CLINICAL EPIDEMIOLOGY OF ECHINOCOCCOSIS IN HUNGARY WITH SPECIAL FOCUS ON EMERGING HUMAN ALVEOLAR ECHINOCOCCOSIS

PhD thesis

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Dedication

For the memory of my grandfather, László Lovász MD, Ph.D who showed me the way how to be a medical doctor and for the memory of my colleague and excellent infectologist, András Máthé MD, Ph.D

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List of abbreviations

2nd second

^{18}F-FDG-PET/CT fluorin18-fluorodeoxyglucose positron emission tomography computerised tomography

ABZ albendazol

AE Alveolar echinococcosis

AIDS acquired immunodeficiency syndrome

AL adventitial layer

ALP alkaline phosphatase

ALT alanine aminotransferase

AST aspartate aminotransferase

BMZ benzimidazole

CE Cystic echinococcosis

CI confidence interval

cm centimeter

CT computed tomography

d ductus

DALY disability adjusted life year

DNA deoxyribonucleic acid

ECDC European Centre for Disease Prevention and Control

EEA European Economic Area

E.g. *Echinococcus granulosus*

e.g. example given

ELISA enzyme linked immunosorbent assay

E.m. *Echinococcus multilocularis*

EPI endoscopic and percutaneous intervention

ERCE European Register of Cystic Echinococcosis

ERCP endoscopic retrograde cholangio-pancreatography

et al. and others (Latin: *et alia*)

FNAB fine needle aspiration biopsy

G genotype

GGT gamma-glutamyltransferase

GL germinal layer

G/l Giga per liter

H&E haematoxylin and eosin

HCC hepatocellular carcinoma

HLA Human leukocyte antigen

ICC intrahepatic cholangiocarcinoma

IFN α interferon alpha

IFN γ interferon gamma

IgG immunoglobulin G

IL 12 interleukin 12

IWGE Informal Working Group on Echinococcosis

kDa kilodalton

kg kilogram

LL laminated layer

mAb monoclonal antibody

MBZ mebendazol

mg milligram

MHC major histocompatibility complex

mm millimeter

µmol/l micromol per liter

MRCP magnetic resonance cholangio-pancreatography

MRI magnetic resonance imaging

mtDNA mitochondrial deoxyribonucleic acid

neg negative

No number (Latin: *numero*)

NUTS Nomenclature of Territorial Units for Statistics

OLT orthotopic liver transplantation

PAIR puncture aspiration injection reaspiration

PAS Periodic acid-Schiff

PCR polymerase chain reaction

pers. comm. personal communication

PT percutaneous treatment

PTC percutaneous transhepatic cholangiography

PTD percutaneous transhepatic drainage

PZQ praziquantel

RIEC Italian Registry of Cystic Echinococcosis

S liver segment

sebi serum bilirubin

s.l. *sensu lato*

SPEM small particles of *Echinococcus multilocularis*

spp. species (plural)

s.s. *sensu stricto*

Th T helper

TNF α tumor necrosis factor alpha

tx treatment

U/l Unit per liter

US ultrasound

v vena

viz. namely (Latin: *videlicet*)

vs. against (Latin: *versus*)

WW watch and wait

WHO World Health Organization

I. Introduction

I.1. Endemic helminth infections in Hungary with severe and potentially lethal outcome

Even nowadays, easily treatable helminth infections with high prevalence and generally good prognosis can cause severe or life-threatening complications if left unnoticed and thus untreated. For example, enterobiosis is currently the most prevalent human helminthosis in Hungary based on unpublished data derived from database management systems of selected reference laboratories for parasitic diseases (National Reference Laboratory for Human Parasitic Diseases, National Public Health Center, Budapest; Laboratory of Clinical Microbiology, Central Hospital of Southern Pest, Institute of Hematology and Infectious Diseases, Budapest). In a recent case of unnoticed enterobiosis, egg-laying female *Enterobius vermicularis* ascending in the genital tract of a woman led to granulomatous salpingitis and tuboovarial abscess necessitating a total hysterectomy with bilateral salpingo-oophorectomy (1). Historically, trichinellosis was the most dangerous and potentially lethal helminthosis in Hungary due to its neurological and cardiological sequale (2,3,4). The last lethal case was reported in 1970 (4) and the last Hungarian outbreak in 2009 (5). The presence of this nematode in wild animals in the territory of Hungary (6) and imported human cases from highly endemic neighbouring countries (e.g. Romania) (7) still have to be considered. Among the currently notifiable helminth infections (8), strongyloidosis also has a long history in Hungary (8,9). Nosocomial outbreaks in pediatric institutions caused the deaths of many children as late as the 1950s (10,11). Although the annual incidence of strongyloidosis has decreased significantly in Hungary during the past several decades (4), sporadic autochtonous cases in adult patients in the recent past are well documented (12,13,14). The significance of strongyloidosis has also increased in parallel with the growing number of immunocompromised patients in connection with its poor outcome in this cohort of individuals (15,16,17,18). Until the first recognition of alveolar echinococcosis (AE) in a Hungarian patient (19), the medical term ″Echinococcosis″ in Hungary was synonymous with cystic echinococcosis (CE) caused by *Echinococcus granulosus sensu lato* (*E.g.s.l.*). CE has been a well-known parasitic disease in Hungary since the 19th century (20,21) and currently remains the most prevalent reportable zoonotic helminthosis in the country, being endemic in several

regions with considerable disease burden (4,22). The last presumably CE-associated death was reported in 2004 (4), and CE is persistently the major helminthic infection in our country requiring hospital admission and treatment (4,22). Much less is known about the Hungarian occurence and characteristics of alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* (*E.m.*), one of the most pathogenic zoonoses in the temperate and arctic region of Europe (23) with a potentially lethal course.

I.2. Microbiology of *Echinococcus granulosus sensu lato* and *Echinococcus multilocularis*

E.g. and *E.m.* are small (just a few millimeters long) tapeworms belonging to the Phylum Platyhelminthes, Class Cestoda, Order Cyclophyllidea, Family Taeniidae, Genus *Echinococcus*. The human pathogen *Echinococcus granulosus sensu lato* is a species cluster, which unites ten genotypes: *E. granulosus sensu stricto* (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6/7, G8, G10), and *E. felidis*. The G6/7 cluster is also called *E. intermedius* (24). These genotypes have different ranges of host infectivity and biological features, and several (but not all) of them can cause human CE. The great majority of human CE infections are caused by *E.g. sensu stricto* (*E.g.s.s.*). *E.m.* is a distinct species causing AE (25,26). All Echinococcus species complete an indirect two-host life cycle in which the sexually reproducing adult is a predominantly self-fertilizing (27,28) hermaphrodite, and the larval metacestode stage proliferates asexually. After ingestion of viable protoscoleces by the carnivorous definitive host, the adult worm develops in the small intestine, attaching in the crypts of Lieberkuhn. All metabolic interchange of the worm takes place across the syncytial outer covering, - the *tegument* – which anteriorly possesses a specialized attachment organ called, the *scolex*, with two rows of hooks and four muscular suckers. A narrow neck region separates the scolex from the segmented *strobila*, which consists of a number of reproductive units (*proglottides*) containing fertilized eggs (25). Each egg is immediately infectious to the intermediate host and contains a fully developed embryo (oncosphere), when they are voided with the faeces of the definitive host. Maximum diameter of spherical or ovoid taeniid eggs usually ranges from 30 to 50 micrometers, they possess several outer membranes (e.g. the *embriophore*) (29,30,31) affording protection to the *oncosphere* (25) and extreme resistance, supporting the survival of eggs in the environment (32,33,34,35). *E.m.* eggs, however, are specifically sensitive to

desiccation and high temperature (35), an adaptation strongly associated with the epidemiology and geographical distribution of AE. After the ingestion of viable eggs by the intermediate host, they hatch in the stomach and small intestine. The oncosphere attains the liver, - its site of predilection - by the portal venous and lymphatic circulation, and postoncospheral development subsequently proceeds, leading to the formation of the metacestode (25).

The metacestode of *E.g.* is typically a single-chambered, unilocular, ovoid or spherical, fluid-filled cyst which consist of an inner germinal layer (GL) supported externally by a thick acellular laminated layer (LL). This larval endocyst is surrounded by a host-produced fibrous adventitial layer (AL), - the so-called pericyst. The cyst shows expansive growth by concentric enlargement. Asexual proliferation of the GL and brood capsule formation take place entirely endogenously. Pouching of the cyst walls may occur, giving rise to secondary chambers communicating the central cavity (36). Eventually, incomplete septa separates secondary cyst chambers, and the primary cyst can collapse and, form groups or clusters of small cysts of different sizes. In a human host where large cysts may develop, daughter cysts may form within the primary cyst (37). The undifferentiated cells of the GL are proliferative and are responsible for the formation of brood capsules. Within the lumen of these capsules, a repetition of the asexual budding process takes place, leading to the production of numerous protoscoleces. Fully developed protoscoleces are characterized by the possession of hooks on the invaginated rostellum (25).

In contrast to *E.g.*, the metacestode of *E.m.* is a multivesicular, infiltrating structure without a limiting host-tissue barrier (adventitial layer, or AL), consisting of numerous small vesicles embedded in a dense stroma of connective tissue (38). The larval mass usually contains a semisolid matrix rather than fluid. Proliferation occurs both endogenously and exogenously, and is attributable to the GL (37,39,40). The metacestode consists of a network of filamentous solid cellular protrusions of the GL which are responsible for infiltrating growth, transforming into tubelike and cystic structures (40,41,42). The detachment of germinal cells from infiltrating protrusions and their subsequent distribution via the lymph or blood can give rise to the distant metastatic foci characteristic of *E.m.* (40,41,43,44). *E.m.* develops rapidly in its natural

intermediate host, producing protoscoleces in only two to four months, an adaptation to the short-lived arvicoline rodents it utilizes (45,46). Thereafter, proliferation of vesicles is curtailed and there is little if any further increase in size (47). In humans, as in aberrant intermediate hosts, proliferation continues indefinitely although there are few if any protoscoleces produced (37,47). The larval mass proliferates peripherally and at the same time regressive changes occur centrally (47,48), and an enlarging mass of necrotic tissue with a thin zone of viable proliferating parasite is produced. The term 'alveolar hydatid' was initially used to describe this form of growth (25).

I.3. Life cycle patterns of *Echinococcus granulosus sensu stricto* and *Echinococcus multilocularis*

E.g.s.s. [G1-G3] is transmitted predominantly in domestic life cycles involving dogs (*Canis lupus familiaris*) as definitive hosts and livestock animals as intermediate hosts. Sheep, cattle, and domestic pigs are among the most important livestock species, but numerous other species can contribute as competent intermediate hosts. Infection of dogs occurs by purposeful feeding of contaminated offal after home slaughter (49). Concerning domestic transmission, the incidence of human infection with *E.g.s.s.* coincides with regions of extensive and traditional sheep farming in the Middle East, northern Africa, and southern Europe (50,51) a fact that highlights the importance of the sheep-dog cycle for the abundance and infection pressure of the parasite (49).

E.m. The sylvatic life cycle of *E.m.* most typical for rural areas in central Europe involves the red fox (*Vulpes vulpes*) and the common vole (*Microtus arvalis*) as the principal definitive and intermediate hosts. This host-parasite system seems to be most frequent in landscapes fragmented by traditional agricultural land use, which is typical for most parts of central Europe (52). The red fox has settled populations living in close proximity to humans in urban and periurban areas (53,54).

Transmission to humans Humans are intermediate hosts for *Echinococcus* parasites and become infected by the eggs of *Echinococcus* spp. when exposed to infected definitive hosts (eggs on animal hair), via the contaminated environment (hand-to-mouth transmission), or by consuming contaminated food (vegetables, water) (55). In the synanthropic cycle of *E.g.s.s.*, close contact with infected dogs is evidently a risk

factor for acquiring CE. The domestic dog is a proven definitive host of *E.m.* as well (56,57). Despite the extremely low *E.m.* prevalence in the general dog population (58), the large number of dogs has led to estimates, that they may contribute up to 19 % of the *E.m.* egg production in urban or periurban areas of Central Europe (59).

I.4. Overview of the epidemiology of cystic echinococcosis and alveolar echinococcosis in Hungary

I.4.1. Echinococcus granulosus sensu lato infection in animal host species

There is no available data in Hungary on the prevalence of *Echinococcus* spp. in domestic dogs as a major definitive host of *E.g.*, or as a minor definitive host of *E.m.* (pers. comm. Sréter, 2019). Regarding intermediate hosts, during the 2015-2018 parasitological investigation of samples derived from Hungarian slaughterhouses, 62 livestock animals (cattle, sheep, swine) were found to be infected with *Echinococcus granulosus s.l. Echinococcus intermedius* [G6/G7] (n = 31) and *Echinococcus granulosus s.s.* [G1-G3] (n = 2) were identified in swine. In cattle, only *E. granulosus s.s.* (n = 20) was detected. *E. granulosus s.s.* (n = 7) was the dominant species in sheep; nevertheless, *E. intermedius* was also identified in two animals (60). The genetic variability of *E. granulosus s.s.* has been described previously through the identification of Hungarian haplotypes by mitochondrial deoxyribonucleic acid (mtDNA) analysis (61). The genetic diversity and haplotype network of *E. granulosus s.s.* were similar to that observed in some other countries of Eastern Europe. Based on the number of animals butchered in the slaughterhouses involved, the rate of infection was 0.013% in sheep, 0.007% in cattle, and 0.001 % in swine (60).

I.4.2. Echinococcus multilocularis infection in animal host species

Throughout temperate zone of Europe, red foxes are considered the principal definitive hosts of *E.m.* (57, 62). Following political changes in 1990, considerable changes in land use were observed in the former communist Central Eastern European countries due to the disintegration of large state farms. A decrease in the annual hunting

index resulting from the fall of fox fur prices, and the initiation of antirabies vaccination for foxes in Hungary caused a corresponding increase in the fox population (63,64,65) and probably the coincidental increase of *E.m.* prevalence. Urbanization of foxes has also been observed in Hungary (64) as well as other European countries (53,54) which is a known risk for human infections. *E.m.* infection of red foxes in Hungary was first confirmed by Sréter et al. in 2002, in two counties of the Northern Mountain Range: Nógrád and Borsod-Abaúj-Zemplén, close to a known *E.m.*–endemic region: the Muránska Planina Mountains in Slovakia (65). Later systematic studies were conducted on the prevalence and geographical distribution of *E.m.*-infected red foxes in Hungary between 2008 and 2013, showing an overall *E.m.* prevalence of 10.7 % in 2008-2009 and 7.9% in 2012-2013. By 2013 the parasite had been detected in almost every Hungarian county, with a higher prevalence in the northern ones (66,67,68). Environmental determinants influencing *E.m.* distribution and genetic characteristics of isolated strains were also studied. *E.m.* eggs are sensitive to high temperature and desiccation (35), thus the climate of Hungary as characterized by its warmer mean annual temperature and low annual precipitation, is considerably different and less favourable for *E.m.* than that of the historically known endemic Alpine areas of Europe (67). Molecular genetic analysis supported that Hungary should be considered as a peripheral area of a single European focus, where the dispersal of foxes resulted in the spreading of the parasite from one county to another (66).

The golden jackal (*Canis aureus*), a definitive host of *E.m.*, occurs predominantly in southeast Europe (69) but has persistently been detected in Hungary as well. *E.m.*-infected golden jackals were repeatedly reported from Somogy county, southwest Hungary (70,71) with an *E.m.* prevalence similar to what was seen in red foxes during a period between 2008 and 2009 (9.1% vs. 10.7%) (66,70,71). A recent study conducted between 2016 and 2020 in the same territory found a 15.6 % *E.m.* prevalence in golden jackals (71) which coincides with newly detected human AE cases in Somogy county (72).

The major intermediate host for the *E.m.* is the common vole (*Microtus arvalis*) which is widespread in Europe and is a preferred prey of foxes and golden jackals (73,74,75,76,77,78,79). The field vole (*Microtus agrestris*) and other Arvicolid vole species are also susceptible to *E.m.*, their role in the life cycle is confirmed, and these

vole species occur widely in Hungary (80). Indirect evidence suggests the role of the European water vole (*Arvicola amphibius*) in natural *E.m.* transmission in Hungary (67). To date, a comprehensive study of the prevalence of *E.m.* among natural intermediate hosts in Hungary has not been conducted. Among livestock, swine were found to be infected with the parasite in Hungary. Swine cases are possible indicators of environmental egg contamination and the risk of human exposure to *E.m.* eggs (60).

I.4.3. Officially reported human Echinococcosis cases in Hungary between 2000 and 2014

Regarding human infections, Echinococcosis is listed among the reportable helminthic diseases, such as schistosomiasis, taeniasis, strongyloidosis, trichinellosis, and ancylostomiasis (8) and is subject to surveillance according to European legislation (81). Data on annual numbers of Echinococcosis cases are available beginning from 1960 (82). Between 2000 and 2014, a total of 116 cases of Echinococcosis were officially reported to Hungary's National Public Health Centre. Beginning in 2007, cases have been reported based on the application of European Centre for Disease Prevention and Control (ECDC) "Echinococcosis" case definitions (83). After the official report on the first confirmed AE case in 2006, two retrospectively confirmed and one probable AE case have been registered up to 2014. The remaining 112 cases were regarded as confirmed or supposed CE, as with all „Echinococcosis" cases before 2000. Between 2000 and 2014, Echinococcosis was the most prevalent reportable helminthic disease in Hungary, with an average of 7.73 cases per year. Most of the officially reported cases derived from Hajdú-Bihar county (n=20), Jász-Nagykun-Szolnok county (n=13) and Baranya county (n=12). We have data available on the number of patients requiring hospital care from 2000 to 2010. The annual hospitalization rate of Echinococcosis cases was over 50 % (range: 50%-85.7%) (4).

