CASE REPORT

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First cases of alveolar echinococcosis in dogs in Poland

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Abstract

Alveolar echinococcosis caused by Echinococcus multilocularis is common parasitic disease among humans and animals in the northern hemisphere. Dogs, foxes, and other wild canids are definitive hosts, whereas small rodents play the role of intermediate hosts. In rare cases, after incidental ingestion of tapeworm eggs, dogs can become an intermediate host. The study describes briefly two cases of alveolar echinococcosis in dogs in Poland, including clinical management, diagnostic, treatment and molecular confirmation. Diagnostic procedures included laparotomy, cytology, histopathology and molecular analysis. Obtained sequences data were 100% homologous to E. multilocularis dehydrogenase subunit 1 gene sequences in GenBank®. To the authors' knowledge, alveolar echinococcosis has not been reported previously in a dog in Poland.

Key words

Poland, dog, Echinococcus multilocularis, molecular biology, first case, canine alveolar echinococcosis

INTRODUCTION

Cases of human alveolar echinococcosis caused by Echinococcus multilocularis and cystic echinococcosis, in turn caused by Echinococcus granulosus sensu lato are reported as one disease entity – 'echinococcosis'. According to the European Centre for Disease Prevention and Control, echinococcosis is among the most commonly reported zoonoses in Europe [1]. Both, alveolar and cystic echinococcosis are caused by the larval stage of Echinococcus tapeworms. Wild and domestic canids are mainly the definitive hosts, shedding eggs of tapeworm into the environment [2, 3]. In rare cases, dogs can develop larval echinococcosis after ingestion of other canids feces containing tapeworm eggs. Developing alveolar echinococcosis in canids causes damage in the abdominal cavity, infiltrating tissues and organs [4]. The current study presents the clinical findings and molecular diagnosis for the first two canine cases of alveolar echinococcosis in Poland.

CASE 1

In August 2022, a mixed breed female dog, aged one year and eight months, was presented to a small clinical practice in Mszana Dolna, Lesser Poland Province in southern Poland. The dog's owners reported that for three weeks it had suffered depression and abdominal enlargement. No such abnormalities had been observed in the previous year. During the physical examination the dilated rectum was palpable, and body temperature was 38.7 °C. Ultrasonography revealed the existence of fluid-filled spherical formations and a minor amount of free fluid in the abdominal cavity (Fig 1). An exploratory laparotomy was recommended. Analysis of blood sample revealed slight leukocytosis 17.4×10^3 [reference interval (RI): 6–16,5×10^3], with lymphocytosis (8,2×10^3; RI: 1–5×10^3), and a high activity of alanine aminotransferase (ALT 431 U/L; RI: 10–118 U/L), alkaline phosphatase (ALP 731 U/L; RI: 40–300 U/L), and a high level of total bile acids (TBA 51,4 µmol/L; RI: 0–15 µmol/L). No intestinal parasites were found in faecal flotation or on examination of a fresh faecal smear.

Diagnostic laparotomy revealed the presence of 100 ml of bloody fluid. The omentum was clogged and thickened. All lobes of the liver had cysts filled with a cloudy fluid. The walls of the cysts were cream-colored and varied in thickness from 2–5 mm. The surfaces of the cysts were irregular, and had invaded the liver parenchyma. A section of a cyst wall was taken for histopathological examination, and the contents one of the cysts was removed for cytological examination. The dog was awakened due to feeling well. The decision was made to postpone further proceedings until the results of cytology and histopathology were received.

The cytological examination of the cystic fluid revealed a moderate number of leukocytes, quite numerous and

