

# Cytology and Genetic Analysis of Liver Echinococcosis in a Woman Came from Bolivia

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## Abstract

Echinococcosis is a global and zoonotic helminthic disease caused by *Echinococcus* sp. A 24-year-old woman, coming from Bolivia two years before, was diagnosed as cystic echinococcosis (CE) by unstained wet mount and cytological findings through aspiration cytology from the liver, suggested as liver abscess by diagnostic images. The patient was classified as CE1, smaller than 5 cm, and was diagnosed as P1N0M0, stage I. The phylogenetic trees of *Echinococcus* spp., based on *cox1* genes showed that the isolates on the patient belonged to *E. ortleppi*. The patient was conservatively treated with two courses of albendazole therapy. *E. ortleppi* has been reported to be in regions including Latin America, except with Japan. Although the patient came from Bolivia before two years, this is the first case report of *E. ortleppi* from liver echinococcosis in Japan.

## Keywords

Echinococcosis, Liver, Aspiration Cytology, Genetic Analysis, *E. ortleppi*

## 1. Introduction

Echinococcosis is a global and zoonotic helminthic disease caused by *Echinococcus* sp. tapeworm larvae [1]. Intermediate hosts, such as wild herbivores, livestock and small mammals, as well as accidentally infected humans receive their infections through ingestion of contaminated food and water with *Echinococcus* eggs from the feces of the definitive hosts, such as dogs and foxes, etc. [2]. Nine

species of *Echinococcus* have been reported globally, with confirmed zoonoses caused by *E. granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarthra*, *E. canadensis* and *E. ortleppi*; while no zoonotic evidence for *E. equinus*, *E. felidis* and *E. shiquicus* [3] [4].

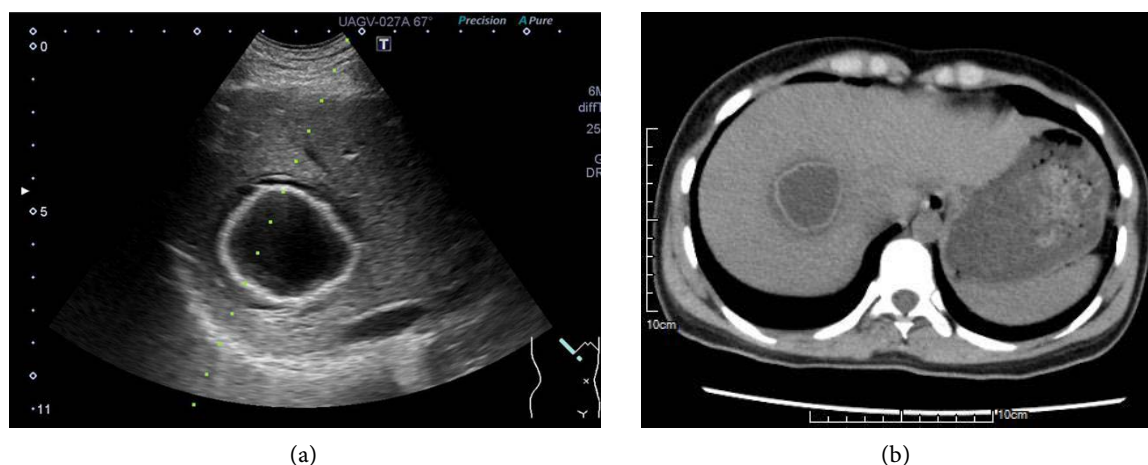
Among those various *Echinococcus* spp., the most detected species in human echinococcosis (CE) is *E. granulosus*, which parasitizes in dogs and livestock globally [5]. Based on the previous molecular taxonomic findings, *E. granulosus* has been regarded as genotype complex, i.e. *E. granulosus* sensu lato (s.l.) comprising genotypes 1 to 10 (G1 - G10). The *E. granulosus* s.l. further split into five species as like *E. granulosus* sensu stricto (s.s.) (G1/G2/G3 genotypes), *E. equinus* (G4 genotype), *E. ortleppi* (G5 genotype), *E. canadensis* group (G6/G7/G8/G10 genotypes), and *E. felidis* (lion strain) [6] [7] [8] [9] [10].

Most cases of liver echinococcosis in Japan have been reported *Echinococcus multilocularis*, and most of which were detected in Hokkaido, north of Japan [11]. Meanwhile, *E. ortleppi* infection was first reported in a herd of cattle in Holland; thus, it was named a cattle strain [6] [7]. Hosts of *E. ortleppi* have been reported from cattle, camels, pigs, goats, sheep and wild animals, and humans in many regions, Europe, Africa, the Middle East, Asia and Latin America [12] [13]. However, *E. ortleppi* infection has not been reported in Japan. Although the patient came from Bolivia, Latin America before two years, this is the first case report including the cytology and genetic analysis of liver echinococcosis in Japan.