In Hungary, suspected Echinococcosis cases require a confirmatory laboratory test from the National Reference Laboratory for Human Parasitic Diseases, and all samples which are positive for *Echinococcus* are transmitted there for confirmation (8). Laboratory confirmation of Echinococcosis in Hungary currently means [I] detecting *Echinococcus* specific antibodies in the patient's sera by a highly sensitivite enzyme

linked immunosorbent assay (ELISA) or indirect hemagglutination test as a first method, and then by the application of a second highly specific Western blot as a confirmatory test (Ldbio Diagnostics, Lyon, France) or [II] the detection of parasite specific structures (protoscolex, hooklets, LL) in a clinical specimen. Most of the officially reported cases were diagnosed using serology and do not include infections confirmed exclusively by histopathology or by imaging methods; therefore, both forms of Echinococcosis are believed to be underreported. Furthermore, seronegative cases remain undetected by the serology-based official surveillance system. Another basic limitation was the lack of an integrated hospital medical information system in Hungary, which hampered nationwide data collection that could have supported the distinction between CE and AE cases and made it possible to estimate the real burden and ratio of the two disease forms (22). Although probable and retroactively-recognized AE has dated back to 2003, the first unquestionably autochtonous case in Hungary was reported in 2016 (84). This recent work by our group, together with pioneering animal studies (60,65,66,67,70,71), aims to confirm that AE is a newly endemic – although still rare - helminthic zoonosis in this country.

I.5. Summary of the clinical course and management of cystic echinococcosis

I.5.1. Clinical course of cystic echinococcosis

The liver is the most frequent location of hydatid cysts (CE), representing approximately 70% of cases; lungs are the second most common location. In the minority of cases CE can present in any organ (e.g. spleen, brain, bone, skin) (26). CE cysts can be solitary or multiple. Cysts may grow 1-50 mm per year or persist without changes for years (85,86,87). Based on imaging studies, the cysts progress from a fluid-filled unilocular cavity to a pseudosolid, eventually calcified lesion through well-characterized stages (Fig 1). The sequence of cyst development, however, is still poorly understood (88). During the course of infection CE can remain entirely asymptomatic. Clinical symptoms usually occur when the cyst compresses or ruptures into neighbouring structures (obstruction of bile ducts, parasitic embolism) (86,87). Bacterial superinfection of ruptured cysts and cysto-biliary fistulas are common

complications which can arise before and after surgical, endoscopic, or percutan interventions (89,90). Secondary CE can develop from spillage of cyst content (e.g. protoscoleces) to the peritoneum after spontaneous or procedure-associated cyst rupture (91).

I.5.2. Diagnosis of cystic echinococcosis

The diagnosis of CE is based on clinical findings (see I.5.1.), imaging techniques, and serology. Confirmation of diagnosis is possible either [I] by the direct visualization of *Echinococcus* specific structures (PAS positive LL, protoscolex, double-refracting hooklets) during microscopic examination of cyst content, or [II] being histopathology compatible with CE, or [III] the detection of pathognomic macroscopic morphology of a cyst in surgical specimens (87).

Ultrasound (US) is the best imaging modality in the diagnostical work-up of echinococcal cysts in abdominal locations (92). Currently the World Health Organization Informal Working Group on Echinococcosis' (WHO IWGE) standardized US classification of CE (Fig 1) is universally applied to distinguish the different stages of CE based on radiomorphological characteristics. In particular stages or documented transitions between stages, US examination itself is enough to confirm CE. Utilization of this system will not only support a standardized diagnosis, but also has major implications on clinical decision-making, treatment, and follow-up of CE during the stage-specific approach (86,87,93).

Serology has a supportive role in diagnosing CE. Major limitations of the recently used serological tests are cross-reactivity with other parasitic (e.g. taeniid) antigens, and highly variable sensitivity depending on the number, size, location, and stage of cysts (93,94).

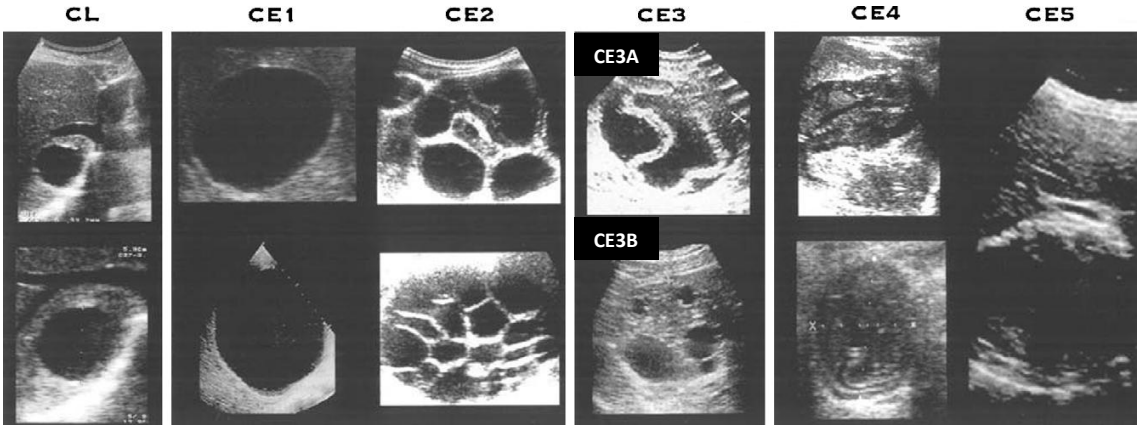

Fig 1 WHO IWGE standardized classification of CE CL – cystic lesion CE1 – unilocular simple cyst with uniform anechoic content CE2 - multivesicular, multiseptated cysts in which daughter cysts fill the unilocular mother cyst CE3A – anechoic content with detachment of laminated membrane from the cyst wall visible as floating membrane CE3B – unilocular cyst daughter cysts and echoic areas CE4 – heterogenous hypoechoic or dyshomogenous degenerative contents CE5 – characterized by a thick-calcified wall which is arch shaped, producing a cone-shaped shadow (87)

I.5.3. Treatment and follow-up of cystic echinococcosis

Treatment of CE is complex and four main modalities (antiparasitic drug treatment, percutan treatments, surgery, watch and wait) can be choosen based on the carefull evaluation of cysts (number, size, location, WHO IWGE stage, neighbouring structures), characteristics of the patient and the capability of the given healthcare setting. Besides these main therapeutic approaches, endoscopic retrograde cholangio-pancreatography (ERCP) with spinchterotomy and stent insertion or nasobiliary drainage is an option in the management of hepatic hydatid cysts with biliary complications, especially for high surgical risk patients (95,96).

Drug therapy Benzimidazoles (BMZs) have diverse biological activity, their favourable selectivity ratio makes them excellent antimicrobials (97). Antimicrobial, and thus antihelminthic activity is based on the inhibition of DNA synthesis through the striking structural resemblance to the tubulin binding colchicine which disrupts mitosis by inhibiting the polimerization of tubulins and deactivating the division spindle (98,99,100,101). *E.g.* and *E.m.* has three major β-tubulin isoforms (Tub-1, Tub-2, Tub-3) as target molecules for BZDs (102,103,104). Albendazol (ABZ) is the preferred drug in the antiparasitic treatment of CE, given orally at a dosage of 10-15 mg/kg/day, in two

divided doses, continuously, for as long as three to six months. An average cure rate of 30% can be expected, and intermittent administration should be avoided (86,87). Mebendazol (MBZ) may be used as an alternative. To increase intestinal absorption, both drugs need to be taken with fatty foods. Hepatic and haematologic toxicities are the most frequent serious adverse effects associated with benzimidazole (ABZ and MBZ) administration. For patients receiving BMZ therapy, it is generally recommended to have liver enzymes and complete blood cell counts monitored. BMZs must be used with caution in patients with chronic hepatic disease and avoided in those with bone marrow depression. Alopecia is a recognized side effect in patients with chronic cholestasis and/or portal hypertension (87).

Percutan treatment methods Percutan treatment methods can also be applied, replacing surgery in selected cases. The classical method is the PAIR technique (105,106): puncture of cyst, aspiration of cyst content with subsequent injection of scolicid agent and reaspiration of this fluid aiming to destroy the germinal layer. PAIR is a successful method in the CE1 and CE3A stages (86,87). Other newly developed percutan methods – the so-called standard catheterization technique (107,108) and the modified catheterization technique (109) - are also employed in some centers.

Surgery In case of liver CE, surgery is indicated for: [I] removal of large CE2-CE3B cysts, [II] single liver cysts, situated superficially that may rupture, [III] infected cysts, or [IV] cysts communicating with the biliary tree if percutaneous treatments (PTs) are not available, and [V] cysts exerting pressure on adjacent vital organs. Parasitic material should be removed as much as possible (87). Total pericystectomy is a non-anatomical liver resection around the cyst wall; cystectomy means cyst de-roofing and cyst content evacuation without removal of the pericyst.

Watch and wait (WW) The so-called WW approach means that some inactive cysts do not require any treatment if uncomplicated, namely CE4 and CE5.

CE follow-up visits, including US examination, should be done every three to six months initially, and annually after stabilisation. One of the major problems of CE is the frequency of relapses (87).

I.6. Summary of the clinical course and management of alveolar echinococcosis

I.6.1. Clinical course of alveolar echinococcosis

Initially, metacestodes of *E.m.* develop almost exclusively in the liver, from foci of a few millimeters to areas of 15–20 cm or more in diameter, sometimes with a central necrosis. From the liver, the larvae spread to other organs by infiltration or metastasis formation. AE is characterized by an initial asymptomatic incubation periodof 5 to 15 years and a subsequent chronic course. Symptoms are primarily cholestatic jaundice (33.3 %) and/or abdominal pain (33.3 %). In 33.3 % of patients, AE is found incidentally on investigation of various symptoms such as: fatigue and weight loss, hepatomegaly and abnormal US, or routine laboratory findings (26, 87). AE symptoms are primarily dependent on the location and secondarily on the size of the lesion. Centrally located hepatic lesions will present with cholestasis, jaundice, and sometimes recurrent cholangitis, whereas lesions located in proximity to the hepatic veins and/or inferior vena cava will lead to a Budd-Chiari-like presentation with or without inferior vena cava obstruction. A parasitic mass in this location, often leads to metastatic lesions in the lungs, heart, or other organs. Lesions located in the periphery of the liver remain asymptomatic for a long time and can become very large prior to becoming symptomatic. Poor vascularization of these large lesions favours the development of large necrotic cavities, which are at high risk for secondary bacterial infection and/or abscess formation. The main causes of death, due to AE, are either septic shock, complications after major liver surgery, hepatic failure, cerebral AE, or gastrointestinal bleeding due to secondary biliary cirrhosis (87,110).

Under the influence of the host's immune system, the metacestode can degenerate and die; calcified dead lesions can be identified during mass screening campaigns (111,112,113,114).

Genetic factors influencing AE The type of immunoreactions, the severity and the natural course of AE infection are associated with major histocompatibility complex (MHC) antigens, and thus the human leukocyte antigen (HLA) haplotype of the human host. HLA-DRB1*11 can protect from AE resulting in abortion of the infection, while HLA-DQB1*02 predisposes to progressive disease (115).

I.6.2. Importance of secondary immunodeficiency in the course of alveolar echinococcosis

AE patients exhibiting an advanced stage of the disease have repeatedly been subjected to liver transplantation as a curative treatment option. Observations of those patients, who received immunosuppressive agents to prevent liver rejection, confirmed the increased susceptibility to *E.m.* growth and metastasis formation in humans upon impaired immune responsiveness (116,117). In *E.m.* infected individuals with impaired immunity (e.g. AIDS), other immunodeficiencies, or undergoing immunosuppressive therapy (following organ transplantation or to treat malignancies or chronic inflammatory disorders), the metacestode proliferation appears uncontrolled, leading to a very rapidly progressing disease status (118).

I.6.3. Diagnosis of alveolar echinococcosis

Diagnosis of AE is based on clinical findings (see I.6.1.), epidemiological data, imaging techniques, histopathology and/or nucleic acid detection, and serology (87).

Imaging Typical US findings (70% of cases) include [I] juxtaposition of hyper- and hypoechogenic areas in a pseudo-tumour with irregular limits and scattered calcification (Fig 4), and [II] pseudo-cystic appearances due to a large area of central necrosis being surrounded by an irregular hyperechogenic ring. Less typical features (30% of cases) include [I] haemangioma-like hyperechogenic nodules as the initial lesion, and [II] a small calcified lesion due to either a dead or a small sized developing parasite (110,119). Computed tomography (CT) gives an anatomical and morphological characterization of lesions and best depicts the characteristic pattern of calcification (26, 87). In cases of diagnostic uncertainty, magnetic resonance imaging (MRI) may show the multivesicular morphology of the lesions, - thereby supporting the diagnosis - and is the best technique for studying extension to adjacent structures. For preoperative evaluation, magnetic resonance cholangio-pancreatography (MRCP) has replaced percutaneous transhepatic cholangiography (PTC) for studying the relationship between AE lesions and the biliary tree (119). Initial radiological examination to exclude pulmonary and cerebral AE is recommended (87). Several advanced classifying systems

have been developed over time for US (120), for MRI (121) and for CT (122), which are based on the characteristic imaging appearance of different types of AE lesions. Recently a natural progression model of AE lesions has been proposed based on a proven correlation between CT characteristics (*Echinococcus multilocularis* Ulm Classification for Computed Tomography) and histological features of different lesion types. Further investigations are needed to determine its real clinical impact regarding therapy and prognosis (123).

Serology Serology is a useful tool in the diagnosis of AE. The use of purified and/or recombinant, or in vitro-produced *E.m.* antigens has a high diagnostic sensitivity reaching 90–100%, and a specificity of 95–100%. Immunoblotting tests may be used for confirmation or as a firstline investigation (87). Currently in Hungary the distinction between CE and AE and the preliminary probable diagnosis of AE is based on the Western blot method (LdBio Diagnostics, Lyon, France). Em18 and Em16 are two putatively specific antigenic components of protoscoleces of *E.m.*, which were detectable from sera from active AE patients (124). The immunoblot assay uses a whole larval extract from *E.m.* as the antigen on the nitrocellulose strip. In the event of AE infection, the sera specifically bind to antigens of 16 kDa and 18 kDa, as sharp bands beside the genus-specific (26, 28 kDa) bands, resulting in a P3 band pattern which is specific for AE (Fig 2). This test allows correct differentiation between CE and AE patients in 76% of cases (125).

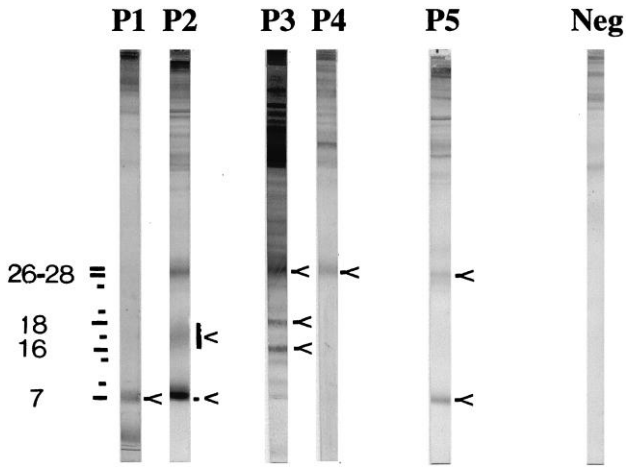

Fig 2 Echinococcus Westernblot IgG. Five immunoblot patterns (P1 through P5) are obtained with cystic and alveolar echinococcosis sera. Most of the significant bands are indicated by arrows. Molecular sizes

(in kilodaltons) are indicated on the left. Neg, represents the negative-control serum. Reprinted by courtesy of LDBIO Diagnostics (123).

Histopathology Histopathological examination of tissue specimens resected or biopsied from AE patients shows characteristic multiple, slender, Periodic acid-Schiff (PAS) positive laminated layers. For *E.m.*, primary vesicles remain small in size and rapidly start to form external buds that proliferate as either still connected or sometimes detached secondary vesicles. Opposite to the protoscolex production of GL experienced in natural intermediate hosts, during human *E.m.* infection, protoscoleces and double-refractering patognomic hooklets are rarely detectable in the AE lesion (126,127,128). Microscopically, typical AE lesions are characterized by an extensive conglomerate of small vesicles, each consisting of an inner GL composed of a thin coat of syncytial cells, mainly primary stem cells; the GL is surrounded by a thin acellular PAS-positive LL (129). Parasite proliferation is usually accompanied by a periparasitic granulomatous host response, as a giant-cell foreign body reaction including vigorous synthesis of fibrous and connective tissue in the close vicinity of the LL of the metacestode. The central part of the metacestode tissue shows necrosis with calcification (128,130). A recent study on the pathology of Echinococcosis identified six morphologic criteria, which sufficiently discriminated between CE and AE on conventionally stained (H&E, PAS) histological samples, the size of the smallest (CE/AE: >2/≤2mm) and largest cysts (CE/AE: >25/≤25mm), thickness of the laminated layer (CE/AE: >0.15/≤0.15mm) and pericystic fibrosis (CE/AE: >0.6/≤0.6mm), striation of the laminated layer (CE/AE: moderate-strong/weak), and the number of cysts (CE/AE: ≤9/>9) (131).