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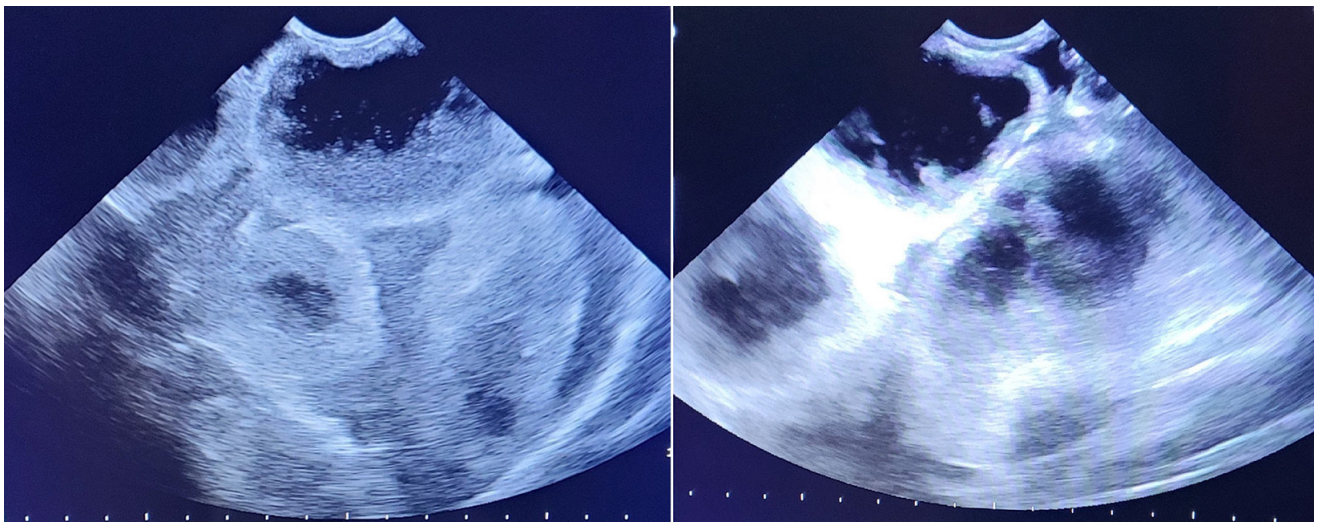

Figure 1. Ultrasound image of spherical structures in the liver of the examined dog with alveolar echinococcosis

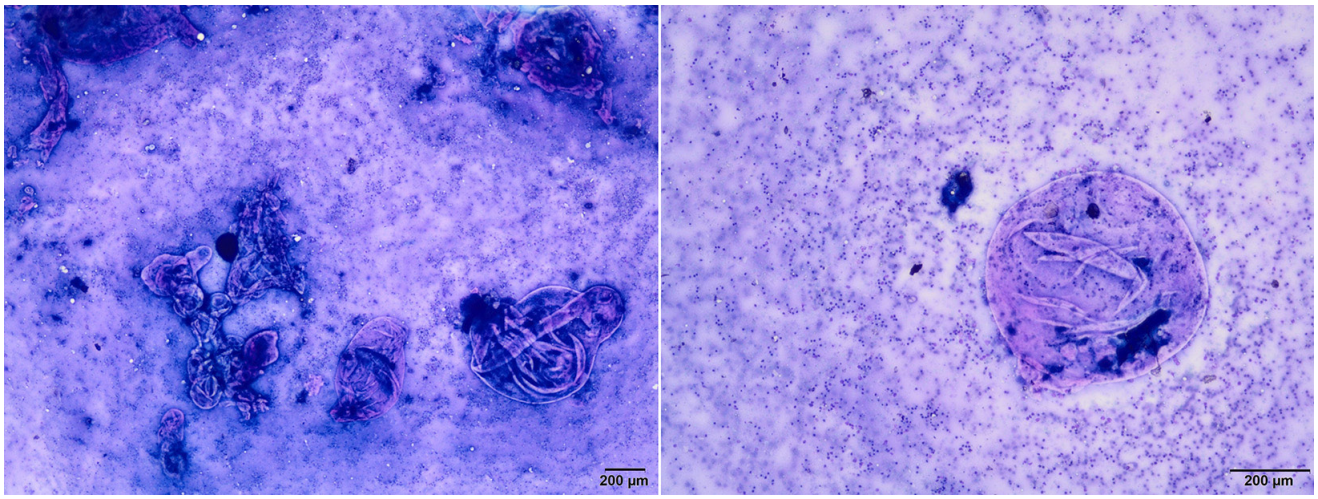

Figure 2. Some of the bulbous structure found in sediment of fluid aspirated from the cyst cavity

with various sizes protein bulbous structures (Fig. 2). The cytological examination suggested parasite infection with a tapeworm larval stage. To reduce and partially absorb the cysts, fenbendazole in a dose 50 mg/kg body weight was recommended orally, once a day

On 20 December, the dog was brought to the clinic again due to a poor appetite and weakness. Since the day of the laparotomy, the abdominal area had grown. One litre of fluid was removed during another abdominal puncture. Fenbendazole administration was maintained.