## 2. Case Report

A 24-year-old woman, coming from Bolivia two years before, visited to our hospital complaining of high fever (37.9°C) and general fatigue. Her laboratory data showed high CRP [14.51 ( $\leq 0.3$ ) mg/dL] and a relatively high WBC [ $9.68 \times 10^3$  ( $4.0 - 9.0 \times 10^3$ )/mL] and a relatively high ratio of eosinophilic 8.2 (0.2 - 6.8)% (Table 1). An abdominal ultrasonography (USG) (Figure 1(a)) and a plain CT showed a cystic lesion, 42 mm in diameter, in right lobe of her liver, suggesting a liver abscess. Calcification at the margins and low concentration bands were also seen (Figure 1(b)). Whole body CT and USG examinations, showed no evidence of extra-hepatic invasion or metastasis. After then, fine-needle aspiration cytology (FNAC) was performed with the help of a 22-gauge needle under USG. FNAC material was smeared on glass slides followed by wet mount, fixation in 95% ethanol for Papanicolaou stain and air-dried for May-Giemsa stain. Many protoscolexes were found (Figure 2(a)) and hooks and suckers on the tip of the scolex were also recognized in unstained wet mount smears (Figure 2(b)). Basophilic stained protoscolexes were commonly seen in May-Giemsa stain (Figure 3(a)). In Papanicolaou stain, orange and purple stained protoscolexes were also observed (Figure 3(b)). From the above findings, the patient was diagnosed as CE in her liver [14].

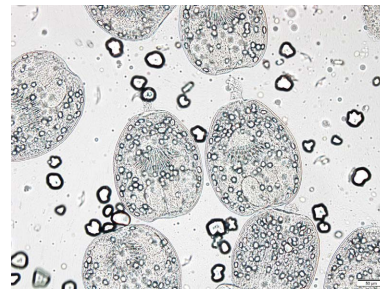
According to the WHO informed working group on echinococcosis international classification [15], the patient was classified as CE1, smaller than 5 cm.



**Figure 1.** (a) An abdominal ultrasonography showed a monocystic lesion, measuring 42 mm in diameter with coating formation of high-intensity echoes were recognized in right lobe of her liver. (b) A 42 × 37 mm lesion at the S4/S8 boundary area in her right liver lobe was observed. Calcification at the margins and low concentration zones around it were recognized.

**Table 1.** Laboratory data of the patient on admission.

	patient value	reference range		patient value	reference range	
TP	6.5	6.7 - 8.3	g/dl	WBC	$9.68 \times 10^3$	↑ 4.0 - $9.0 \times 10^3$ /mL
TB	0.3	0.2 - 1.2	mg/dl	Neu	60.8	40 - 71.9 %
AST	10	13 - 33	IU/L	Lymph	24.6	26 - 46.6 %
ALT	6	8 - 42	IU/L	Eosino	8.2	↑ 0.2 - 6.8 %
LDH	145	119 - 229	IU/L	Baso	0.4	0.0 - 1.0 %
ALP	88	38 - 113	IU/L	Mono	6	2.3 - 7.7 %
gGT	23	9 - 32	IU/L	RBC	$416 \times 10^4$	$376 - 500 \times 10^4$ /mL
CK	35	45 - 163	IU/L	Hb	11.6	11.3 - 15.2 g/dl
AMY	53	40 - 126	IU/L	Ht	35.2	33.4 - 44.9 %
Na	142	135 - 147	mEq/L	MCV	84.6	79 - 100 fL
K	4.3	3.4 - 4.8	mEq/L	MCH	27.9	26.3 - 34.3 pg
Cl	106	98 - 110	mEq/L	MCHC	33	30.7 - 36.6 %
Cr	0.65	0.40 - 0.80	mg/dl	PLT	$36.7 \times 10^3$	$15 - 35 \times 10^3$ /mL
Glu	80	70 - 109	mg/dl			
CRP	14.51	↑ ↑ -0.3	mg/dl			
HBsAg	(-)					
HCVAb	(-)					
HIV	(-)					
TP antibody	(-)					
RPR	(-)					
SARC-COV2Ag	(-)					

