Human Hydatid Disease in Peru Is Basically Restricted to *Echinococcus granulosus* Genotype G1

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Abstract.   A molecular PCR study using DNA from 21 hydatid cysts was performed to determine which strain type is responsible for human infection in Peru. The mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene was amplified in 20 out of 21 samples, revealing that all but 1 sample (19/20, 95%) belonged to the common sheep strain (G1). The remaining samples belonged to the camel strain (G6). The G1 genotype was most frequently found in human cases of cystic hydatid disease (CHD) in Peru. Local control measures should focus primarily on decreasing dog and sheep infection rather than intermediate reservoirs.

INTRODUCTION

All the 5 recognized species within the genus *Echinococcus* require 2 hosts to perpetuate their life cycle: a carnivore as the definitive host, which carries the adult egg-producing tapeworm, and a herbivore as the intermediate host in which larval metacestode stages establish and develop, causing hydatid disease. *Echinococcus granulosus* causes cystic hydatid disease (CHD), *Echinococcus multilocularis* causes alveolar hydatid disease, *Echinococcus oligarthus* and *Echinococcus vogeli* both cause polycystic hydatid disease, and *Echinococcus shiquicus* causes unilocular minicyst hydatid disease.[1–3] Humans can act as intermediary hosts of the first 4 species, with diverse clinical presentations depending on the affected organ and type of larvae.

Cystic hydatid disease is an important and widespread zoonosis, especially in sheep-raising areas of Europe (Mediterranean countries), Asia (Russia, China), North and East Africa, Australia, and South America (Peru, Bolivia, Argentina, Chile, Uruguay, and Rio Grande do Sul state in Brazil). It affects the liver (52–77% of cases), lung (9–44%), and other organs such as brain, heart, and bones.[4–6] CHD is a major public health problem in Peru, with a prevalence of 6–9% in many areas of the country and numerous human cases reported every year.[6,7]

Around the world, strain-typing surveys have shown that human infection is mostly often by the common sheep strain (G1) in mainland Australia, Tasmania, Jordan, Lebanon, Holland, Kenya, China, and Spain.[8–11] G1 may coexist with other strains, such as cattle strain (G5) in Holland; camel strain (G6) in Nepal, Iran, and Mauritania; porcine strain (G7) in Poland and Slovakia; and cervid strain (G8) in the United States. When multiple strains are present, they may infect atypical intermediate hosts; e.g., G5 infection in sheep and goats in Nepal and G7 beaver infection in Poland.[10,12] In Argentina, human infections are caused by strains G1, G2, G5, and G6.[13–16] There is little information available on strain

composition of hydatid disease in other Latin American countries.[17,18] We carried out a survey using a PCR analysis and CO1 sequencing of *E. granulosus* isolates collected from humans to determine the *E. granulosus* strains that infect humans in Peru.

MATERIALS AND METHODS

This study was performed in Lima, Peru, at the Hospital Nacional Dos de Mayo (a government referral center for treatment of hydatid disease), using cyst material excised from patients who had surgery for CHD during the period March 2006–January 2007. Immediately after excision, the specimen was placed in ethanol (70%), stored at 4°C, and processed within 2 days of collection.

Macroscopic information on the appearance, size, and status of the larvae was collected from surgical reports. The nature and fertility of the sample were confirmed by microscopic observation of *E. granulosus* protoscoleces. Each cyst was separated into membrane and intracystic fluid with protoscoleces (hydatid sand). The germinal layer was washed 3 times in ethanol to remove any contaminant (debris, blood, host tissue), and both membrane and hydatid sand were preserved submerged in 70% ethanol and stored at −20°C. Samples were sent to Departamento de Parasitología, Instituto Nacional de Enfermedades Infecciosas, ANLIS, in Buenos Aires, Argentina, for strain identification. There, total *E. granulosus* DNA was extracted using the DNeasy Tissue kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Purified DNA samples were stored at −20°C until their use in PCR reactions. *E. granulosus* genotype was determined by mitochondrial cytochrome *c* oxidase subunit 1 (CO1) sequencing, as previously described.[15] The sequences were determined at the Facultad de Ciencias Exactas y Naturales, UBA, in Buenos Aires (USFCEyN).

