization and unusual clinical course of these parasitic diseases, in a patient from a low-incidence region, make this case instructive, which may help when diagnosing similar patients in the future.

2. Case Description

A 71-year-old female patient came to the University Hospital for Infectious Diseases in June 2024, looking for a second medical opinion, after several medical examinations performed by a general practice doctor, surgery specialists and an infectious disease specialist at the local County Hospital, due to chronic symptoms present on her left lower leg.

Ten months earlier, in August 2023, a slowly progressing, increasingly painful swelling appeared on her left calf (Figure 1). Despite absence of fever, a local infection was suspected, and the

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patient was treated with several broad-spectrum antibiotic courses (ciprofloxacin, amoxicillin / clavulanic acid twice, piperacillin-tazobactam, doxycycline) during the 8-month period, including one in-hospital treatment.

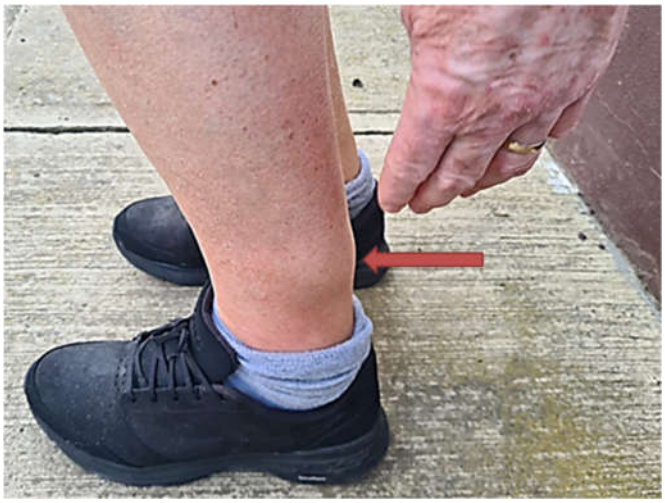

Figure 1. Status of the patient's left lower leg at the early stage of the disease (slightly painful, gradually growing tumorous swelling is pointed by the red arrow).

Despite applied antibiotic therapies, neither significant nor permanent improvement appeared. Contrary to expectations, 6 months after the initial symptoms, in February 2024, local pain exacerbated and local status worsened, leading to surgical incision, drainage and biopsy, which was performed at the local County Hospital, under clinical suspicion of a calf abscess. According to the patient`s words, after the wound was squeezed, numerous „whitish, rubbery balls, up to 1-1.5 cm in diameter", were expelled through the incision wound. Although they were not sent for microscopic analysis, the histopathology of the biopsy sample revealed fragments of amorphous, eosinophilic material without parasitic elements. Afterwards, a post incisional fistula developed, through which „whitish membranes" would occasionally pour out. At that time, the patient's laboratory findings were unremarkable, except of a slight, transitory eosinophilia of 700 eosinophils per μL of peripheral blood, recorded shortly after surgical intervention, which spontaneously withdrew. The patient had a history of arterial hypertension, hyperlipidemia, chronic gastritis, hypothyroidism, and in 1990 underwent neurosurgical removal of a meningioma. Due to those chronic diseases, she regularly took appropriate therapy. Additionally, for a couple of years, a simplex liver cyst of 2 cm in diameter, multiple small simplex cysts of the kidneys, and a Backer's cyst in the left popliteal region were known, showing no progression on the control imaging.

Our patient was a retired woman who lived in a house with a garden, in a small town in eastern continental Croatia (45°35′N 18°28′E / 45.59°N 18.46°E), who had a dog for over a year, denied having contact with other animals, forest environment and has not travelled abroad within the last 15 years.

The examination at the University Hospital revealed slightly elevated blood pressure of 140/90 mm/Hg, and a 2.5 cm long dry incision wound without local signs of inflammation and without any secretion after pressure, situated on the lateral, middle to distal third of the left calf. Other physical findings were normal.