Six weeks later, the owner decided to euthanize the dog due to its significantly deteriorating health. After euthanasia, an autopsy was performed to collect fresh tissues for further study. On opening the abdominal cavity, a significantly altered and enlarged liver was found (Fig 3). Sections of fresh liver tissue with cysts were taken for histopathologic and molecular tests. Fluid collected from the cyst was centrifuged, and the supernatant tested biochemically with the following results: total protein 2.6 g/dL; albumin 1.2 g/dL; ALT 5448 U/L, AST 1226 U/L; ALP 28.5 U/L; GGTP 107 U/L; LDH 10925 U/L, CK 7023 U/L.

In the fluid sediment, protoscolex filled with calcareous bodies and a chain of hooks were found (Fig 4). Tissue sections were fixed in a 10% formalin solution. Histological slides were stained with haematoxylin and eosin (H&E) dyes, using the standard procedure (Fig 5).

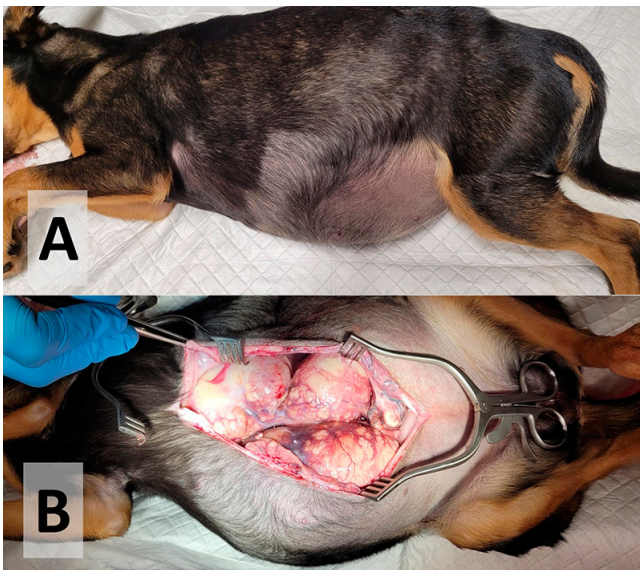

Figure 3. Euthanized dog with a visibly enlarged outline of the abdominal cavity (A); autopsy image – visible irregular surface of the liver with cream-coloured cysts (B)

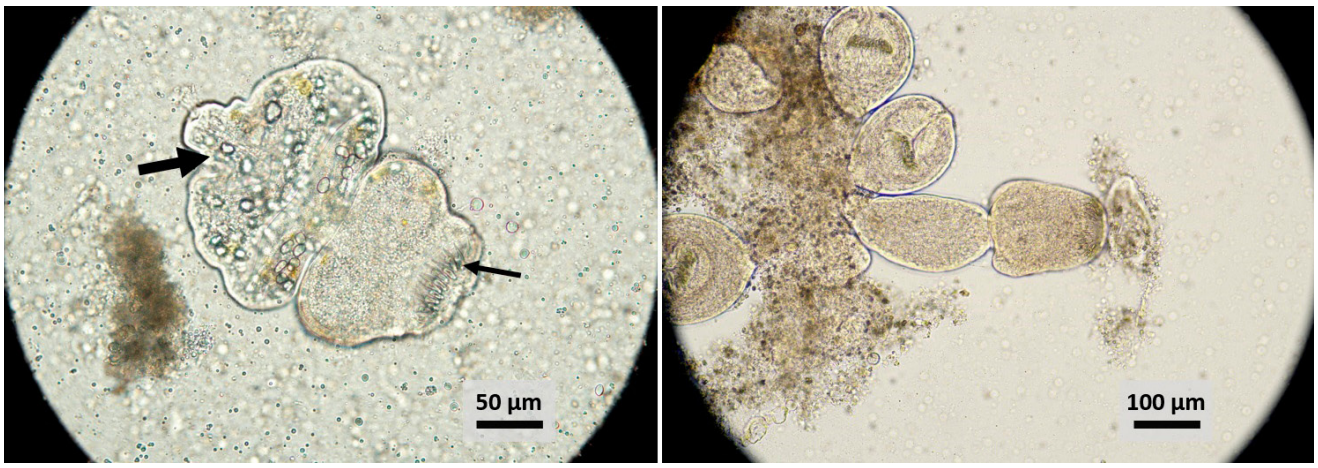

Figure 4. Protoscolex of Echinococcus tapeworm found in fluid sediment aspirate from cysts. Round calcareous bodies (thick arrow) and chain of hooks are present (thin arrow)