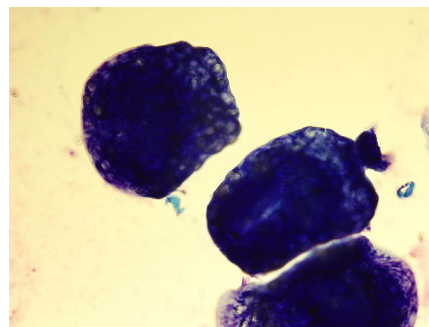


(a)

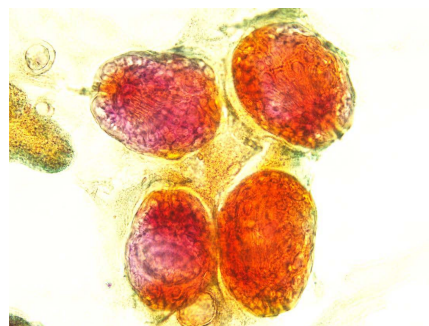


(b)

**Figure 2.** (a) Many protoscoleces were found in unstained wet mount smears ( $\times 200$ ). (b) The scolex was found with growing head. Hooks and rounded suckers on the tip of the scolex were recognized in unstained wet mount smears. Many calcareous corpuscles were found in the scolex ( $\times 400$ ).



(a)



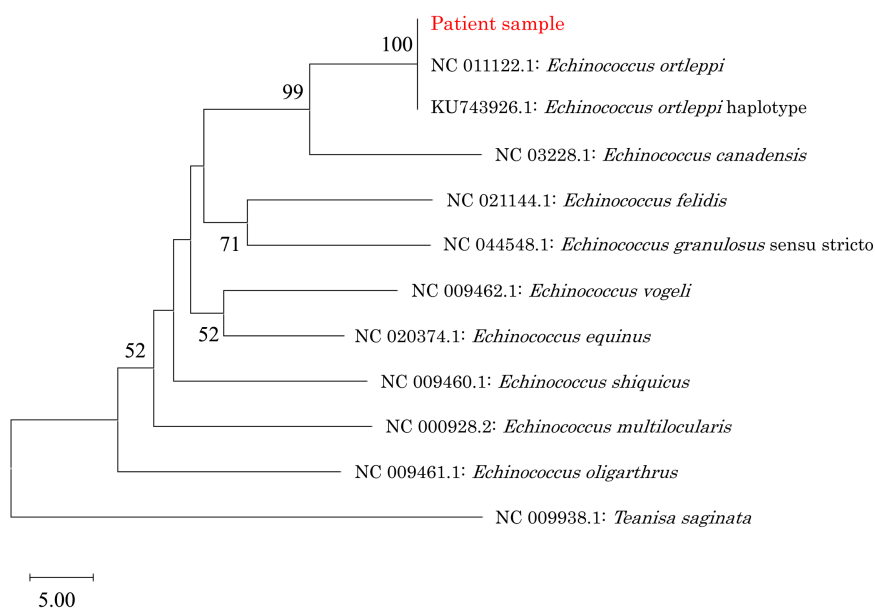
(b)

**Figure 3.** (a) Basophilic stained protoscoleces were seen in May-Giemsa stain ( $\times 400$ ). Many calcareous corpuscles were observed. (b) Orange and purple stained protoscoleces were also observed in Papanicolaou stain ( $\times 400$ ). Many calcareous corpuscles were recognized.

Also, modified according to the WHO informed working group on the PNM classification and staging of alveolar echinococcosis [16], the patient was diagnosed as P1N0M0, stage I.

We determined the parasite identification using molecular methods as follows. For DNA extraction, we extracted DNA by using QIAamp DNA Mini (Qiagen) from parasite protoscolices collected from hydatid sand of the patient. We amplified approximate 450 bp of mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene by using JB3: 5'-TTTTTTTGGGCATCCTGAGGTTTAT-3' for forward primer, and JB4.5: 5'-TAAAGAAAGAACATAATGAAAATG-3', for reverse primer [6]. PCR was run with T100 thermal cycler (Bio-Rad Laboratories) and then we conducted *cox1* gene sequencing. SimpliFi HS Mix (Meridian Bioscience) was used for amplification. PCR protocol was as follows: initial denaturation at 95°C for 30 s, 40 cycles of denaturation at 95°C for 15 s, Annealing at 51°C for 15 s, extension at 72°C for 30 s, and final extension at 72°C for 5 min. The DNA sequence of PCR product (396 bp) was read and then used for homology search at NCBI web site (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and phylogenetic analysis with MEGA 11 software. Basic local alignment search tool (BLAST) results were shown in Table 2. The patient sample was matched over 99% to *E. ortleppi*. The phylogenetic trees of *Echinococcus* spp. based on *cox1* gene showed that the isolates on the patient's sample belonged to *E. ortleppi* (Figure 4).