The magnetic resonance imaging (MRI) of the left lower leg was indicated on that occasion, and it was performed in June 2024. It showed two cystic/solid morphologically diverse lesions, mostly hypointense with discreet postcontrast ring enhancement (Figure 2). Multi-slice computer tomography (MSCT) of lower legs, taken one month earlier, was in accordance with the MRI finding. Although MRI has better contrast resolution than the MSCT scans, in our patient, the two described lesions were not distinctive enough, so that a CE and AE coinfection could not have been suspected

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at this point. MRI of the head in January 2024, chest x-rays in May and MSCT of abdomen and pelvis in June 2024 showed no suspicious parasitic lesions, which was in accordance with the absence of any additional focal symptoms in our patient.

After parasitic origin of the lesions was profoundly suspected in June 2024, molecular diagnostics was performed retrospectively on the previously stored surgical paraffin block sample collected in March 2024. On that occasion, the presence of *E. granulosus* was confirmed by positive PCR and sequencing of the *COI* gene [13] while specific PCRs for *E. multilocularis* that amplify *nad1* [14] and *12S rRNA* gene [15] were negative in that sample.

In May 2024, serological testing performed 9 months after the onset of clinical symptoms, revealed positive enzyme linked immunoassay (EIA) for the semiquantitative determination of IgG class of antibodies against *Echinococcus* spp. (test NovaLisa *Echinococcus* IgG ELISA, manufacturer Gold standard diagnostics Frankfurt GmbH), and positive *E. multilocularis* semiquantitative detection of IgG antibodies against Em2 and Em18 specific antigens (*E. multilocularis* ELISA test, manufacturer Bordier Affinity Products, Crissier, Switzerland), accompanied by positive confirmatory qualitative test *(Echinococcus* western blot IgG, manufacturer LDBIO Diagnostics, Lyon, France) for serological diagnostics of alveolar and hydatid echinococcosis. Since bands common to both species were found, the dual infection was suspected for the first time in June 2024.

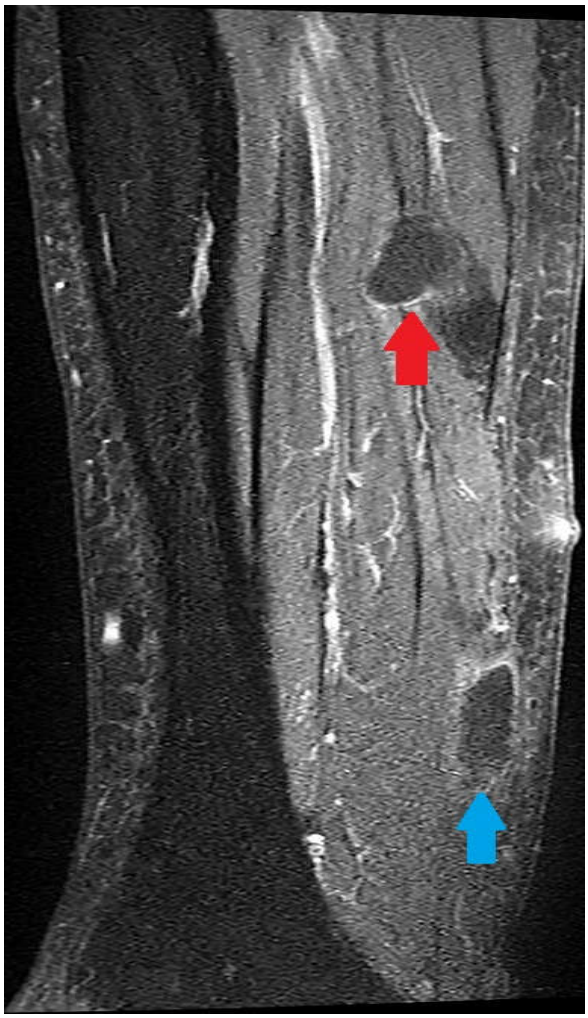

Figure 2. Postcontrast magnetic resonance imaging (MRI) scan of the patient's left calf, performed on June 18, 2024, after surgical incision, revealed two focal lesions with different morphological features (red and blue arrows). The first one was situated in the proximal part of the calf, inside the soleus muscle. At the postcontrast T1 WI MRI scan, the lesion was dominantly hypointensive compared with the surrounding muscle with discreet

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postcontrast ring enhancement, with defined contours (red arrow). The second lesion was extramuscular, subfascial collection of dense fluid; located in the distal third of the left calf, near the dorsal part of the soleus muscle and the dorsal part of the Achilles tendons. On postcontrast T1 WI MRI scan, the lesion was hypointense with postcontrast ring enhancement and no clear border (blue arrow).