Additional PCR reactions performed were amplification of the DCO1 mitochondrial fragment using the set of primers DCO1F and DCO1R as previously described by Cabrera and others[19]; amplification of the *E. granulosus* actin gene as described by da Silva and others[20]; and amplification of an *E. granulosus* repetitive DNA element as described by Abbasi and others.[21]

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TABLE 1

Localization and characteristics of the hydatid cysts related with *Echinococcus granulosus* strain

HP	Organ affected	Geographic location	Type	Daughter cyst	Volume (mL)	Strain
1	Lung* (LLL)	Pasco	Hyaline	No	810	G1
2	lung (LLL)	Junin	Hyaline	No	441	G1
3	Lung (LLL)	Ayacucho	Broken	Yes	2250	G1
4	Liver (RHL)	Pasco	Hyaline	No	100	G1
5	Lung (RUL)	Junin	Hyaline	No	384	G1
6	Lung (LLL)	Huancavelica	Broken	No	90	G6
7	Liver (RHL)	Junin	Infected	No	216	G1
8	Lung (RUL)	Lima	Broken	No	96	G1
9	Lung* (LLL)	Junin	Hyaline	No	595	G1
10	Lung (RUL)	Ayacucho	Hyaline	No	576	G1
11	Lung* (LUL)	Pasco	Infected	Yes	420	—
12	Lung (RLL)	Pasco	Hyaline	No	2085	G1
13	Lung (LLL)	Lima	Hyaline	No	125	G1
14	Lung (RUL)	Pasco	Hyaline	Yes	448	G1
15	Lung (LLL)	Huancavelica	Hyaline	No	1500	G1
16	Lung (RUL)	Junin	Broken	No	770	G1
17	Lung (RLL)	Junin	Broken	Yes	80	G1
18	Lung (RLL)	Junin	Hyaline	No	576	G1
19	Lung (ML)	Lima	Hyaline	No	8	G1
20	Lung (LUL)	Junin	Hyaline	No	175	G1
21	Lung* (LLL)	Ayacucho	Hyaline	No	576	G1

LLL = left lower lobe; RHL = right hepatic lobe; RUL = right upper lobe; LUL = left upper lobe; RLL = right lower lobe; "—" = strain could not be determined.
* Patients without abdominal ultrasound or CT scan.

RESULTS

We analyzed a total of 21 cysts from 21 individuals. The majority of individuals ($N = 18$) came from villages in the Central Peruvian Highlands, with altitudes varying between 3000 and 4500 m above sea level. Villages in the area have similar ecology, agriculture, and livestock. Of the 21 cysts, 19 were lung cysts and 2 were liver cysts. Seven cysts showed evidences of complication (2 infected and 5 ruptured), and 4 cysts had daughter cysts. The mean volume was 586.68 ± 627.46 mL (range 8–2250 mL) (Table 1). Preserved protoscoleces were seen under the microscope in 8 cysts. In the other 13, parasite cells, degenerated protoscoleces, and/or parasite structures—e.g., hooks—were observed. The CO1 gene was amplified in 20 out of 21 samples (Figure 1).

A second reaction of PCR-CO1 with addition of an internal *E. granulosus* DNA control was carried out in the nonamplifying sample. Because a control band of the expected size was obtained, we ruled out the presence of inhibitors in the sample. Also, a second reaction to amplify a more internal
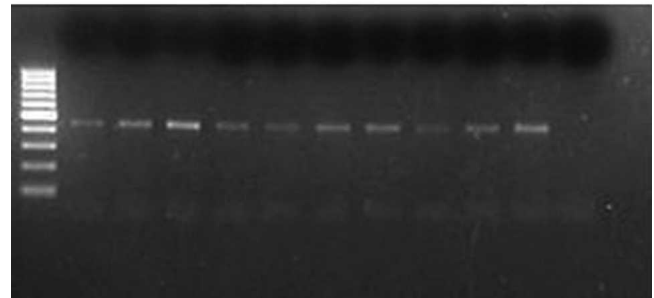

FIGURE 1. PCR amplification of mitochondrial cytochrome c oxidase subunit 1 (CO1): Lane 1, size marker; lane 2, HP1; lane 3, HP2; lane 4, HP3; lane 5, HP4; lane 6, HP5; lane 7, HP6; lane 8, HP7; lane 9, HP8; lane 10, HP9; lane 11, positive control; lane 12, negative control.

region of the cytochrome *c* oxidase subunit 1 gene was performed by using DCO1 primers to determine if the absence of amplification was produced by substitutions in the CO1 annealing primers site. Again, no amplification products were obtained. To confirm the identity and quality of the extracted DNA from this sample, 2 reactions using different primers were performed (1 for the constitutive gene actin and 1 for an *E. granulosus*-specific repetitive DNA element). In both cases, we obtained the expected amplification product (Figure 2). Details on these reactions are provided in the supplemental online material at www.ajtmh.org.

Sequencing of the mitochondrial CO1 gene confirmed that all the 20 cysts whose material was amplified were *E. granulosus* metacestodes. All but 1 sample (19; 95%) belonged to the common sheep strain (G1). The remaining sample belonged to the camel strain (G6) (Table 1).