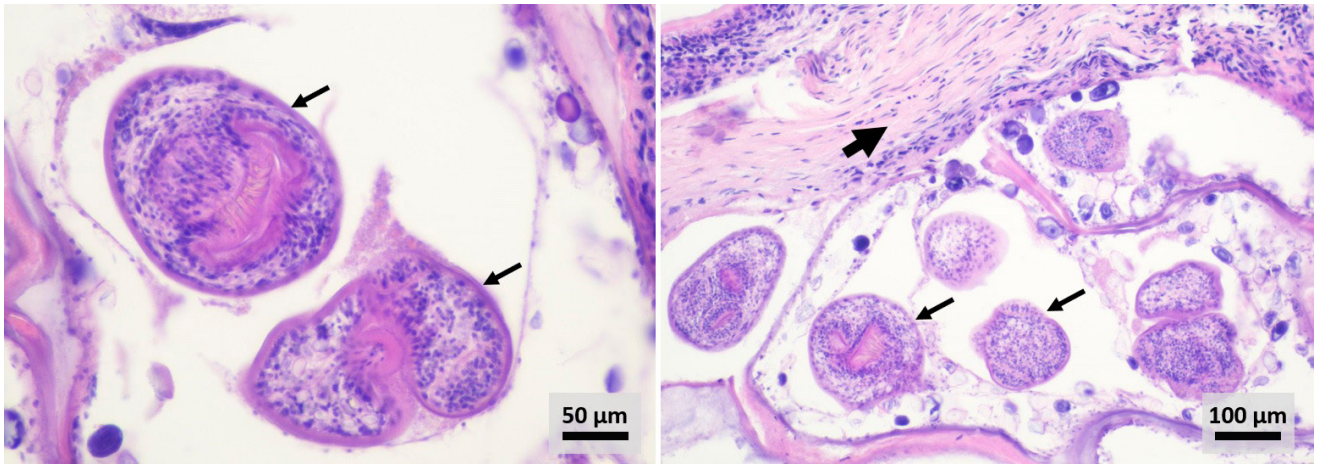

Figure 5. Histopathology of liver with alveolar echinococcosis. Tapeworm protoscolex (thin arrow) and tissue fibrosis (thick arrow) are present

Infiltrated liver parenchyma, the wall of the cyst, and fluid sediment were frozen and homogenized separately.

CASE 2

In June 2023, a 6-year-old male German Shepherd was presented to a small clinical practice in Człuchów, Pomeranian Province in northern Poland,. The dog had the problem of frequent and erratic urination. After a series of physical examinations, blood and urine tests, a diagnostic laparotomy was performed, during which a tissue mass the size of a child's head was found. The tissue mass had a rough surface with visible cysts of varying sizes below. The mass was found in the abdominal cavity and had infiltrated the posterior wall of the cavity as well as the urine bladder. A tissue section was taken for further examination. Blood sample analysis showed slight eosinophilia 1.44×10^3 [reference interval (RI): 0.1–1.3×10^3], and monocystosis (1.0×10^3; RI: 0.1–0.8×10^3). Biochemical profile revealed a low level of albumin (2.19 g/dL; RI: 2.5–4.4 g/dL) and calcium (8.28 mg/dL; RI: 8.8–11.6 mg/dL). The concentration of total bile acids was slightly increased (TBA 7,41 µmol/L; RI: 0–5 µmol/L). No intestinal parasites were found in faecal flotation or a fresh faecal smear. The dog received fenbendazole in a dose of 50 mg/kg body weight orally, once a day since 12 June to reduce the *Echinococcus* cyst before the planned excision of the cysts.

DNA isolation and molecular identification. In both Case1 and Case 2, tissue samples and fluid from cysts were performed to nucleic acid isolation. The Manual DNA extraction method was performed according to the manufacturers' instructions (Sherlock AX, A&A Biotechnology). The obtained DNA was frozen for further tests.

A multiplex PCR was performed in a 25 µl reaction mixture containing 12.5 µl StartWarm HS-PCR Mix (A&A Biotechnology), 1 µl each primer specific for *Echinococcus granulosus*, *E. multilocularis* and *E. canadensis* 5.5 µl ddH2O and 1 µl genomic DNA [5]. PCR was carried out by an initial denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 15 min in a PCR thermocycler (MultiGene optiMAX, Labnet International, Inc.).

Amplified products were analyzed on 2% agarose gel. 457 bp PCR products were sequenced and then aligned, analyzed with MEGA 11 (Molecular Evolutionary Genetics Analysis Version 11), and compared with the GenBank® database.