Since the patient`s first visit to the University Hospital for Infectious Diseases in June 2024, a continuous antiparasitic therapy with albendazole (2x400 mg tablets per day) was started, and radical surgical excision was advised.

Due to comprehensive and complicated relations between the parasitic lesions and local anatomic structures, surgery was performed by a plastic surgeon in September 2024. On that occasion, half of the m. soleus, subcutaneous tissue with the fascia of m. gastrocnemius together with the skin and subcutaneous tissue of the distal calf, were removed. Nerves and blood vessels were preserved. The surgical site was rinsed with disinfecting sodium hypochlorite and hypochlorous acid solution, and profusely soaked with hypertonic saline for 30 minutes, before closing.

Histopathological examination of multiple intraoperative samples from both locations (1. tissue of m. soleus with cyst formation and 2. tumorous/cystic tissue with skin and muscle from the distal calf) revealed nonspecific granulations. They contained acellular eosinophilic masses which correspond to the hydatid cyst walls. Elements of parasites were not found by this method, which made the involved *Echinococcus* species impossible to determine.

However, two macroscopically different types of samples were sent for molecular diagnostics.

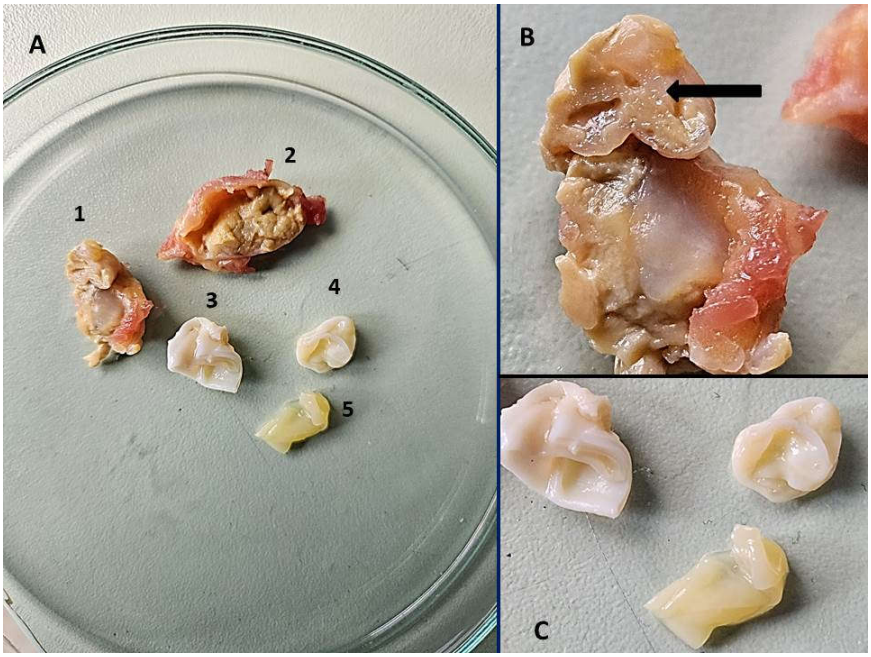

Figure 3. Cysts and tissue sections (1-5) removed from the patient's left calf during surgery in September 2024 which were used in molecular diagnostics (A). A large portion of the sample that tested positive for *E. multilocularis* labeled with black arrow (sample 1) (B). Cysts positive for *E. granulosus* (samples 3-5) (C).