The patient was diagnosed as stage I, P1N0M0 with CE1, smaller than 5 cm, and thus conservatively treated with albendazole (ABZ, 600 mg/day) for 28 days and the withdrawal period for 14 days, according to the WHO informed working group on echinococcosis international classification [17]. The ABZ therapy



**Figure 4.** The phylogenetic trees of *Echinococcus* spp. based on *cox1* genes showed that the isolates on this patient's sample belonged to *E. ortleppi*.

**Table 2.** BLAST result.

Accession No.	Species or genotype	Matches	Identity
MH428013.1	<i>Echinococcus granulosus</i> G5 ( <i>Echinococcus ortleppi</i> )	396/396	100%
MN058591.1	<i>Echinococcus ortleppi</i>	395/396	99.7%
MH428014.1	<i>Echinococcus granulosus</i> G5 ( <i>Echinococcus ortleppi</i> )	393/396	99.2%
LC184605.1	<i>Echinococcus canadensis</i> genotype: G8"	379/396	95.7%
MN787562.1	<i>Echinococcus equinus</i> isolate D18o	365/396	92.1%
EF558356.1	<i>Echinococcus felidis</i>	360/396	91.0%

was repeated for two courses, and then the laboratory data showed no abnormalities and the cystic lesion in her liver almost disappeared. Five months after the start of ABZ therapy, the patient returned to Bolivia.

### 3. Discussion

In diagnostic imaging, calcification findings at the margins and low concentration zones around it are recognized by her plain CT, suggesting the parasitic or tuberculous diseases [18] [19]. Meanwhile, the unstained wet mount smears and cytological findings showed hydatid protoscolex [14]. The unstained wet mount smears were useful for diagnosis of echinococcus in the liver, although they could not be recorded permanently [14]. In diagnostic imaging, hydatid disease is similar to malignant [18] [19]. FNAC helps in eliminating the dilemma and gives conclusive diagnosis [20] [21]. Her relatively high ratio of eosinophilia was also consistent with parasitic disease infection.

CE is caused by *Echinococcus granulosus* s.l., which including 5 species as mentioned above. The highest homology to the DNA sequence of the PCR product of this case was observed with *E. ortleppi* (99.2% - 100%), while others as follows; *E. granulosus sensu stricto* (96.4%), *E. canadensis* (95.7%), *E. equinus* (92.1%), and *E. equinus* (92.1%). In South America, main causative agent of human CE is *E. granulosus sensu stricto*, and only 1.9% of human cases were caused by *E. ortleppi* [22]. There have been no reports of echinococcosis caused by *E. ortleppi* in Japan. In Bolivia, although *E. ortleppi* in cattle was reported [23], the human case of this species has not reported also. *E. ortleppi* can parasitize various host mammals such as cattle, camels, pigs, goats, sheep and human [17]. This is the first case of echinococcosis caused by *E. ortleppi* in Japan. Considering the incubation period of echinococcosis *i.e.*, month to years [12] [13], this case might be imported from Bolivia.

Most cases of liver echinococcosis in Japan have been reported *Echinococcus multilocularis* [11], while the current cases were caused by *Echinococcus granulosus*, especially *E. ortleppi*. For the diagnosis for this case was useful for the unstained wet mount smears and cytological findings showing hydatid protoscolex

[14]. As this case was diagnosed relatively early stage, stage I, P1N0M0 with CE1, smaller than 5 cm, the only ABZ therapy was useful and prognosis was good.

#### 4. Conclusion

Echinococcosis is a global and zoonotic tapeworm disease caused by *Echinococcus* spp. A 24-year-old woman, who came from Bolivia, was diagnosed as CE by unstained wet mount and cytological findings through FNAC from the liver abscess. The patient was classified as CE1, smaller than 5 cm, and diagnosed as P1N0M0, stage I, and treated with albendazole therapies. The phylogenetic trees of *Echinococcus* spp., based on *cox1* genes showed that the isolates on the patient belonged to *E. ortleppi*. Although the patient came from Bolivia before two years, this is the first case report of *E. ortleppi* in liver echinococcosis in Japan.

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#### Consent

Verbal consent was obtained from the patient before writing this case report.

#### Ethical Approval

This was obtained from the ethical committee of Gujo City Hospital before writing this case report (21102901).

#### Conflicts of Interest

The authors declare that they have no competing interests.

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