RESULTS AND DISCUSSION

320-bp (Case 1) and 317-bp (Case 2) products were 100% homologous to *E. multilocularis* NADH dehydrogenase subunit 1 (NAD1) gene sequences in GenBank® (e.g. MH259778, KY094609, AB668376). The sequences obtained have been submitted as OQ470332 and OR166500.

Two species of mammals are required to complete the life cycle of *Echinococcus* tapeworms. In Poland, red foxes, dogs, cats, and raccoon dogs have been recognized as definitive hosts for *Echinococcus multilocularis* [6, 7, 8]. A recent publication did not confirm wolves as a definitive host [9]. Among intermediate hosts in Poland, *Echinococcus multilocularis* infection was reported in Norway rat (*Rattus norvegicus*) and horses [10, 11].

Infected humans are aberrant intermediate hosts and play no role in the life cycle of the tapeworm. Humans become infected by the ingestion of eggs shed in the feces of definitive hosts. Infection is spread through soil, unwashed vegetables or water contaminated with tapeworm eggs. Soil is one of the main sources of zoonotic parasitic infections, including helminths infective eggs and protozoan's cysts or oocysts [12]. The first environmental study carried out in the Warmian-Mazurian Province, north-east Poland, showed the presence of *Echinococcus multilocularis* DNA in 11.3% of examined soil samples in wild areas and around households [13]. In France 26 (10.4%) out of 250 soil samples from rural vegetable gardens were positive for *E. multilocularis* DNA, in contrast to urban vegetable gardens where no *E. multilocularis* positive soil was detected [14]. In the Pomerania Province in north-west Poland, among 104 environmental fruit, vegetable, and mushroom samples, DNA of *E. multilocularis* was found in 5/49 (10,2%) samples from forests and 2/34 (5.9%) samples from kitchen gardens [15].

Rodents from the Arvicolidae family, including the common vole (*Microtus arvalis*), tundra vole (*Microtus oeconomus*) and bank vole (*Myodes glareolus*), play the main role as intermediate hosts [16]. A study performed in Turkey confirmed infection with *E. multilocularis* in 17 out of 843 rodents, and 15 of 17 infected animals belonging to the *Microtus* genus [17]. The metacestode stage develops in internal organs of rodents after incidental ingestion of tapeworm eggs.

Dogs and other canids, mainly red foxes, have been identified as definitive hosts [9, 16]. In North America, the prevalence of *E. multilocularis* among urban coyote (*Canis latrans*) in Canada exceeds 65% [18]. In rare cases, dogs become an intermediate host after incidental ingestion of invasive eggs [19, 20, 21]. Diagnostics imaging, typically ultrasonography or radiography of the body cavity, are used to detect lesions related with infection. [19, 22, 23]. Ultrasound changes are difficult to distinguish from proliferative lesions, including neoplasia [19, 22, 24]. The most common ultrasound findings in dogs with alveolar echinococcosis are large cavitary liver masses, sometimes with cyst wall mineralization [23]. Specimens aspirated from liver masses can be helpful in diagnostics. In fluid sediment from cyst cavities or liver lesions, protoscolex of *Echinococcus* can be found [25]. The morphology of tapeworm protoscolexes are characteristic of the *Echinococcus* genus; therefore, the final recognition of the species is based on the PCR reaction [5, 9, 26].

In humans, albendazole is the drug of choice in the treatment of alveolar echinococcosis [27, 28]. Dogs can be treated with albendazole orally at a dose of 10 mg/kg body weight daily [19, 20, 21]. The survival rate of untreated dogs was 50% and decreased over time to 16%, in contrast to treated dogs, whose survival rate was 82% initially, and decreased to 46% over time [21]. In the presented cases, however, both dogs were treated with fenbendazole orally at a dose 50 mg/kg body weight once a day. The effectiveness of fenbendazole and albendazole against *Echinococcus multilocularis* metacestodes has been compared and found to be very similar in *in vitro* and the murine infection model [28]. The diagnosis of alveolar echinococcosis in dogs might be possible when the tapeworm metacestode causes enlargement of the abdominal cavity.

Euthanasia is commonly necessary as a result of inadequate diagnosis and delayed treatment [4, 19, 20]. Serological diagnostics may be a chance for early diagnosis of echinococcosis in dogs, although more research is necessary [29, 30].

CONCLUSIONS

To the best of the authors' knowledge, the presented cases are the first to be published on alveolar echinococcosis in dogs in Poland. Since there are highly endemic areas of echinococcosis in Poland, it seems important to include the diagnostic of alveolar echinococcosis in the differential diagnosis of proliferative changes in the abdominal cavity of companion animals.

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