Echinococcus canadensis G7 (Pig Strain): An Underestimated Cause of Cystic Echinococcosis in Austria

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Abstract. Anamnesis data of 104 patients with Cystic Echinococcosis were correlated retrospectively with the detected species/strain of *Echinococcus*. Ninety-two percent ($N = 23$) of autochthonous Austrian and 33% ($N = 9$) of patients with former Yugoslavian (YU) origin were infected with *E. canadensis* G7, the pig strain. All patients originating from Turkey harbored *E. granulosus* G1, the sheep strain. All *E. canadensis* G7-infected patients showed small liver cysts (ϕ 5.9 cm), only one of them an additional lung cyst. The median age at the time of operation of the Austrian patients was 55 years, of the Turkish patients 30 years, and of the former YU patients 23 years in the *E. canadensis* and 42 years in the *E. granulosus*-infected patients, respectively. The unexpected high number of *E. canadensis* G7-infected patients and the immigrants' young age show the importance of *E. canadensis* as a cause of human Cystic Echinococcosis in Central Europe and accordingly this new species has to be included into future echinococcosis control programs.

INTRODUCTION

Cystic echinococcosis (CE), caused by the metacestode stage of *Echinococcus granulosus* (sensu lato) belongs to the most serious helminthozoonoses and is considered an emerging disease throughout the world.¹ Dogs are the definitive host, livestock the intermediate, and humans the aberrant intermediate host. Although the incidence in Austria is rather low, 0.4 human cases are diagnosed per 100,000 inhabitants every year,² decreasing numbers of autochthonous human cases face rising numbers of imported cases.³ After some years of controversial discussion about the taxonomic classification, the 10 strains (G1–G10) of the former species *E. granulosus* have recently been split into four species of different pathogenicity to humans;^{4,5} *Echinococcus granulosus* (G1–G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), and *Echinococcus canadensis* (G6–G10).

In a previous study, we identified *E. canadensis* G7 as the major causative agent of human CE in the autochthonous Austrian population.⁶ However, little is known about the clinical presentation of CE in humans caused by *E. canadensis* G7 (“pig strain”). Therefore, we evaluated molecular biological, clinical, and anamnesis data of 104 CE patients operated on in Austrian hospitals between 1978 and 2008. Twenty-four percent of the patients were of Austrian origin, 35% of Turkish, 26% originated from the former Yugoslavia (YU), and 15% from various other regions. Because the majority of Austrian immigrants originated from Turkey and the former YU, we focused our interest on those geographical regions on one hand and on the Austrian patients on the other.

MATERIAL AND METHODS

Patients. In 66% ($N = 69$) of the 104 CE patients only formalin-fixed, paraffin-embedded tissues (FFPT) were available for molecular biological studies (kindly provided by Prof. Friedrich Wrba, Medical University Vienna), in 34%

($N = 35$) fresh, frozen, or 70% ethanol preserved cyst material was used for strain/species determination.

Details on cyst localization, sex, age range, country of origin, and results of the molecular biological studies of all 104 patients were logged into an Excel spreadsheet (Microsoft, Redman, WA) and analyzed (Table 1). Although the documentation of clinical symptoms, cyst diameter, and number of cysts per patient was incomplete, we analyzed the available data in the same way in relation to the results of the molecular biological study (Table 1). Because the international classification of ultrasound images in cystic echinococcosis, as proposed by the World Health Organization (WHO), is not yet established in Austrian hospitals, we could not include these data.⁷

DNA extraction and polymerase chain reaction. For DNA extraction from fresh, frozen, or ethanol preserved specimens, we chose a commercially available DNA extraction kit (Master Pure DNA Purification kit; Epicentre, Madison, WI) and followed the manufacturer's tissue protocol. The extraction procedure for FFPT was described previously.⁶

The polymerase chain reaction (PCR) with the primer pairs SH172F/SH467R and SW163F/SW367R was performed as described previously for all FFPT samples⁶ and modified for the fresh, frozen, and ethanol preserved cyst material in the following way. The 25 μ L reaction mixture consisted of 1 $\times$ Thermophilic DNA Polymerase Reaction Buffer (Promega, Madison, WI), 0.01% BSA (Roche, Mannheim, Germany), 200 μ M dATP, 200 μ M dGTP, 200 μ M dCTP (Promega), 400 μ M dUTP (Applied Biosystems, Foster City, CA), 2.5 mM MgCl₂, 0.2 μ M of each primer (MWG Biotech, AG, Germany), 0.25 U UNG (Applied Biosystems), 1.5 U aTaq DNA Polymerase (Promega), and 2.5 μ L of sample DNA.