Immunohistochemistry (IH) and polymerase chain reaction (PCR) In addition to the above mentioned histopathological features, confirmation of AE from tissue samples and clear differentiation from CE is possible by detecting the *E.m.*-specific antigen with IH using the monoclonal antibody Em2G11 (128). The monoclonal antibody Em2G11 recognizes an epitope of a mucin-type carbohydrate antigen called Em2 (132) which is a major antigen of the LL of *E.m.* metacestode (133). Confirmation of diagnosis is also possible by the detection of *E.m.*-specific nucleic acid sequence(s) with PCR (134); DNA for this purpose can be extracted from formalin-fixed and paraffin embedded tissues (135).

I.6.4. WHO classification of alveolar echinococcosis

The WHO IWGE PNM classification system of AE, based on imaging findings denotes the extension of the parasitic mass in the liver (P), the involvement of neighbouring organs (N), and metastases (M) (Table 1). It enables the standardized diagnostic evaluation of an AE lesion over time and is useful for the quality control of treatment strategies and patient follow-up. Application of the PNM system makes study results comparable on an international level (26,87,136).

Table 1 PNM classification of alveolar echinococcosis (87)

P	Hepatic localisation of the parasite
PX	Primary tumour cannot be assessed
P0	No detectable tumour in the liver
P1	Peripheral lesions without proximal vascular and/or biliar involvement
P2	Central lesions with proximal vascular and/or biliar involvement of one lobe[a]
P3	Central lesions with hilar vascular or biliar involvement of both lobes and/or with involvement of two hepatic veins
P4	Any liver lesion with extension along the vessels[b] and the biliary tree
N	Extra-hepatic involvement of neighbouring organs [diaphragm, lung, pleura, pericardium, heart, gastric and duodenal wall, adrenal glands, peritoneum, retroperitoneum, parietal wall (muscles, skin, bone), pancreas, regional lymph nodes, liver ligaments, kidney]
NX	Not evaluable
N0	No regional involvement
N1	Regional involvement of contagious organs or tissues
M	The absence or presence of distant metastasis [lung, distant lymph nodes, spleen, CNS, orbital, bone, skin, muscle, kidney, distant peritoneum and retroperitoneum]
MX	Not completely evaluated
M0	No metastasis[c]
M1	Metastasis

[a] For classification, the plane projecting between the bed of the gall blader and the inferior vena cavadivides the liver in two lobes.
[b] Vessels mean inferior vena cava, portal vein and arteries.
[c] Chest X-ray and cerebral CT negative.

I.6.5. Treatment of alveolar echinococcosis

Treatment of AE requires a multidisciplinary approach with careful evaluation of imaging data. In the treatment of AE, benzimidazoles are mandatory in all patients, temporarily (for at least two years) after complete resection of the lesions, and for life in unresectable cases. Interventional procedures should be preferred to palliative surgery

whenever possible and radical surgery is the first choice in all cases suitable for total resection of the lesion(s) (87).

Drug treatment Long-term BMZ treatment for several years is mandatory in all inoperable AE patients and following surgical resection of the parasite lesions. The first drug of choice is ABZ given orally at a dosage of 10–l5 mg/kg/day, in two divided doses, with fat-rich meals. In practice, a daily dose of 800 mg is given to adults, divided into two doses; intermittent treatment should not be used. MBZ is an alternative. Because of the known adverse effects of BMZ (detailed previously in I.5.3), monitoring of blood cell count and liver enzymes is mandatory in all cases. A more than five-fold increase in amino transferase levels and/or a leukocyte count lower than 1 G/l indicates hepato- and myelotoxicity respectively and warrants withdrawal of drug treatment (87,137). Previously ABZ was thought to be parasitostatic (138), thus regression and nonprogression due to the suppresive effect of continuous ABZ administration is regarded as a treatment success (137). In fact, some newer evidence suggests the possible parasitocid effect of longterm ABZ treatment in vitro and in vivo (139) and in disseminated (140) and/or immunosuppressed AE cases (141).

Surgery As the parasite's growth resembles a malignant tumour, only radical surgery can be curative. Excision of the entire parasitic lesion is recommended with procedures and techniques generally applied in oncological surgery with a 2 cm safety margin, and requires a team specialized in hepatobiliary surgery (87,138,142,143). After radical resection, surgical specimens should be graded as complete resection (R0), or incomplete or palliative resection (R1: microscopic residuals at resection margins; R2: macroscopic residuals). Disseminated disease with multiple organ involvement does not rule out radical liver resection, but curative treatment has to meet the criteria for R0 resection. The patient should receive two years of postoperative antiparasitic ABZ therapy after radical surgery and should be followed at least for ten years. Preoperative ABZ is not recommended. In fact, ABZ is often given after diagnosis for a longer time prior to surgery (87, 138, 142, 143). Palliative non-radical (R1, R2) resection of AE lesions should be avoided; survival chances of these patients are worse than of those who were treated with long-term BMZ because of unresectable lesions. In certain cases (e.g. abscessing lesion, complete biliary blockage) non-radical resection may be

necessary if percutaneous or endoscopic interventions – as a first recommended choice - fail to solve the AE related-condition (87, 138, 144).

Endoscopic and percutaneous interventions (EPIs) EPIs are indicated for AE-associated complications (eg. abscessing lesion, biliary obstruction) if surgery is felt to be too high a risk and total resection of the lesions cannot be safely performed (87).

Liver transplantation Rescue liver transplantation is a therapeutic option for AE patients with inoperable lesions and/or chronic liver failure (87,117). An inherent problem associated with orthotopic-liver transplantation (OLT) is AE recurrence (116). Therefore BMZ treatment pre- and post-OLT as well as low level immunosuppression are strongly recommended (117). Considering the good outcome associated with medical treatment of nonresectable AE lesions, OLT should only be considered in patients nonresponsive to medical treatment. These patients include those with outflow problems (Budd-Chiari syndrome) or recurrent life-threatening cholangitis with secondary biliary cirrhosis (87, 138).

I.6.6. Follow-up management of alveolar echinococcosis

E.m. stem cells (145) have the potential, as do cancer cells, to reinitiate AE lesions, as recurrence, in the liver or other organs, even years after an apparently successful treatment (138, 146). Therefore, long-term follow-up by US at six to twelve month intervals and by CT and/or MRI at one to three year intervals is recommended after diagnosis and initiation of treatment (87, 138). The course of AE lesions on conventional imaging, can be assessed as follows: *recurrence/no recurrence*, after radical resection, *regression* (decreased size and increased percentage of calcification), *progression* (increase in size and/or extension to neighboring organs and/or metastases), or *stabilization* (no change in lesions) (136). In the majority of patients, AE lesions lost their metabolic activity on fluorin18-fluorodeoxyglucose positron emission tomography computerised tomography (^{18F}FDG-PET/CT) during long-term BMZ treatment (147,148) thus this imaging method can be useful during the follow-up of AE patients (87,141). Currently in Hungary this modality is only available for patients with malignant diseases based on individual licensing. Beside imaging investigations regular follow-up of AE patients also consists of the laboratory controll of liver enzymes and

blood cell count 1, 4, and 12 weeks after starting treatment and then every 6 months if no complications arise. Monitoring ABZ sulfoxide blood level is an optional method to prevent ABZ toxicity in settings where it is available (87, 138).

I.6.7. Outcome and prognosis of alveolar echinococcosis

Historically, 10 to 15 years mortality in nontreated AE patients was 90-100% (139). In some countries of the historical endemic area of Europe (Switzerland, France, Germany) mainly due to an earlier diagnosis, availability of advanced surgical and interventional treatment methods, and efficient antiparasitic drug therapy with ABZ, the prognosis of AE patients has significantly improved in recent years (140). In Switzerland, by 2005 an average 54-year-old patient diagnosed with AE would have their, life expectancy reduced by only approximately 3.5 years for men and 2.6 years for women (149). In France, by 2007, the life expectancy of AE patients one year after diagnosis was found to be similar to that of people without AE (150). In Germany, for AE patients diagnosed after 2000 and treated in the specialized clinical center for AE in Ulm, the ten-year survival rate reached 90.6 % and 17% of patients were cured (151). Globally, the survival of AE patients was still lower than the expected survival of the general population. A poor prognosis has been associated with older age and invasion of the hilar region of the liver, with subsequent biliary complications. Conversely, medical treatment with BMZs (with or without surgery) has been associated with better survival (150,151,152). Use of the PNM classification can help to determine a patient's prognosis (136,151).

II. Objectives

The aim of our studies was to give a first-time comprehensive description of the epidemiological and clinical characteristics of CE and AE patients in Hungary.

1. The first goal of our studies was to collect demographic data (gender, age, place of residence, place of birth) of Hungarian patients suffering from Echinococcosis and to identify potential epidemiological risk factors, partially by revealing a correlation between geographical distribution of human cases and of the infected domestic and wild animals performing as intermediate or definitive hosts.
2. The second goal of our studies was to collect and systematize clinical data (diagnosis, treatment, follow-up, outcome) of CE and AE patients in Hungary.In terms of diagnosis, our purpose was to apply the criteria of the internationally accepted WHO-IWGE classification systems retrospectively to cases in order to make results comparable on an international level and to reveal inadequacies in the diagnosis of Echinococcosis on the national level. We also aimed to compare Hungarian therapeutic and follow-up practices to the current recommendations of the international expert consensus.
3. As AE was hypothesized as a newly endemic but still rare disease, we aimed to collect all recognized and presumably autochtonous human AE cases up until 31.12.2018 in the territory of Hungary. Our main objective was to prove that AE as a newly autochtonous parasitic infection is currently the most dangerous one in Hungary with high local lethality and mortality. A further objective was to search for a correlation between inappropriate clinical management (diagnostic delay, misdiagnosis, inappropriate therapeutic strategies) and the currently poor outcome of AE.
4. By presenting available data on long-term outcomes of indigenous AE cases we aimed to provide useful feed-back on recent Hungarian clinical practice. This has great importance, especially in developing the management of this potentially lethal human parasitosis on the national level.

III. Results

III.1. Epidemiological and clinical characteristics of cystic echinococcosis patients from a single center cohort

III.1.1. Epidemiological features of cystic echinococcosis patients

Based on the diagnostical criteria of WHO-IWGE expert consensus, a total of 45 CE cases were confirmed between 2000 and 2014 in a single pathological center (2nd Department of Pathology, Semmelweis University, Budapest) were confirmed. Among the 45 patients, 19 (42.2 %) were males and 26 (57.8%) were females (sex ratio: 0.73). The age of patients at the time of diagnosis ranged from 5 to 82 (mean 51.4) years. Only five cases (11.1%) were children (1-19 years). The patient's place of residence was known in 44 of the 45 cases. According to Nomenclature of Territorial Units for Statistics (NUTS) 3 level, CE patients were detected in 11 of the 19 Hungarian counties with a total of 44 cases; 2 of which originated in Transylvania (Romania). Concerning national cases, 15 patients (34.1%) originated from the capital Budapest and Pest county; 6 (13.6%) from Jász-Nagykun-Szolnok; 6 (13.6%) from Fejér; 5 (11.4%) from Bács-Kiskun; 3 (6.8%) from Borsod-Abaúj-Zemplén; 2 (4.5%) from Heves and 1 each (2.3%) from Somogy, Szabolcs-Szatmár-Bereg, Nógrád, Komárom-Esztergom, and Veszprém counties. In our series we found the highest CE incidence in Jász-Nagykun-Szolnok county (1.49 cases per 100 000 inhabitants). Multiple CE cases were detected in four towns: Budapest (n=6), Kunszentmiklós (n=3), Jászárokszállás (n=2), and Tököl (n=2) with the highest CE incidence recorded for Kunszentmiklós (33.9 cases per 100 000 inhabitants), a typical small town in the Great Plain with a long history of agriculture. Sheep- and swine-breeding remains the major sector of its economy; data on potential risk factors for CE in this region were limited to only one female patient who was living on a sheep-farm in Kunszentmiklós. According to NUTS2 level, CE patients were detected in 6 out of 7 regions in Hungary (Fig 3), with a total of 8 patients originating from Central Transdanubia (0.72 cases per 100 000 inhabitants), 15 from Central Hungary (0.52 cases per 100 000 inhabitants); 6 from Northern Hungary (0.48 per 100 000 inhabitants); 7 from Northern Great Plain (0.46 per 100 000 inhabitants); 5 from Southern Great Plain (0.37 per 100 000 inhabitants); and 1 from Southern Transdanubia (0.10 per 100 000 inhabitants) (Fig 3). With regards to places of birth,

data were available in 21 cases (47%). Analysis of this data showed that the vast majority of patients originated from the Great Plain (n=10) and from Eastern Hungary (n=13).

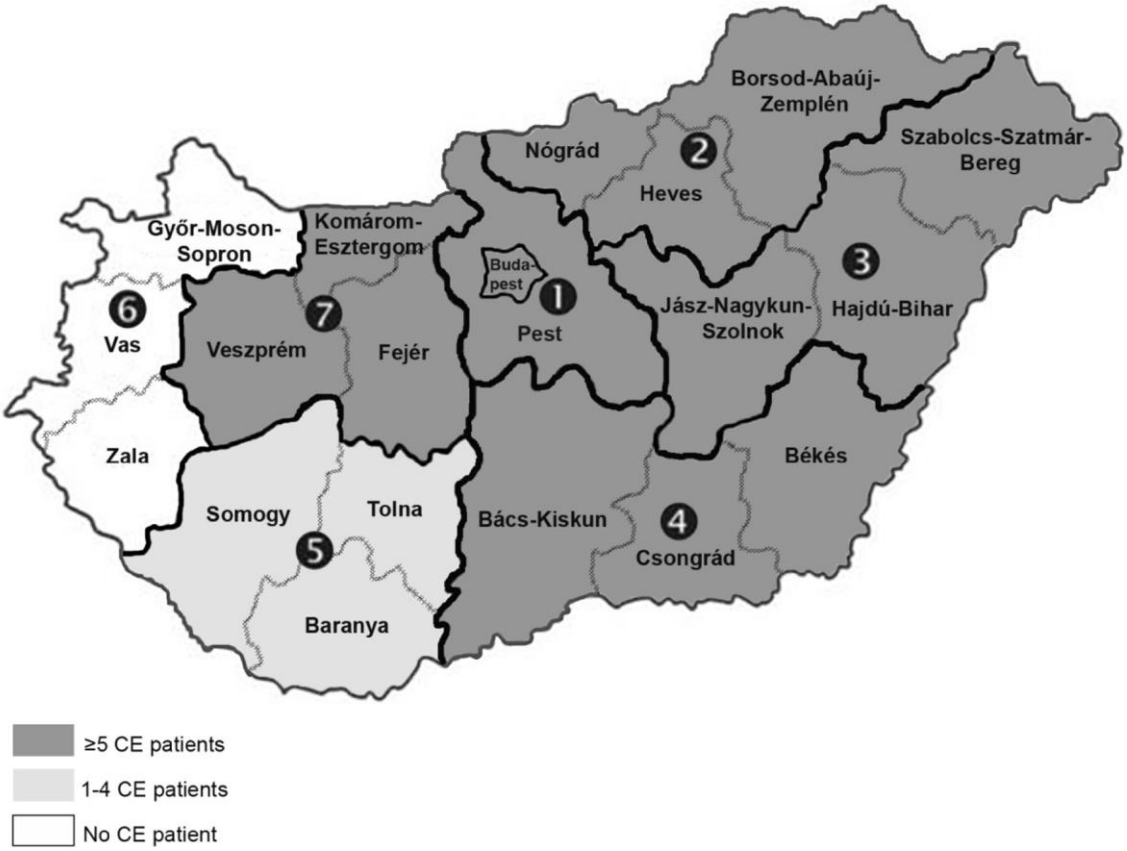

Fig 3 Geographical distribution of CE patients confirmed by histology (2nd Department of Pathology, Semmelweis University, Budapest, Hungary, 2000-2014) using place of residence data. Planning and statistical regions (NUTS2) of Hungary with counties (NUTS3); 1 – Central Hungary (Pest and capital Budapest); 2 – Northern Hungary (Nógrád, Heves, Borsod-Abaúj-Zemplén); 3 – Northern Great Plain (Jász-Nagykun-Szolnok, Hajdú-Bihar, Szabolcs-Szatmár-Bereg); 4 – Southern Great Plain (Bács-Kiskun, Csongrád, Békés); 5 – Southern Transdanubia (Somogy, Tolna, Baranya); 6 – Western Transdanubia (Győr-Moson-Sopron, Vas, Zala); 7 – Central Transdanubia (Komárom-Esztergom, Veszprém, Fejér) (23)

III.1.2. Diagnostic features of cystic echinococcosis patients

The analysis of clinical data showed that the majority of CE patients had a liver CE infection, whereas only one lung manifestation was recorded. Of the 45 CE pathological specimens gained by invasive procedures, 29 (64.4 %) were obtained by surgical methods, 9 (20%) by fine-needle aspiration, and 7 (15.6 %) by conventional liver

biopsy. Histological analysis revealed acellular LL in 42 cases; protoscolex or hooklets were evident in 34 cases, whereas both LL and protoscoleces were identified in 32 cases. Using available data and WHO-IWGE diagnostic criteria, preliminary clinical diagnosis was revealed in 20 cases. Twelve CE patients (26.7%) were identified by targeted investigations performed to clarify the etiology of a certain hepatic cystic lesion. In eight (17.8 %) cases, diagnosis of CE was an incidental histopathological finding during diagnostic work up of aortic aneurysm, liver abscess, acute pancreatitis, acute coronary syndrome, acute cholecystitis, gastric adenocarcinoma, and a pancreatic intraductal papillary mucinous neoplasm. Most patients (34; 75.6 %) were diagnosed in surgical departments; further departments involved were internal medicine, infectious diseases, radiology, and pediatrics. Using the WHO-IWGE classification for ten patients, prior to treatment six patients had CE5 calcified cysts, three patients had CE2 cysts and one patient had a CE3A cyst. *Echinococcus* serology was performed in 27 cases (60%); in further cases, the lack of serological examination was documented. Seropositivity was detected in 17 cases (62.96 %), whereas 10 cases (37.04 %) were seronegative.

