Fasciola hepatica Infection in an Indigenous Community of the Peruvian Jungle

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Abstract. *Fasciola hepatica* is a zoonotic infection with a worldwide distribution. Autochthonous cases have not been reported in the Amazon region of Peru. Operculated eggs resembling *F. hepatica* were identified in the stools of five out of 215 subjects in a remote indigenous community of the Peruvian jungle. Polymerase chain reaction targeting *Fasciola hepatica* cytochrome oxidase subunit 1 (*COI*) gene and sequencing of the products confirmed *Fasciola* infection.

Fasciola hepatica is a zoonotic infection with a worldwide distribution.¹ The parasite and its snail hosts are adapted to a range of different environments. Fascioliasis has been reported in tropical environments like Egypt, the Caribbean, Brazil, and Vietnam.^{2–4} It has also adapted to very high altitude environments like in the Andes of Bolivia and Peru.⁵ The snails actually live longer and produce large numbers of cercariae at higher altitudes, which increases transmission.⁶ In fact, it is estimated that half of the subjects infected with *F. hepatica* worldwide live in the Andes of South America.⁷ In Peru, cases have been described in 17 out of 24 regions, all of which are located at high altitude.⁸ Autochthonous cases have not been reported in the Amazon regions of Peru. We report five cases of *F. hepatica* infection in an indigenous population from a remote area of Manu National Park.

A community health intervention for soil-transmitted helminths was carried out in Yomibato community (S-11.76085, W-71.87385) during November 2012.⁹ Subjects in this community belong to the Matsigenka ethnic group and live in a remote area of the rainforest in Manu National Park in the Madre de Dios region of Peru. The health intervention was part of a nongovernmental organization (NGO) evaluation of ongoing water and sanitation programs. Participation in the intervention was voluntary for subjects in Yomibato community and its surroundings. Stool samples were preserved in formalin for helminth egg identification as previously described.⁹ Stools were also preserved in ethanol for DNA extraction and real-time polymerase chain reaction (PCR) testing.

DNA was obtained from stool samples containing operculated eggs using the commercial stool DNA extraction kit (Qiagen, Valencia, CA). Purified DNA was evaluated by SYBR green real-time PCR using primers targeting the *F. hepatica* mitochondrial cytochrome oxidase subunit 1 (*COI*) gene (F-ACGTTGGATCAT AAGCGTGT, R-CCTCATCCAACA TAACCTCT). DNA extracted from adult *F. hepatica* and from stools with *Fasciola* eggs obtained in Cusco were used as positive controls. DNase-free water was used as the negative control. The PCR products of positive samples were reamplified by PCR using *F. hepatica* *COI* specific primers again. An ~492 bp fragment of DNA was purified with the QIAquick Gel Extraction Kit (Qiagen). The DNA concentration was determined by spectrophotometry (NanoDrop 2000; Thermo Scientific,

Wilmington, DE). Approximately, 20 ng of DNA from each sample was sequenced using an ABI Prism™ 3130XL DNA sequencer (Applied Biosystems, Foster City, CA). *COI* DNA sequences from other trematodes were obtained from the GenBank *Fasciola gigantica* (AB983857.1), *Fascioloides magna* (GU599863.1), *Echinostoma* sp. (FJ477201.1), *Paragonimus* sp. (KC562293.1), and *Schistosoma* sp. (BE505118.1). *Fasciola hepatica* *COI* DNA sequences from different countries were used for comparison (Iran: GQ398054.1), (Poland: KR422380.1), (Peru: KJ716924.1), (Egypt: AB553813.1), (Egypt: AB553812.1). Multiple alignment analysis was conducted using the ClustalW software (European Bioinformatics Institute, Cambridgeshire, United Kingdom), and the phylogenetic neighbor-joining tree was generated with the Phylogeny.fr viewer (Information Génomique et Structurale, Marseille, France). The sequence obtained was submitted to the GenBank.