The PCR was performed in a PTC-200 gradient thermocycler (MJ Research Inc., Waltham, MA) with an initial denaturation step of 95°C for 10 minutes, followed by 40 cycles 95°C for 30 s, 57°C (shPCR) and 58°C (swPCR) for 45 s and 72°C for 60 s. The PCR was terminated with a final extension step at 72°C for 7 minutes. After amplification 10 μ L of the amplification products were resolved on a 1.5% ethidium bromide stained agarose gel and the amplified DNA fragments of specific sizes were visualized by UV fluorescence. Their sizes were verified by a standard DNA ladder (Bio-RAD, Hercules, CA)

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TABLE 1
Personal, epidemiological, and clinical data of patients suffering from *E. canadensis* G7, *E. granulosus*, or *E. canadensis* G6 infection

	Species/strain	Species/strain	Species/strain
	<i>E. canadensis</i> G7	<i>E. granulosus</i>	<i>E. canadensis</i> G6
Number of patients	33	68	3
Sex (female:male)	20:13	36:32	2:1
Mean age	45.8 (5–72) years	39.6 (8–76) years	39.0 (29–48) years
Origin	Austria (23) Former YU* (9) Hungary (1)	Turkey (36) Former YU (18) Romania (3) Austria (2) Albania (1) Greece (1) Italy (1) Chile (1) Lebanon (1) Tunisia (1) India (1) Thailand (1) Bulgaria (1)	Iran (1) Afghanistan (1) Ghana (1)
Organ	Liver (32) Liver and lung (1)	Liver (55) Liver and lung (2) Lung (3) Musculature (3) Diaphragm (2) Kidney (1) Pericardium (1) Heart and brain (1)	Liver (1) Lung (1) Spleen (1)
Average cyst diameter (<i>N</i> = 71)	5.7 cm (<i>N</i> = 23)	10.7 cm (<i>N</i> = 46)	5 cm (<i>N</i> = 2)
Minimal–maximal cyst diameter	3–10 cm	5–21 cm	5 cm
Single cyst	18	33	2
More than 1 cyst	5	13	
Calcified cyst	4	6	
Clinical symptoms (<i>N</i> = 68)	Upper abdominal pain (10) Asymptomatic (4) Suspected tumor (7) Biliary symptoms (2) Fever (1), nausea (1) Accident (1), anaphylactic shock (1)	Upper abdominal pain (18) Asymptomatic (2) Suspected tumor (2) Biliary symptoms (3) Relapse (4) Fever (3) Hemoptysis (2) Nausea (1), pregnancy (1) Ascites (1), renal colic (1) Thrombosis (1), stenocardia (1) Echinococcosis in the family (1)	

*YU = former Yugoslavia.

run simultaneously. The target sequences for the PCRs were located on a 471-bp segment of the mitochondrial ND1 gene,⁸ and the sizes of the PCR products were 295 bp for *E. granulosus* and 204 bp for *E. canadensis* G6/7. Control DNA from the reference strain and negative control were included in each reaction. Because the sequences of *E. canadensis* strain G6 and G7 are very similar, PCR product sequencing was necessary to discriminate between the closely related strains.⁶

RESULTS

Geographical distribution, sex, and age range. The majority of our patients were infected with *E. granulosus* (65%, *N* = 68), 32% (*N* = 33) with *E. canadensis* G7, and 3% (*N* = 3) with *E. canadensis* G6. From the 68 *E. granulosus*-infected patients, PCR product sequencing was performed from 44 (65%) and all of them turned out to belong to *E. granulosus* strain G1. The patients' age range was 5–76 (average 42) years, 56% were women and 44% men. The patients infected with *E. canadensis* G7 derived from Austria, the former YU (Serbia and Macedonia), and Hungary, those infected with *E. granulosus* from Turkey, the former YU (Bosnia, Serbia,

Kosovo, Macedonia), Romania, Austria, and various other countries (Table 1). The patients who harbored the camel strain G6 were immigrants from Iran, Afghanistan, and Ghana, respectively.