III.1.3. Therapeutic features of cystic echinococcosis patients

Benzimidazoles (BMZ) treatment was applied in six cases (13.3 %). A PAIR technique was applied in four cases (8.9 %) combined with other modalities (PAIR+WW; PAIR+WW+BMZ+surgery). In 25 cases (55.6 %), the therapy involved surgical intervention. The registered types of interventions were: liver resection (segmentectomy, hemihepatectomy), pericystectomy, cystectomy, and laparoscopic cyst fenestration. The WW approach was applied in four cases (8.9 %). In two cases, a combination of surgery, PAIR, and drug administration was used; in three cases, the combination of surgery and drug administration was used. A total of six CE5 cysts were identified. In this group, surgery was applied in two cases, PAIR in two cases, BMZ treatment in five cases, and WW approach in one case. As the suggested treatment in CE5 calcified cysts is WW, only one-sixth of these patients were treated using the recommended procedures. In one case, a CE2 large cyst surgery combined with PAIR and BMZ was used; the approach for a small CE2 cyst was simple follow-up. In the case of one large CE2 cyst, the therapy was unknown. A medium sized CE3A cyst was

removed by resection, whereas the WHO-IWGE suggests treatment with PAIR and BMZ administration.

III.1.4. Follow-up of cystic echinococcosis patients and outcome of cases

Post-treatment information was collected for 29 patients with a time period of up to 16 years (mean period: 8.03 years). Among these, 18 patients (62%) had been cured of CE. Thirteen (44.8 %) were treated exclusively with surgery and two (6.8 %) with PAIR. Two patients (6.8 %) had relapses, and six (20.7 %) patients died of causes unrelated to CE.

III.2. Epidemiological and clinical characteristics of all recognized alveolar echinococcosis patients in Hungary until 2018 from a multi-centre cohort

III.2.1. Epidemiological features of AE patients

Between 2003 and 2018, a total of 16 AE patients were reported to the National Public Health Center in Hungary. Based on clinical and laboratory diagnostic criteria (WHO-IWGE expert consensus), ten were diagnosed as confirmed (62.5%) and six as probable (37.5%) AE patients. The sex ratio was 0.5. The mean age of patients at the time of first symptoms or findings related to AE was 53 years (Range: 24-78 years). Patients originated from rural areas (n=7; 43.8%), suburban areas (n=4; 25%), and urban areas (n=5; 31.2%). Data regarding potential risk factors were collected from 12 out of 16 AE patients. The two leading potential risk factors explored in our series were whether the patients had a kitchen garden (91.7%) or went to the forests for vocational reasons (83.3%).

III.2.2. Diagnostic features of AE patients

Five patients (31.3%) were asymptomatic at first examination, and AE lesions were incidental imaging findings. In symptomatic cases, the most frequent clinical signs were epigastric and/or right hypochondriac pain. Hepatomegaly, vomiting, weight loss, and pruritus were observed in six patients (37.5%), while palpable liver mass was detected in one patient (6.3%). Further physical signs were urticaria and anasarca. One patient (with stage P3N0Mx) had jaundice at first presentation (6.3%). Nine patients (56.3%) had elevated liver enzymes (ALP and GGT). Conventional imaging studies (US, CT,

MRI) revealed typical hepatic AE lesions in 14 patients. Hepatic localization of the parasite and parasitic infiltration of surrounding organs at the time of diagnosis could be observed in 15 patients (93.8%). The disease stage was P1 in four patients (25%), P2 in three (18.8%), P3 in two (12.5%) and P4 in six patients (37.5%). Extrahepatic involvement of neighboring organs (N1) was detected in three patients (18.8%). Subphrenic abscess (n=1; 6.3%), dissemination along falciform ligament (n=1; 6.3%), and dissemination along omental peritoneum (n=1; 6.3%) were present. The absence or presence of distant metastasis was evaluated completely in only six patients (37.5%), and metastasis were not found (M0) (n=6; 37.5%). Pulmonary metastasis at the time of diagnosis could be excluded in 15 patients (93.8%). Based on radiological findings, the following preliminary diagnoses were made: Echinococcosis, CE, liver metastasis, sarcoidosis, granulomatous hepatitis, haemangioma, hepatocellular adenoma, hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), cystic neoplasm of the liver, and fibrolamellar carcinoma. Anti-Em antibodies were detected in 13 out of 16 patients. Based on histopathological findings, the following preliminary diagnoses were made: granulomatous hepatitis, chronic hepatitis with fibrosis, helminthosis, and Echinococcosis. In the ten histopathologically confirmed patients, protoscoleces or hooklets were evident in two patients (20%) (Fig 11). In the first (No.3) and second (No.4) proven Hungarian AE cases, PCR was used as a confirmatory method (n=2; 12.5%). *E.m.* specific DNA sequences were isolated from formalin-fixed paraffin embedded tissue samples in collaboration with Medical University Vienna, Vienna, Austria. In the first proven autochtonous case (No.8) and in one further case (No.11) IH was applied for confirmation (n=2; 12.5%) within the framework of an international AE research cooperation with the University of Ulm, Ulm, Germany. Monoclonal antibody Em2G11 was used on tissue slides from original formalin fixed and paraffin embedded blocks. Diagnostic delay ranged from 1 to 122 months (mean latency of diagnosis 33 months) (Table 2)

III.2.3. Therapeutic features of alveolar echinococcosis patients

Drug treatment 13 out of 16 AE patients (81.3%) received ABZ. Three patients (18.8%) received no ABZ treatment due to misdiagnosis (Table 2). During the whole study period, five AE patients (31.3%) received uninterrupted ABZ treatment in a daily

dose of 800 mg continuously from the time of diagnosis because of multiple and/or extended unresectable AE lesion(s). In one patient (6.3%), AE was recognized at an advanced stage (P4N1Mx), and the patient died after three months of treatment. One patient with unresectable lesion (6.3%) was treated with ABZ for a total of 12 months with long interruptions during his 124-month-long follow-up period. Three patients (18.8%) with unresectable AE lesion(s) received ABZ from the time of diagnosis for less than four months. The reasons for ceasing therapy were drug-related hepatotoxicity, allergic reactions, virtual stabilization of AE lesions, and propagation of liver lesions (assumed ineffectivity of ABZ). Four patients (25%) received adjuvant ABZ therapy following removal of AE lesion(s) by radical (R0) liver surgery. Four patients (25%) received an incomplete concomitant ABZ treatment with a total of five months duration (Table 2).

Surgery Surgery was performed in 9 out of 16 AE patients (56.3%). The established diagnosis of AE as an indication and a major impact on choosing the proper method of surgical intervention was only confirmed in one out of the nine patients (6.3%) prior to surgery. In three patients (18.8%), explorative laparotomy was carried out for diagnostic purposes to assess resecability and gain tissue-samples for histopathological analysis. Unresecability was detected in three patients (18.8%) due to extrahepatic peritoneal dissemination (n=1; 6.3%), central localization compressing the ductus hepaticus communis (n=1; 6.3%) and both peritoneal dissemination and compression of the ductus hepaticus communis (n=1; 6.3%). Radical resection aiming to remove the entire parasitic lesion with safety margin (R0) was done in five patients (31.3%) as follows: extended right hemihepatectomy with feeding catheter jejunostomy (n=1; 6.3%), right hemihepatectomy with hepaticojejunostomy (n=2; 12.5%), and segmentectomy (n=2; 12.5%). In two patients (12.5%) fenestration and marsupialisation of AE lesions were performed as palliative methods. In one case, marsupialisation was chosen as palliation of a confirmed and unresectable AE lesion, localized in the dichotomy of the common hepatic duct, causing biliary obstruction. In the other probable patient a giant pseudocystic AE lesion was misdiagnosed as abscessing cystic echinococcosis, thus marsupialisation and drainage were chosen as therapeutic interventions. ERCP was performed in 5 patients (31.3%) because of AE-associated biliary obstruction.

Endoscopic and percutaneous interventions Endoscopic biliary stents were inserted five times in three patients. In one patient, nasobiliary stent placement was also necessary to facilitate bile passage. A total of four percutaneous transhepatic drainages (PTD) were performed of AE lesions in two patients. Among the five patients (31.3%) who needed EPIs, the following stages were determined at diagnosis: P4N1Mx (n=2; 12.5%), P4N0Mx (n=2; 12.5%), and P3N0Mx (n=1; 6.3%) (Table 2).

III.2.4 Follow-up of alveolar echinococcosis patients and outcome of cases

The clinical follow-up period ranged from 1 to 177 months (mean 52.4 months). Because of a misdiagnosis, the probable first documented Hungarian AE patient was left untreated. Based on the laboratory findings, the hepatic AE lesions seem to have progressed, but the lack of pathological investigations did not allow any conclusions to be drawn on this very first patient who eventually died of causes unrelated to AE. In four patients (25%), no recurrence of AE was detected after radical surgery and concomitant ABZ treatment. In these patients, the disease-free period from the curative surgery to the date of final imaging ranged from 10 to 74 months. With continuous ABZ treatment, stabilization of unresectable AE lesions with continuous ABZ treatment was achieved in three (60%) out of five patients with unresectable lesions. One probable AE patient with multiple hepatic AE lesions received ABZ for only three months. Ten years later, no progression was detected despite the lack of continuous ABZ therapy. In one probable and unresectable patient, intermittent low-dose (2x100 mg per day) ABZ therapy had to be discontinued because of drug-related hepatotoxicity and allergic reactions. After 26 months without treatment, hepatic AE lesions stabilized, but pulmonary microlesions developed. Unfortunately, the patient was permenently supplemented with hydrocortisone for hypadrenia, which may have influenced the course of AE. Progression of AE during the study period was detected in seven patients (43.8%). In our series, progression was not generally accompanied with PNM upstaging of cases but increasing size of AE lesion(s) and/or worsening clinical condition directly related to AE. In two (33.34%) out of six patients, short-term or interrupted ABZ treatment could have been a plausible explanation for disease progression. In one case the size of an AE lesion increased despite of adequate continuous ABZ treatment. In this patient, a disseminating malignant neuroendocrine tumor with liver metastases was

diagnosed and the patient was simultenously treated with a probable liver AE lesion. Malignancy, targeted radionuclide therapy, and classical radiotherapy, could have played a role in the course of AE as immunocompromising factors. We registered three AE-related deaths (18.8%) in our study.

In patient No.5 (P4N0Mx), giant pseudocystic AE was presumably misdiagnosed as abscessing CE. ERCP, stent implantation, surgical marsupialisation, and drainage were performed. Central biliary obstruction, bilioperitoneal fistula, injury of bile ducts with subsequent bile leakage, complete lack of ABZ treatment, cachexia and advanced age were possible factors which contributed to the lethal course of the infection.

In patient No.15 (P4N1Mx), imaging studies and explorative laparotomy revealed a central unresectable AE lesion (120mm) occupying the left lobe and compressing the common hepatic duct with ascites and parasitic invasion along the falciform ligament. AE was confirmed by histopathology from the surgical sample. Peritonitis and cholangiogen sepsis were the causes of death in this advanced case.

In patient No.16 (P4N0Mx), imaging studies revealed a 120 mm AE lesion misdiagnosed as cystic echinococcosis, abscess or tumor of the liver occupying the right lobe and compressing the common hepatic duct. ERCP, biliary stent implantation, repeated PTD, and eventually right hemihepatectomy were performed with hepaticojejunostomy. Postoperative bleeding, liver failure, and septic shock led to the death of the patient. AE was confirmed by histopathology postoperatively from a surgical sample. The mean diagnostic delay in the three patients who died of AE patients was close to 10 years (116 months) (Table 2).

Table 2. Clinical characteristics of human alveolar echinococcosis cohort patients in Hungary (2003-2018) (68)

case no.	**1**	**2**	**3**	**4**
onset of symptoms or first findings	09.2001	09.2003 focal hepatic lesions during imaging studies	10.2004	08.2008
initial symptoms and physical findings	epigastric pain, vomitus	asymptomatic, hepatomegaly	epigastric and right hypochondriac pain	jaundice, pruritus, right hypochondriac pain
liver function tests: liver enzymes (U/l); sebi (µmol/l)	normal	-	elevated GGT (104)	elevated ALP (1254), GGT (570) and sebi (202)
initial US/CT/MRI (date) radiomorphology largest diameter of AE lesion(s) in mm	US (09.2001) – 15mm hyperreflective area in SIV CT (03.2003) – echinococcal cysts in both lobes, number, size, localization unknown	US (04.2005) and CT (08.2009) – 10 typical AE lesions in SIV, SV, SVI, SVIII 10-30mm, largest lesion 50mm	US (10.2004), CT (11.2004) and MRI (06.2005) – one typical AE lesion – 100mm – in SV, SVI, SVII, SVIII	US (08.2008) – typical central AE lesion – 110mm – in the dichotomy of hepatic common duct, SIV, SV
preliminary diagnosis	Echinococcosis	liver tumor, Echinococcosis	liver tumor, HCC, liver metastasis	liver tumor, adenocarcinoma
serology Westernblot (Ldbio) P3 *E.m.*	positive	positive	positive	positive
core biopsy/surgical sample/autopsy	-	-	core biopsy (2x) surgical sample (1x)	core biopsy during PTC, surgical sample
histopathology/IH/PCR	-	-	histopathology and PCR	histopathology and PCR
type of diagnosis	probable	probable	confirmed	confirmed
month.year of diagnosis	04.2003	04.2004	07.2005	09.2008
latency of diagnosis (in months)	20	8	10	2
extrahepatic localization at the time of diagnosis	no pulmonary lesion	no pulmonary lesion	peritoneal dissemination no pulmonary lesion	no pulmonary lesion
PNM at diagnosis	PxNxMx	P1N0Mx	P2N1Mx	P3N0Mx
antiparasitic drug treatment (duration in months)	-	ABZ (3) 10.2004 – 12.2004	ABZ (162) continuously since 07.2005	ABZ (12) 11.2008 – 01.2009 and 06.2016 – 03.2017
surgery	-	-	exploration – unresectable	exploration, fenestration, marsupialisation
EPI	-	-	-	PTD (2x), ERCP
follow-up period in months	27	177	162	124
radiomorphology on final control, largest diameter of AE lesion(s) in mm (date)	CT (04.2005) – pseudocystic AE lesion in left lobe 49mm, two more AE lesions in right lobe 35mm and 24mm	US (09.2014) stabilization	MRI (12.2018) stabilization	US (12.2018) residual cavity in SIV 70mm, giant biloma in porta hepatis
PNM at final imaging	P1N0Mx	P1NxMx	P2N1Mx	P3N0Mx
complications	elevated GGT (136), ALP (621), sebi (36,3)	-	-	central biliary obstruction, cholangitis, biloma, bile-leaking
outcome	progression, AE unrelated death	stabilization	stabilization	progression

Table 2. (Continued) Clinical characteristics of human alveolar echinococcosis cohort patients in Hungary (2003-2018) (68)

case no.	**5**	**6**	**7**	**8**
onset of symptoms or first findings	2002 asymptomatic hepatic cyst; patient denied investigations	11.2011	12.2012	11.2012
initial symptoms and physical findings	right hypochondriac pain, vomitus, anasarca, palpable liver tumor (12.2010)	right hypochondriac pain, weightloss, hepatomegaly	asymptomatic, mild hepatomegaly	asymptomatic
liver function tests: liver enzymes (U/l); sebi (µmol/l)	AST (177), ALT (177), GGT (920), ALP (1152), sebi (16)	GGT (105), ALP (543), sebi (7,1)	GGT (335), ALP (999), sebi (7,3)	normal
initial US/CT/MRI (date) radiomorphology largest diameter of AE lesion(s) in mm	US (12.2010) and CT (01.2011) two interconnected pseudocystic AE lesions in both lobes – 130mm and 120mm – dilatated intrahepatic bileducts	US (11.2011) and CT (12.2011) typical AE lesion in SV, SVI – 83mm – and some smaller lesions	CT (04.2013) typical AE lesion in right lobe, 135mm, periportal biliary and vascular involvement (right v. portae, v.hepatica intermedia	CT (11.2012) and MRI (08.2014) multiplying small calcified lesions in SV, SVI, SVII, SVIII
preliminary diagnosis	metastasis, tumor, CE	hemangioma, tumor, CE	cholangiocellular carcinoma	liver metastasis
serology Westernblot (Ldbio) P3 *E.m.*	positive	equivocal	positive	negative (postoperatively 2x)
core biopsy/surgical sample/autopsy	parasitology and cytology from lesion fluid (FNAB) negative	corebiopsy	corebiopsy	corebiopsy and surgical sample
histopathology/IH/PCR	-	histopathology	histopathology	histopathology and IH
type of diagnosis	probable	confirmed	confirmed	confirmed
month.year of diagnosis	03.2011	01.2012	04.2013	10.2014
latency of diagnosis (in months)	111	1	5	24
extrahepatic localization at the time of diagnosis	no pulmonary lesion	no pulmonary lesion	undignified pulmonary lesions	no
PNM at diagnosis	P4N0Mx	P2N0Mx	P4N0Mx	P1N0M0
antiparasitic drug treatment (duration in months)	-	ABZ (5) 02.2012 – 07.2012	ABZ (67) continuously since 06.2013	ABZ (24) postoperatively
surgery	marsupialization, drainage	extended right hemihepatectomy	-	segmentectomy
EPI	ERCP, biliary stent implantation, nasobiliary stent	-	-	-
follow-up period in months	9	84	69	51
radiomorphology on final control, largest diameter of AE lesion(s) in mm (date)	US (11.2011) residual cavity 45mm, atrophy of right lobe	US (02.2018) no recurrence	MRI (10.2018) and US (10.2018), 109mm	US (07.2018) no recurrence
PNM at final imaging	P4N0Mx	P0N0Mx	P4N0Mx	P0N0M0
complications	central biliary obstruction, bile-leaking, bilio-peritoneal fistula, injury of bileducts during surgical manipulation, cachexia	postoperative peritonitis, haematoma, bile-leaking, Kehr-drainage	v. cava inferior compression	-
outcome	progression, AE related death	no recurrence	stabilization	no recurrence