Both *Echinococcus* species were confirmed from morphologically different cysts collected during the surgery (Figure 3). Sequencing of *nad1* [14] and *12S rRNA* gene [15] and comparison with existing sequences in GenBank using BLAST revealed *E. multilocularis* in sample number 1 and *E. granulosus sensu stricto* from the cysts labeled 3, 4 and 5 using *COI* [13]. Furthermore, sequenced *ATP6* gene [16,17] from the cysts labeled 3, 4 and 5 supported its classification as *E. granulosus* genotype G1.

The postoperative course went uneventfully, and the patient recovered well. Only nonsignificant elevation of transaminases due to continuous albendazole therapy appeared postoperatively, which did not require an interruption of therapy.

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3. Discussion

The described patient presents, to the best of our knowledge, the first case of human *E. granulosus* and *E. multilocularis* coinfection localized exclusively in the muscle and soft tissue, without liver involvement. Although being capable to infect virtually any organ or tissue in the human body, due to anatomic reasons, the attack rate for both CE and AE is the highest in the liver, which serves as the filter for venous portal blood flowing from the gut. The mechanism of primary extrahepatic infection is still being hypothesized to this day. An important factor could be the ability of oncospheres to directly invade the lymphatic vessels and bypass the liver [18]. The pathogenesis of coinfection also remains allusive, since it is believed that there exist some mechanisms which could stop the simultaneous growth of both species [19]. However, musculoskeletal involvement by each type of echinococcosis in humans is rare. In case of CE, it is estimated to account for 0.5-4% of cases [20]. So far, the lesions of CE have been described in hamstrings and adductor muscles [21–23], vastus lateralis [24], infraspinatus muscle of the shoulder [25], suprapubic, pubic bone, and left pectineus muscle [26], calf muscles [20], gluteal region [27] and psoas muscle [28]. All of these cases have shown to be primary localizations of CE, with an exception of recently reported pelvis muscle involvement, developed after retrovesical hydatid cyst surgery, which was possibly iatrogenic [26]. Primary cases of extrahepatic soft tissue AE seem to be even less frequent than those of CE [18,29,30]. The extrahepatic finding of AE alone without liver involvement is extremely rare. It has been described in the spleen [18], the psoas muscle [29,31], parotid gland [32], and in lumbar spine with spondylodiscitis – later with a lethal outcome [33]. The cases of CE and AE coinfection in humans can be extremely rarely found in the literature. They are all reported form highly endemic areas such Tibetan plateau in China, where high environmental pressure favors coinfections [7,8]. As far as we are aware, in every up to now described case of coinfection, the liver was involved, either affected by both *Echinococcus* species, or in conjunction with another organ involvement by second species [34–37].

Although the exact incidence of human CE in Croatia is not known, it can be estimated as low, according to clinical experience at our University Hospital Centre, to which all severe or unusual clinical cases gravitate. However, the presence of human AE in Croatia has been only recently recognized, but the number of diagnosed patients seems to increase continuously, reaching 15 by December 2024 [38]. The region with high incidence of 2.94 cases per 100,000 inhabitants in 2022 has been found in central continental Croatia [12]. So far, the liver has been identified as the affected organ in all Croatian AE patients. Interestingly, the patient we presented lives in east Croatia region, has no direct contact with foxes and has no obvious epidemiologic risk for AE.

It is difficult to guess how our patient became infected with both species of *Echinococcus*, as the reservoirs are usually different, although dogs and golden jackals can harbor both species of *Echinococcus* as main hosts. Since our patient, like many of her neighbors, had a dog, we hypothesize that the dog could be the source of her infection. Infection of dogs with *E. multilocularis* has been demonstrated experimentally [39], and "being a dog owner" has been recognized as a risk factor for *E. multilocularis* infection in humans [1,40]. The same is true for the golden jackal [41], an opportunistic, invasive canid that approaches and invades suburban areas to live near humans [42]. Since the golden jackal has become the dominant wild canid in the region where the patient lives and an infection with *E. multilocularis* has been found in this species in Croatia [43], one could assume that the source of the double infection in our patient could also be the golden jackal.

In addition to the scarce exposure, which may be completely absent in some cases of human echinococcosis [32], our patient did not have significant comorbidities such as diabetes mellitus or liver cirrhosis that seem to favor extrahepatic involvement [18,33].

