The first locally-acquired human infection of *Echinococcus multilocularis* in the Netherlands

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In the northern part of Western Europe, *Echinococcus multilocularis* is primarily detected and spreading in foxes. The present case marks *E. multilocularis* as an emerging pathogen for humans, as it describes the first human case of probably locally acquired *E. multilocularis* in the Netherlands, with various interesting clinical aspects.

**Case report**

A 55-year-old female, with a history of myxoid liposarcoma in the left leg (1993) and metastases in the spinal column (2001 and 2007), was presented at our hospital with cervical pain. As new metastases of the myxoid liposarcoma were suspected, diagnostic work-up was initiated. Fluorodeoxyglucose positron emission tomography (FDG-PET) was negative, but computed tomography (CT), simultaneously performed with FDG-PET, and magnetic resonance imaging (MRI) showed seven lesions in the liver, with a maximum size of 1.7 cm (Figure 1). These lesions were not present in an abdominal CT performed 6 months earlier. The patients’ laboratory results were as follows: alkaline phosphatase 89 U/l, gamma-glutamyl transferase 28 U/l, aspartate aminotransferase 19 U/l, alanine aminotransferase 23 U/l, lactate dehydrogenase 346 U/l, bilirubin 10.1 micromol/l and white blood cells 4.9x10⁹/L with 62% neutrophils, 22% lymocytes, 15% monocytes, 1% eosinophils and 0% basophils. Without pathological confirmation of these presumed metastasis of her myxoid liposarcoma, neo-adjuvant chemotherapy was started. As this was bilobar liver disease, radical surgery with curative intent was only possible when performing two separate operations in order to prevent liver failure. Therefore, after chemotherapy, partial left hepatectomy and contra-lateral portal ligation was performed, to allow liver regeneration, leaving four lesions in situ in the right liver lobe.
Pathologic examination of the liver tissue revealed three circumscribed intact nodules with central necrosis and a peripheral wall of histiocytes with focal giant cells. Central cysts and budding daughter cysts with a trilayered membrane wall were seen, suiting echinococcosis. No protoscolices, hooklets or calcareous corpuscles were noticed. Protoscolices, however, are often absent in *E. multilocularis* lesions (5). Hence, the findings were compatible with echinococcosis (Figure 2 and 3). There was no evidence of metastases of the liposarcoma. Subsequently, serum of this patient was sent to a Dutch reference center and *Echinococcus granulosus* serology came back weakly positive (IgG 1:80 (in-house enzyme-linked immunosorbent assay, cut-off 1:40), immunoblot IgG1 positive, IgG4 negative (in-house immunoblot(9)). The result of the specific *Echinococcus multilocularis* ELISA (Em$_2^\text{plan}$) was negative.

To finally confirm the diagnosis, isolated DNA from unpreserved liver material was sent to the Center for Infectious Disease Control (Bilthoven, Netherlands). DNA from our patients lesions was compared with DNA isolated from *E. multilocularis* samples from infected foxes originating from Limburg province (The Netherlands); this was the province where the patient lived. A polymerase chain reaction (PCR) amplifying DNA sequences of two mitochondrial targets was used: cytochrome c oxidase subunit 1 (COX 1) and NADH dehydrogenase (nad1) to identify *E. multilocularis* and to distinguish the organism from *Echinococcus granulosus* and other taeniid tapeworms (2, 3). The DNA sequences were compared to DNA sequences from the same target from other *E. multilocularis* and *E. granulosus* strains in Genbank. DNA sequencing of the PCR amplicons of the nad1 (520bp) and cox1 (420bp) genes showed a 99-100% similarity with the European *E. multilocularis* strains, including four *E. multilocularis* fox strains, and only a 86-93% similarity with *E. granulosus*.

After this diagnosis, our patient started with albendazole 400 mg twice daily. Subsequent abdominal CT-scans, 4 and 7 months post-resection, revealed no progression of the lesions nor a
change in aspect. Informed consent was obtained and this report was approved by the human investigations committee of the Maastricht University Medical Centre.