Table 2. (Continued) Clinical characteristics of human alveolar echinococcosis cohort patients in Hungary (2003-2018) (68)

case no.	**9**	**10**	**11**	**12**
onset of symptoms or first findings	10.2013	04.2012	02.2017	03.2017
initial symptoms and physical findings	right hypochondriac pain, urticaria	right hypochondriac pain, hepatomegaly	epigastric pain, vomitus	right hypochondriac pain
liver function tests: liver enzymes (U/l); sebi (µmol/l)	ALP (125), GGT (86)	normal	normal	elevated ALP
initial US/CT/MRI (date) radiomorphology largest diameter of AE lesion(s) in mm	MRI (12.2015) and CT (01.2016) 2 typical AE lesions in the dichotomy of hepatic veins; in SV/IVB 55mm; in SVIII/IVA 53mm	CT (04.2012) and MRI (10.2012) typical AE lesion in SIV 42mm	US (02.2017), CT (02.2017) and MRI (03.2017) two AE lesions in SVIII 44mm and in SVII 12mm	US (05.2017), CT (05.2017) multiplex AE lesions in both lobes, 40mm
preliminary diagnosis	atypical rare malignancy, liver metastasis	hemangioma, adenoma, liver tumor	hemangioma, cholangiocellular carcinoma, fibrolamellar carcinoma	liver metastasis, sarcoidosis, granulomatous hepatitis
serology Westernblot (Ldbio) P3 *E.m.*	positive	positive	Echinococcus genus P5	positive
core biopsy/surgical sample/autopsy	-	-	(FNAB) and surgical sample	corebiopsy (2x)
histopathology/IH/PCR	-	-	IH	histopathology
type of diagnosis	probable	probable	confirmed	confirmed
month.year of diagnosis	01.2016	06.2016	05.2017	07.2017
latency of diagnosis (in months)	28	50	4	5
extrahepatic localization at the time of diagnosis	no	no	no	no
PNM at diagnosis	P3N0M0	P1N0M0	P1N0M0	P2N0M0
antiparasitic drug treatment (duration in months)	ABZ (3) lowered dose intermittently in 2016, finally ceased	ABZ (30) continuously since 07.2016	ABZ (21) postoperatively	ABZ (3) 09.2017 – 11.2017
surgery	-	-	segmentectomy	-
EPI	-	-	-	-
follow-up period in months	36	30	20	18
radiomorphology on final control, largest diameter of AE lesion(s) in mm (date)	MRI (10.2018), CT (10.2018) no progression in liver, new pulmonary micronodules (09.2017)	MRI (11.2018) SIV 70mm, progression	MRI (06.2018), US (10.2018) no recurrence	US (07.2018) AE lesion in left lobe 65mm, AE lesion in right lobe 44mm
PNM at final imaging	P3N0Mx	P1N0Mx	P0N0Mx	P2N0Mx
complications	ABZ hepatotoxicity and allergic reactions, undignified pulmonary microlesions	-	-	-
outcome	stabilization	progression	no recurrence	progression

Table 2. (Continued) Clinical characteristics of human alveolar echinococcosis cohort patients in Hungary (2003-2018) (68)

case no.	**13**	**14**	**15**	**16**
onset of symptoms or first findings	09.2017	09.2016	04.2008	2008
initial symptoms and physical findings	right hypochondriac pain, hepatomegaly	asymptomatic	asymptomatic	right hypochondriac pain
liver function tests: liver enzymes (U/l); sebi (µmol/l)	elevated liver enzymes	GGT (115)	-	-
initial US/CT/MRI (date) radiomorphology largest diameter of AE lesion(s) in mm	US (10.2017), CT (10.2017) typical AE lesion in SV 80mm	MRI (09.2016) 15 mm wide hypodens area in right lobe, CT (09.2017) and MRI (11.2017) 75mm typical AE lesion in SV and SVIII, dilatation of intrahepatic bileducts	US (04.2008), CT (07.2008) typical AE lesion in SV – 54mm – and three small calcified lesions	US (2008) 20mm hyperechoic liver lesion, CT (10.2016) and MRI (06.2017) 120mm typical AE lesion in right lobe (SV-VI-VIII)
preliminary diagnosis	liver tumor	cholangiocellular carcinoma, Klatskin tumor	atypical hepatic cyst	hemangioma, cystadenocarcinoma
serology Westernblot (Ldbio) P3 *E.m.*	positive	positive	positive	positive
core biopsy/surgical sample/autopsy	-(FNAB2x)	surgical sample	surgical sample autopsy	surgical sample
histopathology/IH/PCR	-	histopathology	histopathology	histopathology
type of diagnosis	probable	confirmed	confirmed	confirmed
month.year of diagnosis	12.2017	01.2018	05.2018	08.2018
latency of diagnosis (in months)	4	17	122	115 (+12)
extrahepatic localization at the time of diagnosis	no pulmonary lesion	subphrenic abscess, peribiliar vascular invasion, no pulmonary lesion	falciform ligament, no pulmonary lesion	no pulmonary lesion
PNM at diagnosis	P4N0M0	P4N1Mx	P4N1Mx	P4N0Mx
antiparasitic drug treatment (duration in months)	ABZ (12) continuously since 01.2018	ABZ (12) continuously since 01.2018	ABZ (3) 06.2018 – 09.2018	-
surgery	-	right hemihepatectomy, exstirpation of d. choledochus and cholecystectomy, hepaticojejunostomia	explorative laparotomy	right hemihepatectomy, exstirpation of d. choledochus and cholecystectomy, hepaticojejunostomia
EPI	-	ERCP	ERCP (2x), stent implantation (2x)	ERCP, stent implantation, PTD (2x)
follow-up period in months	13	12	5	1
radiomorphology on final control, largest diameter of AE lesion(s) in mm (date)	MRI (10.2018) AE lesion in SV 79mm	CT (10.2018) no recurrence	US (05.2018) 120mm AE lesion occupying left lobe, ascites, dilatated intrahepatic bileducts	-
PNM at final imaging	P4N0Mx	P0N1Mx	P4N1Mx	P0N0Mx
complications	thrombosis and parasitic infiltration of right v. portae, compression of d. hepaticus dexter	leukopenia, hairloss, haematoma in residual left lobe (32mm) and undignified pulmonary microlesions	compression of d. hepaticus communis, peritonitis, cholangiogen sepsis	compression of d. hepaticus communis, abscessus hepatis, liver insufficiency, septic shock
outcome	stabilization	no recurrence	progression, AE related death	progression, AE related death

E.m. – *Echinococcus multilocularis* AE – alveolar echinococcosis CE – cystic echinococcosis v – vena d – ductus AST – aspartateaminotransferase ALT - alanine aminotransferase GGT –gamma-glutamyltransferase ALP – alkaline phosphatase sebi – serum bilirubin US – ultrasound CT – computer tomography MRI – magnetic resonance imaging IH – immunohistochemistry using monoclonal antibody mAbEm2G11 PCR – polymerase chain reaction tx – treatment EPI – endoscopic and percutaneous interventions ERCP – endoscopic retrograde cholangiopancreatography PTC – percutaneous transhepatic cholangiography PTD – percutaneous transhepatic drainage FNAB – fine needle aspiration biopsy S – liver segment ABZ – albendazole HCC – hepatocellular carcinoma

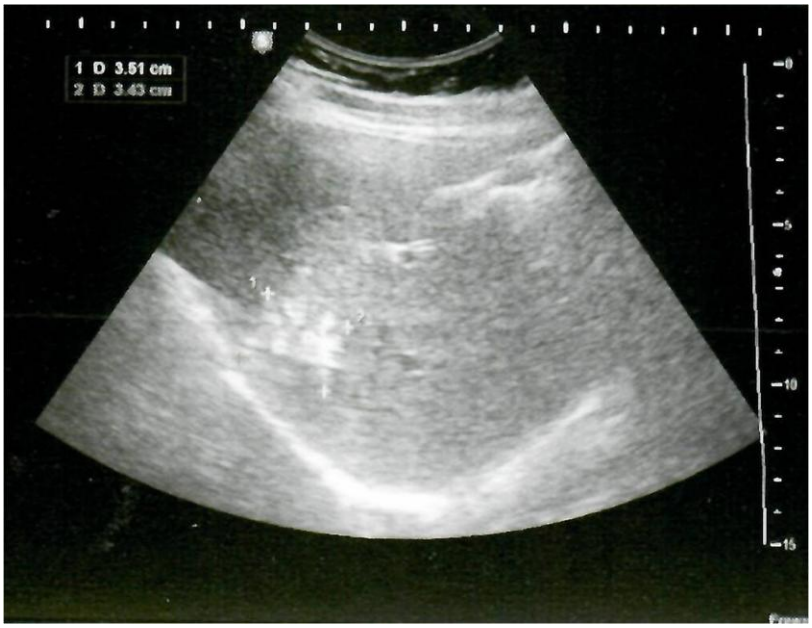

Fig 4. Typical US image of an AE lesion in the liver at first presentation from our cohort (patient No.11). Note the juxtaposition of hyper- and hypoechogenic areas in a pseudo-tumour with irregular borders and scattered calcification

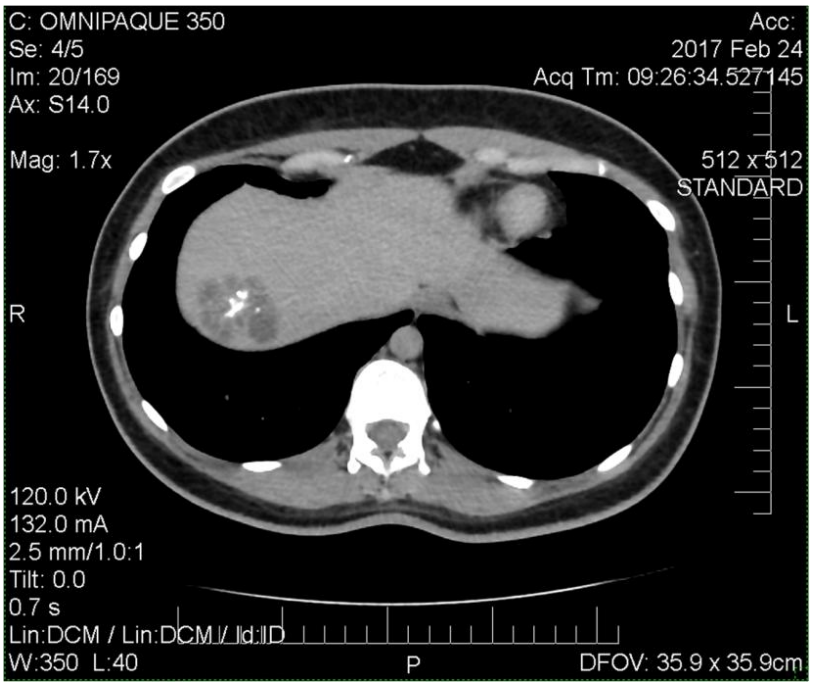

Fig 5 Typical CT image of the same AE lesion in the liver at first presentation from our cohort (patient No.11). No or septal enhancement and central calcifications are characteristic for AE allowing discrimination from CE and ICC.

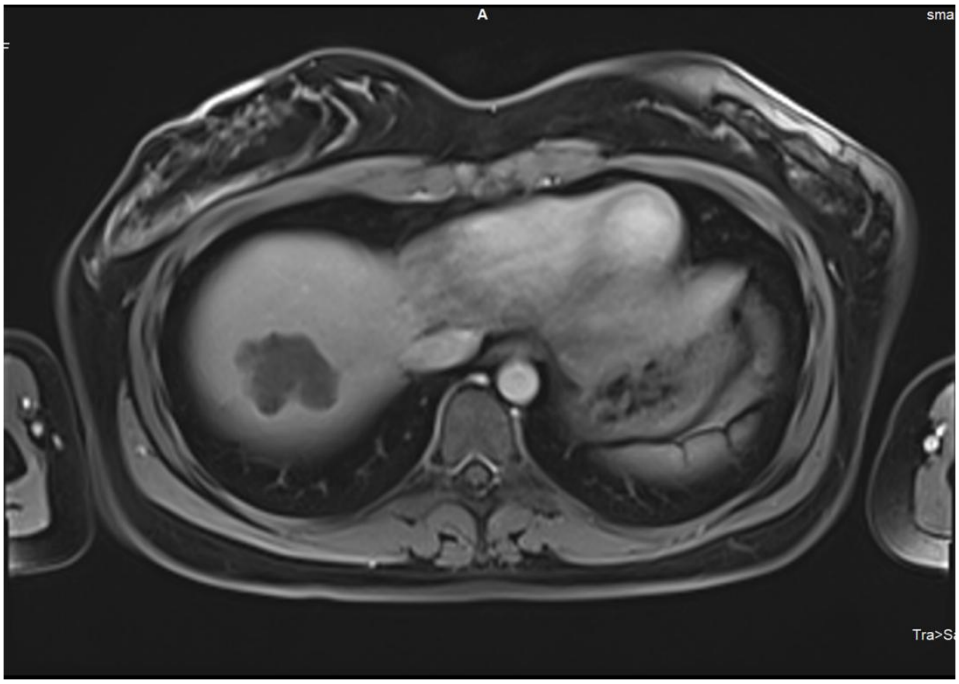

Fig 6. Typical MR image of the same AE lesion in the liver at first presentation from our cohort (patient No.11) Note the multivesicular 'alveolar' morphology of the lesion

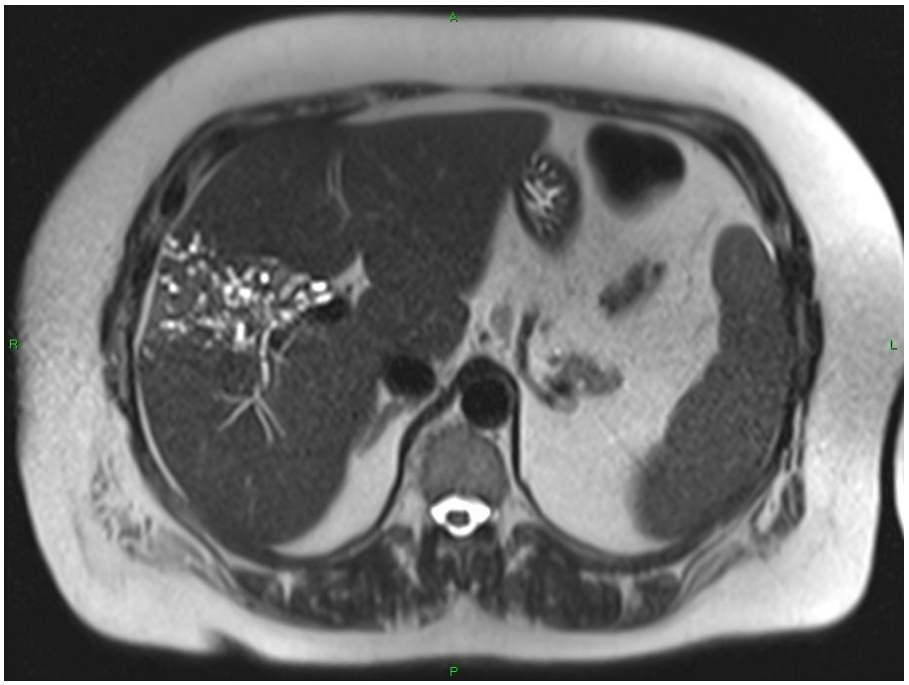

Fig 7. T1 weighted MR image of a typical AE lesion mimicking ICC in the V and VIII segments of the liver from our cohort (patient No.14). Note the cone shaped morphology of AE with dilated bile ducts. (72)

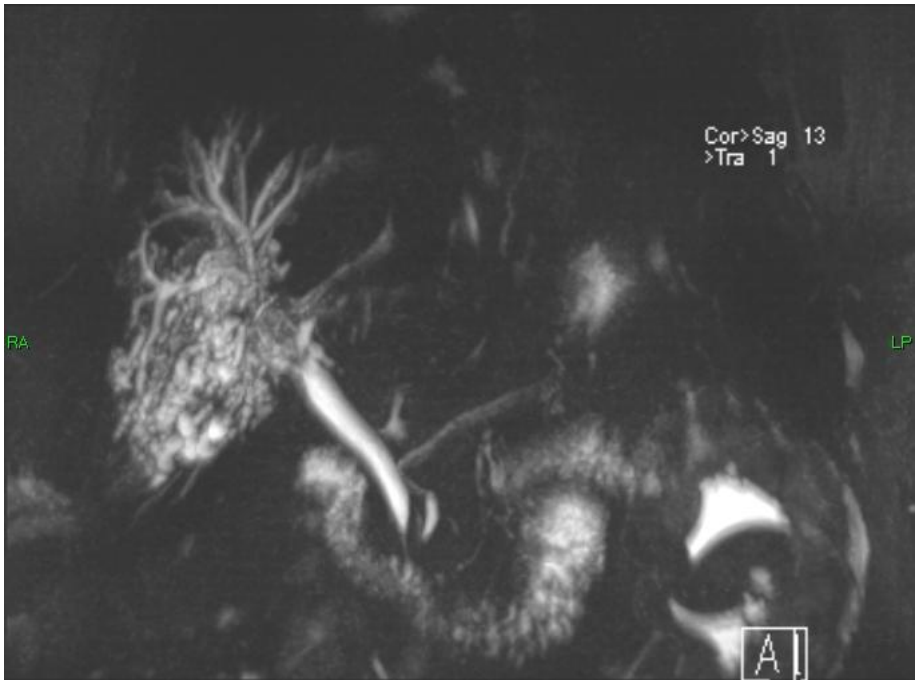

Fig 8. MRCP image of the same AE lesion showing intrahepatic dilation of bile ducts in the right lobe of the liver (patient No.14).