Age range of patients of Austrian, former YU, and Turkish origin in correlation with the detected strain. Statistical analysis (student's *t* test) of the patients' age at the time of operation showed that Austrian patients (*N* = 25) were significantly older than patients deriving from the former YU (*N* = 27, *P* < 0.005) and Turkey (*N* = 36, *P* < 0.0001), the median age of the Austrian patients was 55 (26–72) years, the former YU patients 39 (5–76) years, and the Turkish 30 (8–72) years (Table 2).

All patients of Turkish origin were infected with *E. granulosus* and all Austrian patients except two harbored the "pig strain" *E. canadensis* G7. From the patients deriving from the former YU, the median age of the *E. canadensis* G7-infected patients (*N* = 9) was 23 years, whereas the *E. granulosus*-infected patients' (*N* = 18) median age was 42 years.

Organ manifestation. Fifty-five out of the 68 *E. granulosus*-infected patients suffered from liver cysts, two from liver and lung cysts, five patients showed lung cysts, other localizations

TABLE 2

Echinococcus species/strain distribution and age range of patients from Austria, former Yugoslavia (YU), and Turkey

	Austrian origin (N = 25)	Former YU origin (N = 27)	Turkish origin (N = 36)
<i>E. granulosus</i>	2	18	36
<i>E. canadensis</i> G7	23	9	0
Mean age	53 years	40 years	36 years
Median age	55 years	39 years	30 years
Age range	26–72 years	5–76 years	8–72 years

were musculature, diaphragm, kidney, pericardium, and one patient harbored heart and brain cysts.

All 33 *E. canadensis* strain G7-infected patients harbored liver cysts; one of them (a 5-year-old boy from the former YU) additionally showed a lung cyst. The three *E. canadensis* G6-infected patients showed different organ localizations, the liver, lung, and spleen, respectively.

Cyst diameter. The average cyst diameter of 46 *E. granulosus*-infected patients was 10.7 (5–21) cm, 13 harbored more than one cyst, and in 6 patients the cysts were (at least partly) calcified.

From 23 *E. canadensis* strain G7-infected patients the average cyst diameter was 5.9 (3–10) cm, five harbored more than one cyst, and 4 cysts were calcified. In two of the *E. canadensis* G6-infected patients the maximum cyst diameter was 5 cm.

Clinical signs. Upper abdominal pain was the leading initial symptom ($N = 18$) in the *E. granulosus*-infected patients, followed by symptoms deriving from the biliary system ($N = 3$), fever and a wide range of symptoms reflecting the various organ manifestations (Table 1).

The main initial signs in the *E. canadensis* G7-infected group were upper abdominal pain ($N = 10$), seven were suspected of having a tumor, four patients were asymptomatic, two showed symptoms deriving from the biliary tract, one had fever, one nausea, one an anaphylactic shock, and one 5-year-old boy had a traffic accident. Information about the clinical symptoms of the *E. canadensis* G6-infected patients was not available.

DISCUSSION

The aim of this study was to evaluate the importance of *E. canadensis* G7 ("pig strain") as a causative agent of human CE in Austria for the first time. Accordingly, we analyzed clinical and anamnesis data of 104 CE patients operated on in Austrian hospitals between 1978 and 2008 and correlated them with the detected species/strain of *Echinococcus*. Because the majority of Austrian immigrants originated from Turkey and the former YU, we focused our interest on those geographical regions and the autochthonous population.

The role of the pig strain G7 as a causative agent of human CE was underestimated until recently,^{6,9,10} one possible reason is that small liver cysts might be symptomless for decades or probably for ever.¹¹ Our data confirmed this estimation, showing that the diagnosis of CE patients harboring the pig strain occurred more often incidentally (14.8%, $N = 27$) than in patients infected with the sheep strain (4.9%, $N = 41$, Table 1). Furthermore, another possible reason for the underestimation of the pig strain as a causative agent of human CE in Europe might be the small number of samples that have been examined genetically so far,⁴ and that infection of pigs with the sheep strain occurred frequently.^{12–14}

From the samples of CE patients of Austrian origin ($N = 25$), all except two belonged to *E. canadensis* G7, the pig strain (Table 1). Regarding the two *E. granulosus* G1-infected patients of Austrian origin, our attempts to obtain travel anamnesis data were successful. Both male patients were residents of Vienna, one of them, a 58 years old dentist, reported several travels as an individual tourist, for example to Syria, Tunisia, and other Mediterranean countries and we presume that he acquired his infection with *E. granulosus* G1 there. The other patient, a 34-year-old clerk, reported a few short vacations in Italy; additionally, he had spent parts of his adolescence on a farm with intensive animal contact (dogs, cats, pigs, cattle) in Styria only a few kilometers distant from the Austrian border to Slovenia. A recent Slovenian study reported a relatively high prevalence of human CE in eastern Slovenia, but unfortunately it comprised no molecular discrimination of *Echinococcus* species/strains.¹⁵ In a previous study, we have identified cyst fluid from a pig from Styria as *E. granulosus* G1¹⁴ and accordingly we presume that this case represents an autochthonous human infection with *E. granulosus* G1.