Four lessons can be learned from this case presentation. Firstly, *E. multilocularis* lesions can be rapidly progressive: multiple lesions with a maximum size of 1.7 cm developed within a period of 6 months in our patient. The incubation period after ingestion is estimated to be between 5 to 15 years (5). Liver cysts grow up to 1 cm in the first six months and 2-3 cm per year thereafter, but in our patient one lesion was already 1.7 cm in less than 6 months time (12). When left untreated, the mortality rate is almost 100% within 15 years after diagnosis (5). Recently, a very progressive *Echinococcus multilocularis* infection has been described in a renal transplant patient who was severely immunocompromised (7). Our patient however did not use immunosuppressive drugs in the period before presentation.

Secondly, *E. multilocularis* lesions can be misinterpreted for metastatic lesions, as *E. multilocularis* lesions usually present on CT with irregular borders and various densities, although variation exist (3). Calcifications are present in 82% and are hyperdense, while necrotic areas are hypodense (4). “Hot spots” are often seen with FDG-PET, although lesions can be metabolically inactive as well, as in our case (4, 6, 13). The FDG-PET-negative, multiple circumscript hypodense lesions in the liver seen at regular CT in our patient were not classical for *E. multilocularis* and did resemble liver metastases (15); the initial presumptive diagnosis therefore seemed much more likely.

Thirdly, *E. multilocularis* lesions can develop in patients without reported behavior associated with the ingestion of *E. multilocularis* eggs (10). Our patient’s life-time travel history revealed three short holidays to endemic areas (Switzerland, Italy and Austria in 2006 and 2007) (14), but she had never lived in an endemic area. She was not extensively exposed to forest
environment, did not hunt, did not consume forest fruits, had no contact with (domestic) animals, did not work in her garden and bought her fruits and vegetables in regular supermarkets.

Lastly, serological results do not necessarily correspond with disease. *E. multilocularis* is known to cross-react with *E. granulosus* in serological assays\(^5\) and *E. granulosus* assays are therefore used in the *E. multilocularis* work-up. Serology results in our patient were weakly positive, illustrating that imaging is essential to exclude *E. multilocularis* infection. We do not have an explanation for the weak serology results; it might be due to a difference in the *E. multilocularis* strain in our patient. There is no serological evidence of widespread human contact with *E. multilocularis* in the Netherlands yet\(^11\), but these results should be interpreted with caution.

Although *E. multilocularis* is prevalent in foxes in the southern part of the Netherlands, no human *E. multilocularis* case acquired in the Netherlands has been described so far. Recently three *E. multilocularis* cases have been described in a part of Belgium which borders the southern part of the Netherlands, where our patient resides \(16\). Sequencing results could not define the exact geographical location of acquisition of the *E. multilocularis* strain in our patient but the Netherlands belongs to the possibilities. Although due to the long incubation period (5–15 years), the time and place of infection is hard to determine retrospectively, considering the duration of exposure in this patient, domestically-acquired *E. multilocularis* seems most likely.

This patient represents the first reported case of *Echinococcus multilocularis* acquired in the Netherlands. Surveillance data originating from foxes in the southern part of the Netherlands have shown that *E. multilocularis* spreads by 2.7 km per year in a northern direction and based on the spreading of *E. multilocularis* in foxes in this area in the Netherlands\(16\) the human risk in Limburg province is estimated to be three human cases by 2018\(17\). We therefore believe that *E. 
multilocularis is an emerging pathogen in Western Europe and clinicians should consider this diagnosis when confronted with (asymptomatic) liver lesions.


Figure 1. Computed tomogram of the liver post intravenously-administered iodine containing contrast, scanned in portovenous phase. In this transversal plane of the liver, two hypodens lesions are seen (arrows).
Figure 2. Histopathology of liver lesion (100x, H&E stain). One central cyst and budding daughter cysts with a trilayered membrane wall (arrows). No protoscolices, hooklets or calcaneous corpuscles are seen, however the image is still compatible with echinococcosis.