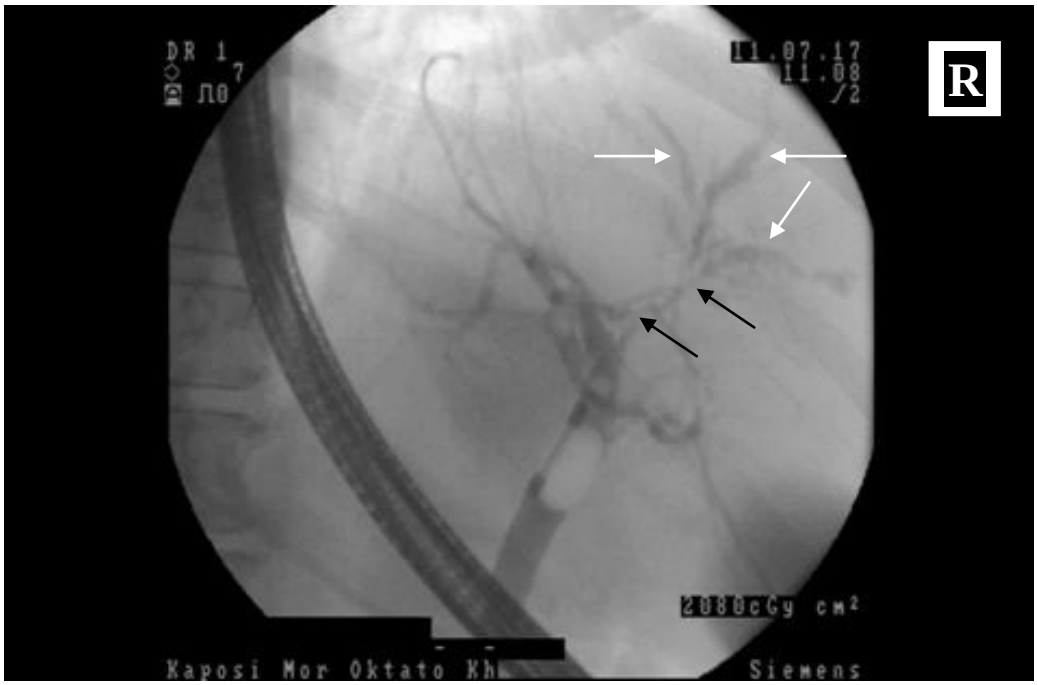

Fig 9. Endoscopic retrograde cholangio-pancreatogramm of the same AE lesion demonstrating dilated intrahepatic bile ducts in the right lobe (patient No 14) White arrows: post-stenotic dilations Black arrows: stenosis R: right (72)

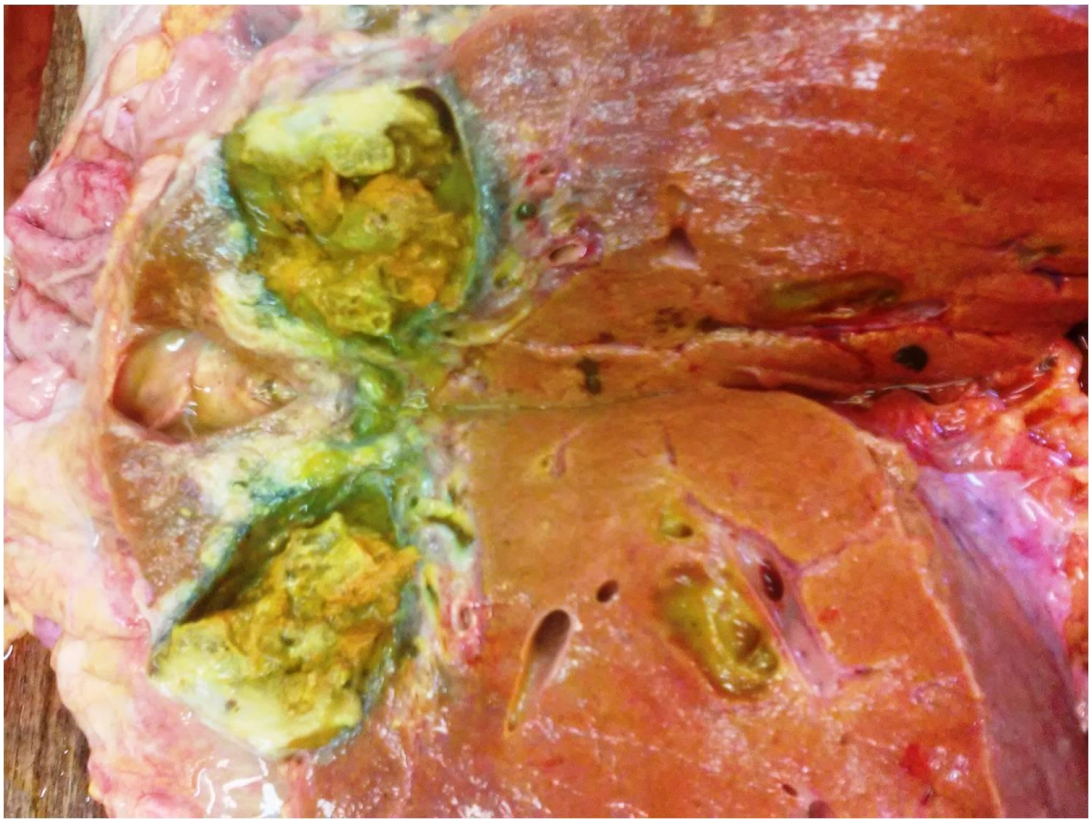

Fig 10. Gross features of resected liver with AE (patient No.14) An irregularly shaped lesion with infiltrative border measuring 10 cm is seen on cut surface. Yellowish green necrotic content is present centrally surrounded by greyish white rim with variable thickness. Infiltration of liver capsule can be detected. (72)

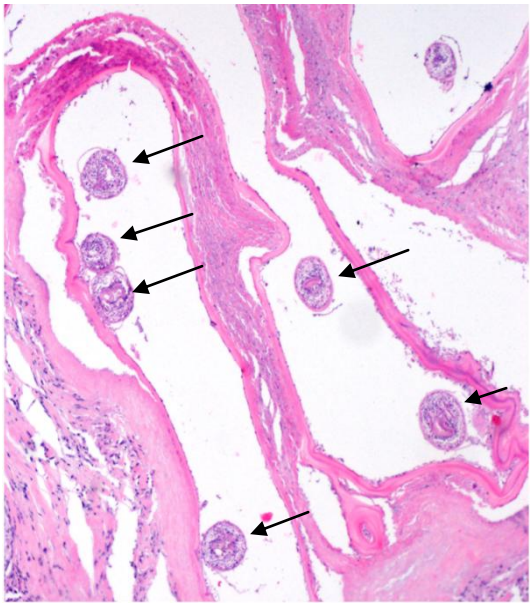

Fig 11 Rare finding of patognomic *E.m.* protoscoleces (arrows) from the same AE lesion H&E, magnification 100x, (No.14) (68)

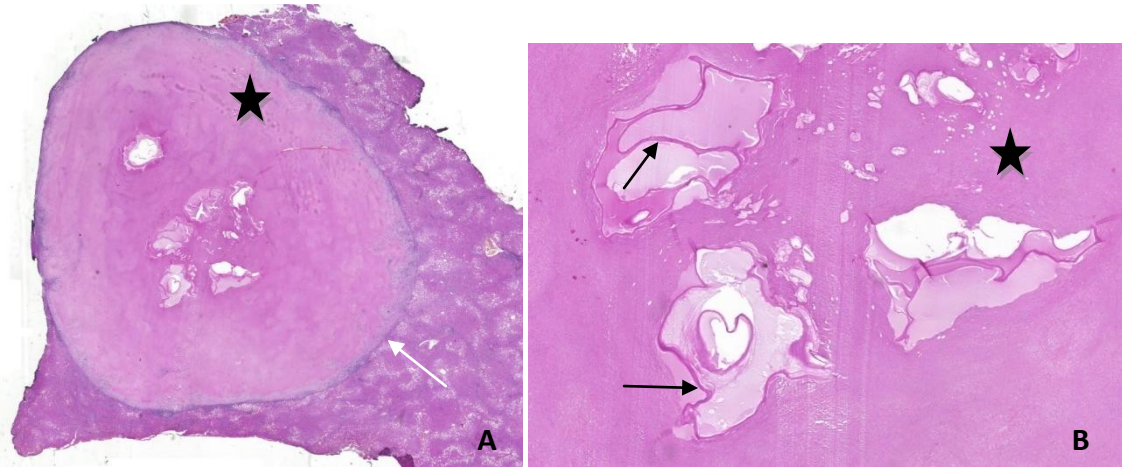

Fig 12. Histopathology of an alveolar echinococcosis lesion from resecated liver (patient No.8). Microscopic view of an AE lesion: **A** (H&E staining,10x magnification) and **B** (H&E staining, 60x magnification) Note the characteristic histopathological features of AE: conglomerate of multiple, small cysts containing slender, weakly striated laminated layer (LL) (black arrows) within the lesion surrounded by abundant necrosis (black asterisks) and thin pericystic fibrosis (white arrow). Protoscoleces can not be found.

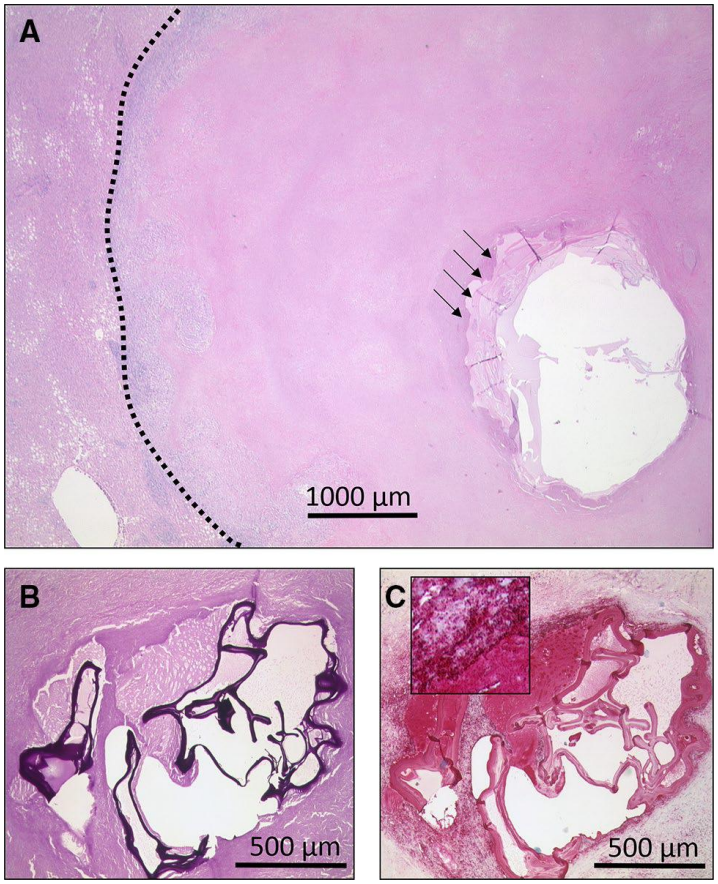

Fig 13. Histopathology performed from the same paraffin embedded block of resecated liver (patient No.8) supplemented with immunohistochemistry **A** Liver parenchyma shows fragments of the LL of the larval state of *E.m.* (see *arrows*) surrounded by a characteristic broad necrotic zone; *dashed line* marks the limits of vital hepatic tissue from parasite-induced necrosis. **B** PAS staining shows the typical slender LL **C** Immunohistochemical staining with the monoclonal antibody Em2G11 shows a strong positive staining of the LL including small fragments of *Echinococcus multilocularis* (SPEMS) (84)

IV. Discussion

Based on data of the National Public Health Center, Echinococcosis is currently the most prevalent zoonotic helminthosis in Hungary among notifiable helminthic diseases (4). Our epidemiological and clinical investigations on echinococcosis cases of the last two decades, are the first comprehensive descriptive studies on human Echinococcosis to date carried out in Hungary. Two major disease forms exist, with distinct biological, epidemiological, and clinical features: cystic echinococcosis (CE) caused by *Echinococcus granulosus sensu lato*, and alveolar echinococcosis (AE) caused by *Echinococcus multilocularis*. Before the first confirmation of human AE disease in 2005 (19), all Echinococcosis cases in the territory of Hungary were regarded as CE and were either registered as CE or simply designated as unspecified Echinococcosis (4). Based on our results, we have shown for the first time that besides the preexisting and prevalent CE disease, AE, - which has a potentially poor clinical prognosis and a lethal outcome, - has also appeared as a newly endemic and emerging helminthosis in this country. We also provide implications for future clinical practice. We studied CE and AE separately.

IV.1. Clinical epidemiology of cystic echinococcosis in Hungary

First we studied the current clinical epidemiology of CE, an endemic parasitic disease well known since the nineteenth century (20,21). Analyzing the places of residence of affected patients, a higher incidence was registered in the Central and Eastern part of Hungary (Fig 3), with one third of the cohort of patients diagnosed with CE living in the Great Plain, a traditional agricultural region with large-scale livestock breeding (22). Considering sheep as the major intermediate host of *E.g.s.s.* and having the highest infection rate (0.013%) among sampled host species in Hungary (49,60), the Great Plain accounts for 67.1 % of the total ewe stock of Hungary (624 180 ewes in 2015)(153). We detected the highest CE incidence (n=3; 33.9 cases/100 000 inhabitants) in Kunszentmiklós, a small town belonging to Jász-Nagykun-Szolnok county of the Great Plain. One patient was living on a sheep-farm in Kunszentmiklós. In our cohort, out of the 11 Hungarian counties where CE was detected, Jász-Nagykun-Szolnok county accounts for 13.6 % of patients (n=6) (22). According to the official registry of

notifiable infectious diseases (4) among the 19 Hungarian counties, Jász-Nagykun-Szolnok had the second highest total case number of CE (n=13) between 2000 and 2014. The highest case number was detected in Hajdú-Bihar county (n=20) which also occupies a large part of the Great Plain. Our cohort does not contain cases from Hajdú-Bihar because patients living there are referred to another university center based on the territorial order of patient care. Nonetheless, these observations suggest that the parasite probably completes the same basic pastoral life-cycle in Hungary involving shepherd dogs and sheep as definitive and intermediate hosts, as it is described and experienced in the highly endemic neighbouring countries (154). Low but detectable CE incidence in children demonstrates the ongoing transmission and autochtonous occurence of *E.g.* (22) which is also proven by animal studies (60). In our cohort CE was an incidental finding in 17.8 % of cases during the histopathological work-up of other well-defined diseases (22). This can be evaluated as one of the contributing factors leading to the underestimation of CE, mainly because of inadequacies in the national official reporting system. Firstly, many cases diagnosed exclusively by histopathology are not reported by pathologists and therefore stay cryptic for the official registry based primarily on serological results. This is an inappropriate notification and reporting practice because in fact histopathology is the confirmatory method in Echinococcosis. Cases identified only by serological methods would always require further confirmation, even after the positive result of a highly specific Western Blot analysis, as typical imaging findings with supportive seropositivity – according to the WHO case definition – only define probable echinococcosis (87). Furthermore, in many cases neither imaging, nor serology provide unequivocal distinction between *E.g.* and *E.m.* infection, if applied as sole diagnostic methods. Secondly, a great proportion of patients with CE having cysts in CE4 and CE5 inactive stages are seronegative (155); in our cohort of patients with CE, anti-*Echinococcus* serology was performed in 27 cases (60%). Seropositivity was detected in 17 cases (63%), whereas 10 cases (37%) were seronegative. Regarding US diagnosis of CE, we experienced in our cohort of patients, that only 22.2 % (10 out of 45) had available and precise US description of CE cysts allowing WHO-IWGE classification (26). US is crucial not just for confirming the diagnosis in cases of particular cyst stages, but also for making decisions about preferable treatment modalities according to the stage-specific approach (87). To date, in Hungary,

radiologists do not apply cyst staging on a regular base as proposed by the WHO (22,26) in 2001 and there is an urgent need to raise awareness, as well as to improve education and training on CE. In accordance with other authors (87), we conclude that neither serology alone, in the absence of evocative imaging, or imaging alone in the absence of pathognomic findings are methods, a CE reporting system should rely upon in Hungary. Serology has only a supporting role in the diagnosis. US imaging itself as a confirmatory method is only useful in the presence of pathognomic findings and in the hands of specifically educated and experienced operators. A CE surveillance and reporting system based on nationwide histopathological results supplemented with unified, patient-associated serological and imaging data by computer mediated synthesis would be more appropriate. As highly organized well developed models to be followed the Italian Registry of Cystic Echinococcosis (RIEC) (156) and the later established European Register of Cystic Echinococcosis (ERCE) (157) need to be mentioned here. These registers process complex epidemiological and clinical data of thousands of CE patients from Italy and from many other countries in Europe – including Hungary - and the Middle-East where CE is endemic (158). Besides their obvious scientific benefit, these registers can also serve local epidemiological purposes.