Although the follow-up of our patients was not the topic of this study, we decided to mention a 24-year-old man of Romanian origin suffering from *E. granulosus* G1 heart and brain cysts, who died after years of treatment as a consequence of inoperable CE cysts of the brain. This case shows very well that CE not only causes severe disease in humans and livestock-associated production losses in relatively poor countries,^{1,16,17} but also possible death, even in countries with an excellent health system like Austria.¹⁸

In general, a younger age at the time of diagnosis is correlated with an increased exposure to *E. granulosus*,^{19,20} which seems to be an explanation for the younger median age of the Turkish (30 years) and the former YU originated patients (39 years). In contrast, the significantly higher median age of Austrian patients (55 years) may reflect the good hygiene conditions, veterinary control systems, and essential improvements in farming conditions over the last decades in Austria. In the former YU the war and the collapse of the state Yugoslavia (1991–1999) might have worsened veterinary controls and hygiene conditions and influenced the epidemiological situation similar to reports from Bulgaria¹⁹ and newly independent central Asian states after the collapse of the Soviet Union.^{20,21} These changes might explain the relatively young median age of the pig strain-infected patients in the former YU but not the divergent age distribution between *E. canadensis* G7 (median age 23 years) and *E. granulosus* (median age 42 years) in the same geographical region.

Although the relatively high mean age (45.8 years) of the entire pig strain-infected patients from different geographical regions seems to be reasonable considering the described cyst localization and diameter and the resultant development of less severe clinical signs, the young age of the *E. canadensis* G7-infected patients from the former YU ($N = 9$) was unexpected and needs further investigation and interpretation. It needs further molecular biological studies comprising *Echinococcus* species/strain differentiation^{6,8,14,22} including more samples of human and pig origin with accurate geographical anamnesis to clarify the epidemiological situation in the former YU.

Because of the long incubation period in the human host, copro-diagnosis in the dog definitive host with differentiation between G1/2 and G5/6/7, as described by Naidich and others,²³ and molecular biological investigation of the intermediate

host including pigs might open new opportunities for the study of transmission routes, especially in regions where different "strains" respectively species of *Echinococcus* cause human CE.

Several groups of researchers assume that the extensive variation in the former species *E. granulosus* may influence life-cycle patterns, host specificity, antigenicity, transmission dynamics, sensitivity to chemotherapeutic agents, and pathology and might therefore have implications for CE control programs and the development of vaccines.^{24–26} At the time the recombinant EG95 vaccine was designed,²⁷ the sheep strain of *E. granulosus* was presumed to be the primary cause of CE in humans and livestock. A recent publication dealt with the problem of the unproved effectiveness of the current EG95 vaccine against *E. canadensis* G6/7.²⁶ Hence, the molecular discrimination of the species of *Echinococcus* and probably also of the strain are major prerequisites for effective control programs.^{25,26} Accordingly, the results of our study show an unexpected high prevalence of *E. canadensis* G7 in Central Europe. The widespread practice of home slaughtering of pigs and the presence of free-roaming domestic dogs in parts of the former YU (e.g., Serbia, Macedonia) until now, and mainly in Austria in the past, are the major prerequisites for the perpetuation of the lifecycle of *E. canadensis* G7. We presume that the dog–pig cycle exists in several other Central European regions where home slaughtering of pigs is customary and hygiene conditions are improvable.

To date, our study seems to be the first one that deals with the different clinical presentation of human CE caused by the only recently described novel species *E. canadensis*, in comparison to the well-described human infection with *E. granulosus*. In addition to the epidemiological consequences, it needs further studies to learn if perhaps some of the elder patients with a Central European geographic anamnesis and small liver cysts could be spared the risky operation. In conclusion, the different appearance of human CE caused by *E. canadensis* and *E. granulosus*, respectively, supports the most recent taxonomic revisions in the genus *Echinococcus*.

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