The bizarre clinical presentation had also misled the clinicians involved, and contributed to a 10-month delay in diagnosis, and a 13-month delay in surgical treatment. Due to the different biology of the metacestode stage between *E. granulosus* and *E. multiloculars* (concentric growth vs. lateral budding, faster vs. slow insidious growth, respectively) [44], the symptoms of progressive, painful swelling of the calf in our patient were most likely caused by the *E. granulosus* lesion. Since the

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"whitish, rubbery balls" expelled at the first incision were not recognized as parasitic cysts, and since the initial histopathological, MSCT and MRI findings gave non-specific results, after *E. granulosus* infection had been confirmed by the retrospective molecular diagnosis of the initial biopsy sample, the first indication of a double infection was provided by serology test results. Serology has proven to be a useful tool in the diagnosis of human echinococcosis, but its results cannot always be used as a definitive diagnosis. The sensitivity of a serological test depends on the stage of the cyst, its size, immunity of the host, local epidemiology and the used test method. Additionally, it should be kept in mind that the manufacturer`s declared sensitivity of a serological test could significantly decrease in case of extrahepatic locations of parasitic lesions, and also the results could be confusing suggesting *Echinococcus* coinfections due to the possibility of cross reactivity [45]. However, in our patient, positive serology on both *Echinococcus* species suggested radical excision of both lesions, from which, in the sample taken from the distal cyst, *E. multilocularis* was clearly proven by sequencing the *nad1, COI* and *12SrRNA* genes, while *E. granulosus sensu stricto* was confirmed in the three samples collected from the proximal cyst, by sequencing the *COI* and *ATP6* genes.

As for hepatic localization, the MSCT, and especially MRI, have shown to be even more important tools in diagnosing extrahepatic CE and AE, including location in the musculoskeletal system [46,47]. But, despite the high resolution of their scans, the results of diagnostic imaging may show uncharacteristic, unusual and in some cases even bizarre findings. Although common radiological features for liver infections for both CE and AE have already been described [8,30,37], even in case of intrahepatic CE and AE coinfections, the radiological findings can become skewed [35]. When it comes to extrahepatic infections of both AE and CE, radiological signs become less specific and depend more on the localization of infection [21,25,29,30,32]. Due to the rarity of extrahepatic infections, the variety of anatomical sites that may be affected, and the uniqueness of the morphology of the lesions, no characteristic sign has been identified as a likely feature of musculoskeletal echinococcosis, and it is impossible to establish a clinically useful classification system for extrahepatic disease. This is especially true for AE, which is often mistaken for a malignant tumor due to its invasive growth, regardless of the anatomic barriers, even when found in the liver. In our patient, both lesions looked similar on MSCT and MRI scans, and based on their morphology, coinfection could not have been suspected, which emphasizes the necessity of applying other direct and indirect diagnostic methods in unusual cases.

4. Conclusions

Our case shows that even in patients with no apparent risk factors, living outside highly endemic regions, *E. granulosus* and *E. multilocularis* coinfection can develop, manifesting solely on extrahepatic location, which should be taken into differential-diagnostic consideration in patients with unusual clinical presentations. In our case, serology has proven as useful diagnostic tool for CE and AE, even without liver involvement. Furthermore, this case emphasizes that in case of multiple lesions of any location, it is necessary to perform multiple sampling of each lesion, and conduct molecular analysis of several samples, since histopathology could reveal nonspecific results. A comprehensive multidisciplinary approach to the patient is essential in resolving such a case. In regions with low incidence of human echinococcosis, additional effort should be placed on education of medical professionals, with the aim of raising the level of clinical suspicion, in order to accelerate diagnostic process and improve disease outcomes.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki. In accordance with local standards, our institution does not require ethical approval or institutional review board approval for reporting individual cases or case series when informed consent is provided by the patient.

Informed Consent Statement: Written informed consent has been obtained from the patient to publish this paper.

Data Availability Statement: The datasets from the current study are available upon request to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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