Medical treatment of echinococcal cysts with ABZ is often incomplete or is lacking in Hungary. Analyzing available data, BMZ treatment was documented in only 6 out of the 45 CE patients in our cohort, which seems to be a suprisingly low number considering that many of our patients underwent invasive procedures (biopsy, PAIR, surgery) which increase the risk of secondary echinococcosis. We have practical unpublished evidence from interviews with CE patients that efficient ABZ prophylaxis supported by some authors (87,159) is currently a missing practice in Hungary. We presume two major causes which lead to short-term or interrupted ABZ administration or complete lack of antiparasitic treatment. Primarily, as mentioned above, information on cyst stage which would help to determine the precise indication and duration of ABZ treatment is often missing. Clinicians, after the introduction of the stage specific treatment approach by the WHO/IWGE in 2010, are rarely aware of this strategy. In five of the six cases of CE5 stage cysts patients received BMZ unnecessarily. Secondarily, the form of ABZ which is recommended for continuous administration in CE (and also in AE) has poor availability due to the lack of marketing authorisation in

Hungary, and thus needs to be imported from EEA based on the licence of the Hungarian National Institute of Pharmacy and Nutrition. This could also contribute to the frequent administration of easily available low-dose MBZ, which in our opinion is meaningless and ineffective in CE. The first documented application of the PAIR technique in the treatment of Hungarian CE patients dates back to 1999. The authors tried to reduce fears of potential complications and pointed out that application of the PAIR technique is a safe and effective treatment method for CE (160). Although professional and material conditions for this practice are available in most of the Hungarian centres specialized in hepatic surgery, - just as in the centre of our study - we experienced that PAIR and other advanced interventional techniques were rarely applied in the treatment of CE. We registered only four cases over a fifteen year period when PAIR was used. It might be explained by persisting unjustified worries of anaphylaxis (161) and disbelief in its curing efficacy.

The oldest treatment modality for CE is surgery. Surgical removal of hydatid cysts had already been documented in Hungary since the end of the nineteenth century (20,21). To date it has remained the most frequent way of treating CE. In our series 55.6 % (n=25) of the patients were treated surgically. Types of intervention were: liver resection (segmentectomy, hemihepatectomy), pericystectomy, cystectomy, and laparoscopic cyst fenestration (22).

The WW approach is recommended in CE4 and CE5 stages (87). In our series it was applied only in four cases, and only in one case of the six with well defined CE5 cysts which suggests that Hungarian clinicians do not follow this recommended stage-specific approach and treat CE patients in cases when surgical and/or medical intervention are unnecessary.

Based on our data, we detected 18 cases (40%) that were presumably cured, although no long-term follow-up was available to assess possible recurrences. The loss or lack of regular follow-up activities are common difficulties hampering the evaluation of the precise cure rate or outcomes of CE cases in Hungary. For example, many cases with well-characterized cysts were treated outside the current international guidelines (22). Another major limitation of our work is that almost three quarters (73.3%) of the CE patients were recruited from a single surgical center specialized in abdominal surgery. This implies that [I] cases of pulmonary CE – its second most common form –

are absent from the study, and [II] possible abdominal CE cases which were not referred to surgery, and [III] patients who have abdominal CE but are not belonging to our center upon the territorial order of patient care were not included in this study. In this context, in Hungary, there are eight surgical centers in total with a similar profile and that are specialized in hepatic surgery; in these we can assume an equal probability to capture abdominal CE. For this reason, we can roughly estimate that the 33 CE cases identified in this single centre study can be multiplied by at least 8, providing the underestimated figure of around 260 patients with abdominal CE during the study period (22) not including cryptic CE patients who were not referred to surgery, thereby suggesting an even higher CE incidence.

IV.2. Clinical epidemiology of alveolar echinococcosis in Hungary

IV.2.1. Historical aspects and first human cases

The clinicopathology of alveolar echinococcosis as a form of human echinococcosis was first described by Buhl and Virchow during the 1850's (162,163). A hundred years later, the causative organism *E. multilocularis* as a distinct *Echinococcus* species and its life- cycle were identified by parasitologists R. Rausch, E.L. Schiller, and J. Vogel (164,165,166,167). Until the end of the 1980's AE in Europe was restricted to the central region composed of Eastern France, Switzerland, Southern Germany, and Western Austria, the so-called historical AE endemic area (23).

We are not aware of whether human AE infection had possibly occurred in Hungary before 2003; it has not been documented in any case. A presumed report of AE was made in 1988 by surgeons (168) but based on the detailed description of the case it can in fact be classified retrospectively as CE (65). Another possible AE case from 2001 in county Békés, with evocative serological results, was explored during our research. Based on the diagnostic criteria of WHO-IWGE it too was re-diagnosed as CE and excluded from the AE study. *E.m.*-infected red foxes were first identified in 2002, proving an eastward extension of the historical endemic area and at new risk of acquiring AE infection in Hungary. The appearence of human AE infection had been predicted by the authors (65). Retrospectively, we revealed seropositive probable AE

cases from 2003, but lacking histopathological confirmation we were unable to draw conclusion about these early cases (68). The first confirmed human AE infection was proven parasitologically in 2005 and was reported in 2008. During the diagnostical work-up of a giant peripheral liver lesion in the right lobe of an asymptomatic patient, core-biopsies and later explorative laparotomy were performed. In possession of the suggestive serological and histopathological results, *E.m.* confirmation was achieved by the PCR method. This first confirmed AE patient was living near the border between Hungary and the AE-endemic Austria, thus the autochthonity of the infection could not be proved (19). The patient is still receiving ABZ and his unresectable AE lesion has stabilized (68).

Meanwhile, according to the animal studies carried out in subsequent years, *E.m.* infection in red foxes has been proven in most Hungarian counties (65,66,67). Autochtonous human infections and an increasing incidence of AE in subsequent years had to be expected. The first undoubtedly autochtonous human AE case was reported in 2016 (84). We have presented the diagnostic challenge of recognizing the cause of multiplying small liver lesions of unknown origin. The primary clinical objective was to rule out malignancy but inconsistency between negative *E.m.* serology and AE suggestive histopathological features has led us to take further steps to confirm the diagnosis. IH using the highly specific Em2G11 monoclonal antibody finally confirmed the *E.m.* infection of the liver (Fig 13). As the patient, an elderly lady involved in agriculture, had experienced red foxes in her kitchen-garden but had never traveled abroad in her life, the autochthonity of her *E.m.* infection was highly probable (not accounting or the possibility that infection was transmitted by imported vegatables or fruits). Eventually, the endemic transmission of *E.m.* in Hungary became evident.

IV.2.2. Epidemiology of alveolar echinococcosis and the emergence of Echinococcus multilocularis infection in Hungary

In 2010 the global burden of AE was calculated at 666 434 DALYs per annum (CIs 331 000-1.3 million). The highest prevalence of AE in the world was registered in China. There were approximately 18 235 (CIs 11 900-28 200) new cases of AE per annum globally with 16 629 (91%) occuring in China, 1 606 outside China and only

109 (0.6%) in the classical endemic region of Europe (Germany, Switzerland, France, Austria). The annual estimated number of AE cases in Hungary was one case per year (169). During the past few decades, autochtonous AE cases have been reported from many European countries where this disease had not been noted previously (154,170). Among the countries neighbouring Hungary, the first cases were reported in Romania in 1999 (171,172), Slovakia in 2004 (173), Slovenia between 2001 and 2005 (174), and Croatia in 2014 (175).

In Hungary, since the first identification of *E.m.* in the major definitive host (red foxes) (65), several studies about the prevalence of *E.m.* in red foxes have been carried out. The presence of the parasite was demonstrated in 18 of the 19 counties, comprising Budapest, between 2008 and 2019, with an average prevalence of 7.6% in 3265 analyzed red foxes (66,67,68). Extensive infection of red foxes can be regarded as a substantial condition leading to environmental egg contamination and a risk of human AE. Furthermore, the urbanization of foxes observed in Hungary (64), is an important additional factor increasing human exposure to *E.m.* infection. To what extent domestic dogs contribute to human *E.m.* infection in Hungary requires future investigation. Although the prevalence of *E.m.* in natural intermediate host Arvicolid rodents has still not been examined in Hungary.

We retrospectively revealed 16 human AE cases between 2003 and 2018 in the territory of Hungary, which represents all the recognized Hungarian AE cases up to the end of our study. The mean age of patients was 53 years (range: 24–78 years). The sex ratio was 0.5. The average annual incidence of AE in the period between 2003 and 2018 was calculated as the average annual number of cases per 10^5 inhabitants, using the national population data for the median year 2009 as a reference (176). AE incidence in Hungary was 0.1595 cases/100 000 inhabitants, which is low in comparison with the estimated AE incidence of other *E.m.* endemic European countries, but suggests a ten fold higher annual case number than the estimated figure of one case per year (169). We experienced a slight increase in annual case numbers during the study period: until 2016 the number of cases did not exceed one case per year; from 2016 more than one AE case was detected each year (4,68). Since the termination of our study four further possible AE cases were registered in 2019. In 2020 AE was not notified based on the serological results of the National Public Health Center (unpublished, pers. comm.

Danka, 2021). As 94% of the cases in our study were recruited by seropositivity for *E.m.*, cases diagnosed exclusively by histopathology are lacking for statistical analysis. Therefore the real number of AE cases during this period in Hungary is thought to be underestimated (68).

By analyzing the places of residence of the affected patients in our study, we found that the residence of human AE patients overlaps the geographical distribution of *E.m.* infected red foxes in Hungary (Fig 14), suggesting that the majority of human cases may be autochthonous, thereby supporting our hypothesis of endemic occurence of AE in Hungary (68). The presence of *E.m.* infection in golden jackals (*Canis aureus*) as definitive hosts in Hungary is also proven, particularly in the core area of their habitat in Somogy county, with a prevalence of 15.6% (71). Three patients from our cohort (n=3; 18.8 %) lived in Somogy county, including the first probable AE patient from 2003 (68). This represents a figure of 0.9311 cases per 100 000 inhabitants, more than five times higher than the overall incidence of AE in the country. More recently, in 2019 two further AE cases were reported from Somogy county (72, unpublished). Although regional case accumulation can be observed in this south-western county of Hungary, and the association of this phenomenon with infected red foxes and golden jackals seems to be reasonable, a statistical correlation between AE cases and the prevalence of infected definitive hosts cannot be made because of the low number of human cases.

The majority of AE patients from our study (n = 11; 68.8%) were living in rural or suburban areas, which corresponds with previous observations (53) and contributes to the notion that living in rural areas may be a risk factor for AE infection (177). Regarding other main potential risk factors, almost three quarters of the patients had kitchen gardens or visited forests for vocational reasons (68).

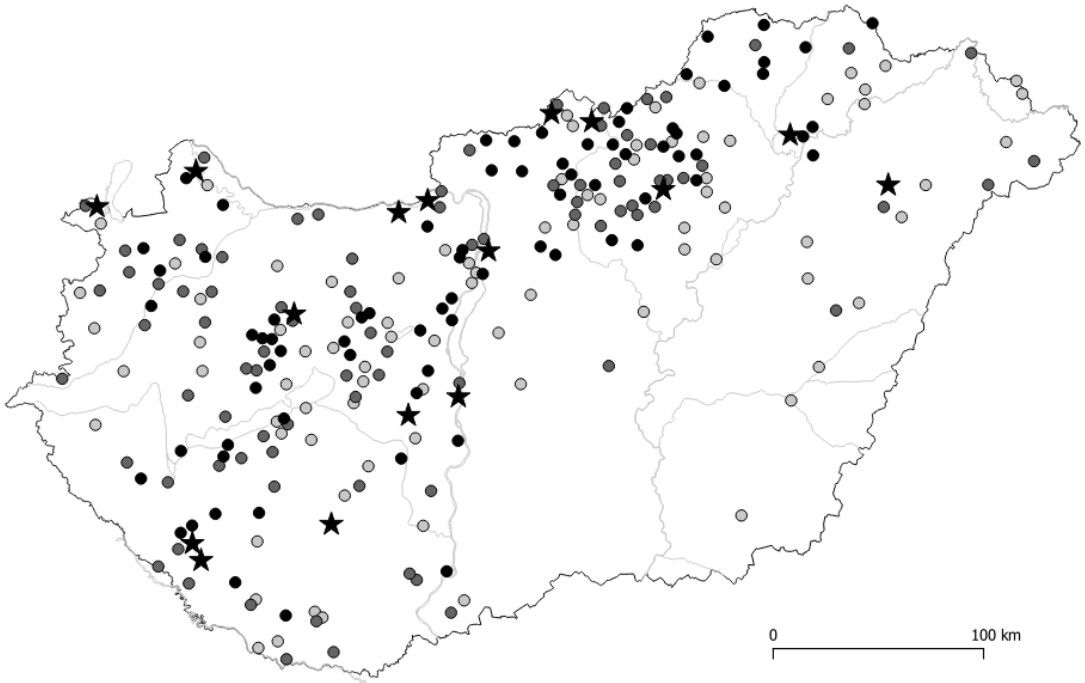

Fig 14. Spatial distribution of 16 AE human cases (stars) and that of 247 red foxes (*Vulpes vulpes*) (dots) infected with *Echinococcus multilocularis* out of 3265 foxes examined between 2008 and 2019. The darkness of the dots reflects the intensity of infection in foxes (light grey: <10 worms; dark grey: 10-100 worms; black: > 100 worms) Approximately 4% of the fox population of each county was sampled (68)

IV.2.3. Challenges of alveolar echinococcosis diagnosis in Hungary

The major diagnostic challenge in AE, just as in other diseases with a chronic course and an asymptomatic nature at the early stage, is to identify the disease during its progression as early as possible. AE of the liver acts like a slowly growing tumor; a peripheral lesion can stay asymptomatic for a long time until it becomes very large and symptomatic. Conversely, a smaller AE lesion located near the porta hepatis can present with obstructive jaundice and vascular complications earlier (87,110). In our series six patients (37.5%) were diagnosed at a stage when AE had already infiltrated the porta hepatis and/or v. cava inferior (P4); in this subgroup lethality reached 50% (68). Among the three patients who eventually died, one had been refusing investigations planned to clarify the entity of her hepatic lesion for almost ten years; in the two other cases the diagnostic delay reached ten years (!). On the other hand, in the cases of the two symptomatic patients with P4 and early diagnosis (less than one year), stabilization of AE was achieved by treatment. The sixth patient with P4 was asymptomatic at first

presentation; AE diagnosis was made after 17 months and no recurrence was detected 10 months after radical surgery and concomittant ABZ treatment.

In summary, our results support that early diagnosis substantially affects the outcome of AE and can improve the prognosis even in advanced cases. In asymptomatic patients AE is usually an incidental imaging finding. During differentialdiagnosis by imaging methods, AE mimics CE, and other benign hepatic lesions (e.g. sarcoidosis, bacterial abscess, hemangioma see - III.2.2, Table 2.) and also a series of malignancies (e.g. ICC, HCC, liver metastasis). If non-malignant disease is suspected, diagnostical work-up can be halted, or may lead to diagnostic pitfalls such as CE, hemangioma, or bacterial liver abscess, rejecting further investigations and delaying the introduction of appropriate treatment (178). Raising suspicion of malignancy has the advantage that it urges for tissue sampling and histopathological evaluation, which can help to recognize and confirm AE in addition to excluding neoplasia. ICC is a frequent misdiagnosis during AE evaluation. Calcification combined with septal or no enhancement of lesions during CT and MRI imaging has 100% specificity for AE (179); in our series 68.8% of patients would have been distinguished from ICC during preliminary imaging based on this characteristic of AE (68). We also found that recommended efforts to search for parasitic metastases in the lungs and brain (87) were carried out in only six cases (37.5%). The main causes of this phenomenon are the failure to discriminate between CE and AE; and clinicians remaining unaware of the possible occurence of AE and its truly invasive and metastatic nature. This is why diagnostical investigations often stop at the level of unspecified Echinococcosis, a practice which has now became particulary dangerous considering the appearance of human AE.