DISCUSSION

Using sequencing of the mitochondrial CO1 gene, we demonstrated a clear predominance of the common sheep/dog strain (G1), with a single isolate of camel/dog strain (G6) of *E. granulosus* in Peruvian CHD human cases. We could not identify the reason why 1 sample did not amplify despite being confirmed as *E. granulosus* DNA by other molecular markers. Because inhibition was shown to be unlikely, a possible explanation would be the presence of a mutation in the CO1 gene.

TABLE 2

Characteristics of different *Echinococcus granulosus* genotypes

Genotype (strain)*	Definitive host	Intermediary host	Human infectivity	Prepatent period
G1 (common sheep strain)	Dog, fox, dingo, wolf jackal, hyena	Sheep, cattle, goat, buffalo, camel, pig, kangaroo.	Yes	45 days
G2 (Tasmanian sheep strain)	Dog	Sheep, cattle	Yes	39 days
G3 (buffalo strain)	Dog, fox?	Buffalo, cattle?	?	?
G4 (horse strain)	Dog	Horse, donkeys	No	More than G1
G5 (cattle strain)	Dog	Cattle, sheep, goat, buffalo	Yes	33–35 days
G6 (camel strain)	Dog	Camel, goat, cattle, sheep	Yes	40 days
G7 (pig strain)	Dog (fox?)	Pig, wild boar, beaver	Yes	34 days
G8 (cervid strain)	Wolf, dog	Moose	Yes	?
G9	?	Pig?	Yes	?
G10 (Finland cervid strain)	?	Moose	?	?

* Genotype (strain), determined by molecular techniques; "?", indetermined or low number of analyzed sample (see Refs. 1, 10, 16, 24, 26, and 34–39).
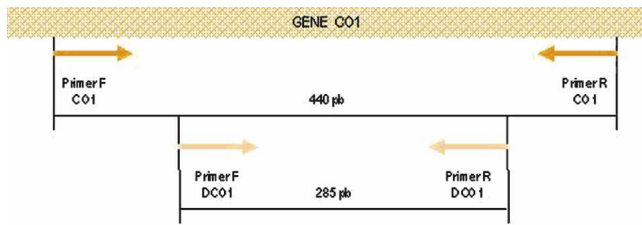
FIGURE 2. Scheme of CO1 and DCO1 attach primers site. This figure appears in color at www.ajtmh.org.

To date, 10 distinct well-characterized genetic intraspecific variants are recognized within *E. granulosus* (genotypes G1–10), based on polymerase chain reaction (PCR) amplification by sequencing mitochondrial markers in *cytochrome c oxidase 1* (CO1) and *nicotinamide adenine dinucleotide dehydrogenase 1* (ND1) genes. Seven of them are infectious to humans[22–25] (Table 2). There appears to be very limited genetic variation within *E. multilocularis*, and there are no available data to assess sequencing variability in *E. vogeli, E. oliganthus*, or *E. shiquicus*. Intraspecific variants or "strains" may play an important role with regard not only to life-cycle patterns and host assemblages but also to transmission dynamics, control of disease, pathogenicity, fertility of developed cysts, and rate of growth.[1,13,16,23,26–31]

Although the number of Peruvian isolates examined was not extensive, the G1 genotype was far more prevalent in humans than the G6 genotype. The common sheep strain, G1, is widely reported as cause of human infection in Southern and Eastern Europe, Northern and Eastern Africa, parts of Asia, Australia, and South America (Argentina). Although it predominantly affects sheep, in a few cases, G1 infection of other intermediary hosts, such as cattle and goat, has been described.[13,15,16,27] On the other hand, G6, typically a camel strain, has also been reported in cattle.[32,33] In Argentina, this strain may contribute for up to 37% of human CHD cases, second to G1 infection with 46%.[13] Our examined samples came from the Peruvian Central Highlands, which comprise approximately 70% of the endemic areas for CHD in Peru. Although it is possible that samples from the Southern Highlands (Puno, Cusco) near Bolivia and Chile could have different patterns, we consider it unlikely given the high similarities in terms of ecology, altitude, behavior, and livestock raised.

G1 is the commonest strain in CHD human cases worldwide. Its predominance supports that the endemicity of *E. granulosus* in the Peruvian highlands is based on a sheep/dog cycle. This is highly consistent with its geographical pattern, overlapping major sheep raising areas between 3200 and 4500 meters of altitude. This information provides support to concentrate control measures in Peru to decrease dog and sheep infection rates in preference to working on other intermediate reservoirs.

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