Besides imaging, - the current primary method in Hungary for distinguishing AE from CE is serology (125). In our series we detected anti-*E.m.* antibodies in 13 patients (81.25%) which supports the diagnostic usefulness of Westernblot serology in combination with other methods (87,138). Differentiation between CE and AE and the identification of AE by histopathological analysis of tissue samples are challenging and important tasks for pathologists. Because of the still low AE incidence and coexistence of the better known CE, preparedness of pathologists to recognize AE with conventional staining methods (e.g. H&E, PAS) and to subtype the two disease forms, can be low. In our series, conventional histopathological analysis of samples gained by core-biopsy,

surgery, or autopsy was performed in the ten confirmed AE cases (62.5%). Here we emphasize that the frequently applied fine-needle aspiration biopsy is an inappropriate method for ruling out AE. If patognomic protoscoleces or hooklets are found in the aspirate, - a rare event in AE (37,47,126,127,128), - cytology without a further confirmatory method can only support the diagnosis of unspecified Echinococcosis, thus cytological results were excluded from our study. AE specifically, with its distinct histopathological features (131) was recognized independently from other diagnostic methods, in only three cases. Considering the low incidence of the infection, such vigilance from the recognizing pathologists can be appreciated, as advanced confirmatory methods (e.g. PCR, IH) are currently unavailable in Hungary.

In summary, misdiagnosis - such as CE and other non-malignancies – and substantial delay in AE confirmation are the major current pitfalls of AE diagnosis in Hungary. Although some of the advanced confirmatory methods are not currently available in Hungary for prompt diagnosis, accurate and combined application of conventional methods (imaging, histopathology) are enough to distinguish AE from CE and for the timely diagnosis of AE.

IV.2.4. Current difficulties of alveolar echinococcosis treatment in Hungary

In Hungary each therapeutical modality is available for the appropriate treatment of AE.

Antiparasitic drug treatment Primarily, we believe that deficiency in sustained antiparasitic drug treatment with ABZ – indicated in unresectable cases and after surgical removal of AE lesions (87) - originates from the lack of proper clinical knowledge on AE infection, as in most cases diagnostic uncertainty or missing histological confirmation has led to short-term/interrupted or a complete lack of ABZ treatment (68,178). Secondarily, deficiency in continuous antiparasitic treatment is mainly attributable to the lack of ABZ marketing authorisation in Hungary, which leads to insufficient ABZ supplies to patients and results in treatment delays and interruptions (detailed above in IV.1). ABZ-related drug toxicity as major complication of drug treatment was reported in one case. A more than tenfold increase in amino transferase levels and the appearance of generalized giant urticarias directly associated with ABZ administration has led to the ceasing of antiparasitic treatment. In one case, hair-loss

and mild leukocytopenia were reported as ABZ-associated minor side-effects which were tolerated by the patient.

Endoscopic and percutaneous interventions EPIs as palliative non-resective procedures were used in five advanced AE cases from our cohort of patients (68, Table 2). At the time of first interventions, which were performed because of the blockage of extrahepatic bile ducts, the exact diagnosis of AE was known in only one case. Preliminary diagnoses during cholangiography were: perihilar extrahepatic bile duct tumor and, abscessing CE. Bile passage was transiently resolved in each case, often by a combination of methods (placement of nasobiliary stent and PTD; endoscopic placement of biliary stent and PTD). Three out of the five patients, who underwent EPI, eventually died of AE-associated complications, and one case (No.4) - with total blockage of the common hepatic duct by AE - was under consideration of OLT until the end of the study. The high fatality in this subgroup can be explained by the fact that these interventions are usually performed for palliative purposes in a fragile group of patients to facilitate bile passage in case of centrally located AE lesions with high PNM stage and unresecability. Prior to EPI, unresecability of the lesion was formally assessed only in one case, and in one advanced case EPIs were applied before radical surgery, resulting in lethal complications. Therefore we experienced considerable deviation from the WHO IWGE recommendations, which suggest EPIs for palliative treatment of defined unresectable cases and should redeem heroic surgical attempts.

Surgery In Hungary there are eight surgical centers specialized in hepatic surgery, thus professional and material conditions are readily available for curative surgery of AE. In our studies, radical (R0) surgery was performed in five AE patients (31.25%). In four patients (25%), no recurrence of AE was detected after radical surgery and concomitant ABZ treatment. In this subgroup of patients, the disease-free period from curative surgery to the date of final imaging ranged from 10 to 74 months (mean 42 months) which corresponds to a 100 % three-year survival rate. In one advanced case (P4N0Mx) the patient died on the thirteenth postoperative day after radical surgery because of its complications (postoperative bleeding, liver failure, septic shock). In two cases AE was presumably mistreated as CE, abscessing CE and fenestration, marsupialisation and drainage were performed without concomitant ABZ therapy according to the clinical descriptions. An AE lesion usually does not have a definitive

parasitic cyst wall, the so-called endocyst. Marsupialization - preparing communication between the external and internal part of the lesion - would be more aptly named surgical drainage here as palliation and can be regarded as a less favourable treatment method in AE (87,138). Numerous complications were registered following these procedures: long-term external bile-drainage, namely biliar fistula, biloma, hematoma, bile-leakage with subsequent bile-acid malabsorption, and cachexia (Table 2). Again we highlight, that diagnostic uncertainty, usually through a clinician confusing CE and AE, and missing histological confirmation led to inappropriate surgical interventions, including radical surgery with severe complications (68,178).

Liver transplantation is a salvage therapy in advanced unresectable AE cases (87,138). In Hungary an average of 73 liver transplantations per year have been performed in the last five years (180). Liver transplantation as a result of AE has not been carried out in Hungary to date. In one AE case (No.4) with total blockage of the common hepatic duct by the lesion, after several palliative attempts, liver transplantation was under consideration until the end of the study. Considering recent trends in the epidemiology of AE and the occurence of advanced, late-presenting cases, Hungarian experts have to be aware of this possible treatment option for AE.

IV.2.5. Follow-up management of alveolar echinococcosis in Hungary

Data regarding the number and quality of follow-up visits of AE patients are limited. Nonetheless, we presume, that the seemingly inappropriate follow-up activity originates from the low incidence and basically unknown status of AE and also from frequent misdiagnoses. We regard this as a further contribution to the high rate of disease progression (43.8%) and lethality (18.8%) among Hungarian AE patients. Although advanced tools as ^{18}F FDG PET/CT and laboratory measurement of ABZ sulfoxide level are not currently available in Hungary for monitoring AE, we believe that better awareness of the disease – through education of clinicians - can increase the frequency of follow-up visits using conventional imaging and laboratory methods. Progression and complications associated with AE could be prevented by the timely recognition of any alteration in the disease stage using the PNM classification (151).

IV.2.6. Outcome of alveolar echinococcosis cases in Hungary up to 2019

Twentyfive percent of Hungarian AE patients have been presumably cured and 18.8 % have been stabilized by treatments based on WHO-IWGE recommendations. In cases of cured patients, after R0 liver resection and concomitant ABZ administration, recurrence of the disease was not detected until the end of the study. In cases with unresectable AE lesion(s), continuous ABZ treatment led to the stabilization of lesions. This experience from the Hungarian cohort supports the previous observations of other researchers that the adequate treatment of AE according to the WHO-IWGE recommendations, results in a favorable outcome (146,149,150,151,181). Some of the patients from our study did not show progression despite the lack of appropriate surgical and/or antiparasitic drug treatment. In the background we can presume the individual immunologic characteristics of the affected patients as certain HLA associated immunoresponsiveness, through the operation of Th1 type immunity characterized by IFN α (182), IFN γ (183), IL 12 (184) and TNF α (185,186) cytokine profile, can lead to the abortion of *E.m.* infection (115). Another possible explanation is the parasitocid effect of ABZ despite the lack of sustained administration (139,140,141). On the other hand, we have found supporting evidence (e.g. case No.10) for the previous observation that immunosuppresion can lead to AE progression despite appropriate antiparasitic treatment (118).

In our series, we have revealed a 43.8 % progression and an 18.8 % lethality rate. This fatality rate is comparable to those experienced in some *E.m.* endemic regions of Asia, 25% has been described in a case series involving AE patients who underwent palliative surgery (187), - and significantly higher than the AE lethality reported recently from historically endemic European countries (150,151). Since 2005, no other autochthonous parasitic infection has resulted in human death in Hungary (4), therefore we can regard AE as the most dangerous, potentially lethal endemic parasitic infection in the country. Fatal cases of this series correspond with historical survival rates of the old-endemic regions before the introduction of benzimidazoles and advanced surgical and interventional techniques, when the 15 year lethality of AE reached 100 % from diagnosis without treatment (139,149).

We have revealed that substantial delays in diagnosis, misdiagnosis – such as

CE, inadequate treatments characterized by short-term/interrupted or complete lack of antiparasitic ABZ dosing, inappropriate surgical procedures containing radical surgery with severe complications, and non-radical palliative surgical interventions were the major contributing factors responsible for the unfavorable outcomes and poor prognoses of Hungarian AE patients.

IV.2.7. Prevention of human alveolar echinococcosis and related complications

For primary disease prevention it is necessary to reduce egg contamination in the environment to avoid human infection. Because of the basically sylvatic life cycle of *E.m.*, control of the parasite in wild animals acting as host species – primarily red foxes - is a challenging task. Controlling fox population size and density by culling and hunting activities could possibly reduce infection pressure but remains a subject of constant debate regarding its efficacy and applicability (188). Some countries – for example the adjacent *E.m.*-endemic Slovakia – have managed to lower infection pressure with *E.m.* eggs by deworming red foxes through regular baiting campaigns using praziquantel (PZQ) as an antihelminthic drug (189). But parasites survive in rodents and in the environment, thus considering the 30 days prepatency period for *E.m.*, monthly deworming would be necessary to prevent reinfections. Therefore the efficacy and the costeffectiveness of this method are highly questionable (188). In regions with high prevalences of *E.m.* infection in dogs there is frequently a high incidence of human AE (190,191,192,193,194,195,196,197). In areas, such as Hungary, where AE and CE are coendemic, regular dosing of domestic dogs with PZQ, more than 4 times per year is reasonable. Considering the shorter time span and prepatency period of *E.m.*, more frequent dosing would also decrease reinfections by *E.m.* and reduce the risk of human AE as well as CE and seems feasible in combination with human hygiene-linked measures (e.g. hand and food hygienic rules) (188). Keeping away wild canids (viz. red foxes, golden jackals) and even untreated domestic dogs from kitchen gardens is warranted. Regarding future prospects, several *E.m.* specific antigens, such as Em 14-3-3 (198), Em 95 (199), EMY 162 (200) and Em Tetraspanins (201) have been proven to be immunogenic in murine studies and have induced protective immune

responses against primary infection, providing a promising model for the development of human vaccines against AE.

For the early detection and secondary prevention of AE, combined abdominal US and serological screening is reasonable (187,202), particularly for professional foresters and/or farmers who are at higher risk of acquiring AE than the average person.

For tertiary disease prevention and, to avoid AE-related complications, the timely confirmation of AE, application of the PNM staging system, regular follow-up visits and treatment according to WHO-IWGE recommendations can be regarded as standard preventive measures (151,152).

V. Conclusions

1. Our study is the first comprehensive research on the epidemiological features of CE and AE patients in Hungary.

2. CE is the most prevalent notifiable helminthic disease in Hungary in recent times. We found that the highest incidence of CE can be detected in the Great Plain of Hungary where large scale livestock breeding occurs traditionally, and the prevalence of *E. granulosus* infection in these intermediate host animals has been proven previously. This observation is in line with the pastoral domestic life cycle of the *E. granulosus s.l.* parasite widely observed in other CE-endemic parts of the world, although prevalence of *E. granulosus* in domestic dogs has not been investigated in Hungary to date. The real burden of human CE is significantly underestimated in Hungary according to our results.

3. AE is an emerging helminthic disease in Hungary; residence of AE patients in Hungary overlaps with the geographical distribution of *E. multilocularis* infected red foxes as definitive hosts. The precondition of acquiring AE is the environmental *E.m.* egg contamination originating from the presence of infected definitive hosts. We conclude that the appearence of human AE cases after 2000 and the increase of AE incidence from a hypothetical zero to sporadic - can be explained by [I] the endemic transmission of the *E.m.* parasite in its sylvatic lifecycle and [II] its synanthropic transmission patterns (infection of domestic dogs and urbanization of infected red foxes) in the territory of Hungary.

4. Our research is the first to reveal current clinical characteristics of the two distinct echinococcosis forms, namely CE and AE, in Hungary. CE and AE are often confused by clinicians (diagnosis, treatment, follow-up, and prognosis). Differentialdiagnosis of CE and AE is a serious challenge for clinicians in Hungary and should not exclusively rely on a single diagnostic method (e.g. serology or imaging) but on a combination of methods. To distinguish AE from the coincidental CE and hepatobiliar malignancies in time is fundamental and influences the treatment and therefore the long-term outcome of cases.

5. Regarding CE, the regular application of WHO-IWGE US classification and identifying different cyst stages during imaging evaluation could help to improve diagnosis and treatment. Application of the stage specific approach accompanied with regular patient follow-up in the management of CE, could also help to improve curing efficacy and, prevent CE recurrence and complications originating from unneccessarily or inappropriately used therapeutical procedures. The clinical practice detailed above is still not generally applied in Hungary.

6. Regarding AE, during the detection of a liver lesion of unknown origin, AE has to be considered in Hungary, and evocative imaging with or without supplementary serological results indicates a need for tissue sampling for histopathological analysis and early confirmation of the infection. Application of the PNM system is useful in treatment planning and in determining prognosis. We conclude that diagnostical delay, frequent misdiagnosis, insufficient antiparasitic drug treatment, and inappropriately applied invasive therapeutical procedures are the major factors contributing to the poor outcome of AE in Hungary to date.

7. Among notifiable helminthic infections in Hungary, AE has the highest lethality and mortality in recent years. Since 2004, no other autochtonous parasitic disease has been reported and identified in Hungary with a fatal outcome, and all three lethal cases of autochthonous parasitic originating between 2004 and 2020 were AE.

8. The rate of progression (43.8%) and lethality (18.8%) in our AE series are inferior to AE outcome properties of other European regions where AE has been endemic for a longer time. We conclude that the minimally neccessary and recommended diagnostic and therapeutic tools are readily available in Hungary to effectively cure AE, therefore the education of clinicians should be introduced to improve the outcome and prognosis of this newly endemic helminthic disease.

9. We propose to create Hungarian AE teams - on the pattern of modern oncoteams – with the extensive collaboration of experts (infectologists, surgeons, radiologists, pathologists). These national teams could help to optimize the clinical management of AE patients, bring it to an evidence based level and edit national/institutional AE guidelines to follow.

VI. Summary

Echinococcosis is the most prevalent notifiable helminthosis in Hungary. Two distinct forms of echinococcosis are cytic echinococcosis (CE) caused by *Echinococcus granulosus sensu lato*, and alveolar echinococcosis (AE) caused by *Echinococcus multilocularis*. The latter is considered the most pathogenic zoonotic helminthosis in the temperate region of Europe. The metacestode of *Echinococcus multilocularis* develops mainly in the liver of infected humans. An evolving AE lesion consists of a parasitic mass surrounded by granulomatous inflammation, and has the potential to invade adjacent organs and to form distant metastases thus behaving clinically like a malignant tumor with potentially poor prognosis. The present thesis unites two separate research projects with retrospective data analysis to describe the current clinical epidemiology of cystic and alveolar echinococcosis in Hungary.

In the first project, the clinical epidemiology of CE was investigated. Cystic echinococcosis has been proven to be prevalent mainly in those regions of Hungary (e.g. Great Plain) where breeding of intermediate host ungulate species is a traditional practice. Inadequacies in the epidemiological reporting system have led to susbstantial underestimation of CE, thus contributing to its neglected nature. As a consequence, clinical management of this infection diverges from internationally accepted current WHO guidelines in many cases regarding diagnosis, treatment, and follow-up.

The second project demonstrates that AE is a newly endemic, emerging infectious disease in Hungary with potentially poor clinical outcome. Appearance of human AE disease can be traced back to the increasing population size of *Echinococcus multilocularis* infected definitive host carnivore species in the territory of Hungary. Analyzing data of all recognized human AE cases in Hungary, we proved that diagnostical delay and frequent misdiagnosis resulted in belatedly introduced and/or inappropriate medical and surgical treatment of AE, contributing to the high rate of progressive and lethal cases. We also proved that AE has now become the most deadly autochtonous parasitic disease in Hungary. Distinguishing AE from the coexisting CE and hepatobiliary malignancies has become necessary in clinical practice, warranting the education of experts and improve the prognosis of AE patients in Hungary.

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VIII. List of Publications

Articles related to the thesis

Dezsényi B, Somorácz Á, Danka J, Kucsera I, Barth TFE, Casulli A. (2018) Human cystic echinococcosis in Hungary (2000-2014): a retrospective case series analysis from a single-center study. Infection, 46: 477-486.

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Dezsényi B, Strausz T, Makrai Z, Csomor J, Danka J, Kern Peter, Rezza G, Barth TFE, Casulli A. (2016) Autochtonous human alveolar echinococcosis in a Hungarian patient. Infection, 45: 107-110.

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Dezsényi B, Dubóczki Z, Strausz T, Csulak E, Czoma V, Káposztás Z, Fehérvári M, Somorácz Á, Csilak A, Oláh A, Almási K, Patonai A, Görög D, Széll Z, Tolnai Z, Sréter T, Danka J, Auer H, Gruener B, Barth TFE, Casulli A. (2021) Emerging human alveolar echinococcosis in Hungary (2003-2018): a retrospective case series analysis from a multicentre study. BMC Infect Dis. 21: 168.

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Other articles

Grimm J, Beck A, Nell J, Schmidberger J, Hillenbrand A, Beer AJ, Dezsényi B, Shi R, Beer M, Kern P, Henne-Bruns D, Kratzer W, Moller P, Barth TF, Gruener B, Graeter T. (2020) Combining Computed Tomography and Histology Leads to an Evolutionary Concept of Hepatic Alveolar Echinococcosis. Pathogens. 9: 634.

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