In March 2014, the same NGO performed a second evaluation among Yomibato villagers collecting stool and blood samples. Dry blood blots were collected in Whatman paper from finger pricks performed for point of care hemoglobin level testing. The dry blots were used for *F. hepatica* antibody testing by Fas2-enzyme-linked immunosorbent assay (ELISA) (Bionoma, Lima, Peru) in suspected cases.

The Universidad Peruana Cayetano Heredia Institutional Review Board approved the use of deidentified subjects' information for secondary data analysis. Subjects found to be infected with gastrointestinal helminths were treated as needed. Subjects with operculated eggs in the stools were treated with a single 10 mg/kg dose of triclabendazole.

Stool samples from 215 subjects (54% female and 83% younger than 35 years)⁹ from 52 extended families were obtained in the first intervention. The stool samples of five subjects (2.3%) from four extended families had operculated eggs of 70 µm × 30 µm morphologically resembling *F. hepatica* in the rapid sedimentation and Kato-Katz tests (Figure 1). The mean number of eggs/gram of stools was 40 (±28.3). Table 1 shows the characteristics of the subjects. None had a history of living in or consuming vegetables coming from the highlands. All were treated with a single 10 mg/kg dose of triclabendazole.

The real-time PCR with primers targeting the *F. hepatica* mitochondrial *COI* gene showed amplicons with melting curves similar to those from the positive controls in four of the subjects. Sequencing of the PCR amplicons was successful in the samples provided by subjects 1, 2, and 4. The analysis of nucleotide sequences (KT869169) showed 99% identity between the subject's sequences and the *F. hepatica* *COI* gene sequences selected from GenBank and less so with that of *F. gigantica* (AB983857.1) (Figure 2).

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FIGURE 1. Trematode eggs in the stools of Matsigenka subjects in Yomibato. (A) Rapid sedimentation test with an operculated egg measuring about 70 μm . (B) Rapid sedimentation test stained with Lugol's iodine solution showing a similar trematode egg. (C) and (D) Kato-Katz tests showing operculated eggs. 400 $\times$, bar line 50 μm .

In the second intervention, stool samples from 194 subjects were obtained and none of the specimens studied had operculated eggs including four from previously infected subjects. The fifth subject did not participate in the second evaluation. In two of the four subjects (subjects 2 and 3) dried blood samples were available for antibody testing and were positive for *F. hepatica* by Fas2-ELISA.

This is the first report of *F. hepatica* infection in the Peruvian jungle. It is unlikely that these infections were acquired outside the reserved area of the Manu National Park. The Yomibato community is extremely isolated from populations outside the park. The site is only reached by traveling for days by river with no other permanent residents in the park other than indigenous populations. There is no commercial trade with the highlands as per park regulations.¹⁰ Hunting and fishing are the main source of protein. No cattle or sheep farming is permitted inside the reserved area. Adult males occasionally leave the park to work in areas adjacent to the reserve that may have commercial trade with the highlands. The demog-

graphics of the subjects infected in this report are not compatible with temporary workers.

Determining the time of introduction of *Fasciola* in the park is difficult from our data. Since the creation of the Manu National Park in the early 1970s, the Matsigenka in Yomibato community have avoided contact with outside populations to prevent diseases and abuse. Thus, introduction could have occurred in relatively recent years through temporary migration of adult males to endemic areas. Some have left the park to obtain money needed to access goods like metal tools and clothes.¹¹ Sociocultural changes in indigenous communities have previously been documented to result in emergent infectious diseases.¹²

Fasciola hepatica has a remarkable capacity to adjust to new hosts and environments.¹³ Previous reports have shown adaptation of the parasite to very high altitudes, different snail species, and wild animal reservoirs.^{6,14,15} *Fasciola* has not been previously described in animals in the Madre de Dios area. However, potential *Fasciola* natural hosts exist in Manu

TABLE 1
Characteristics of the subjects with trematode eggs in the stools in November 2012

Characteristics					November 2012			March 2014		
Subject	Family	Sex	Age (years)	Nutritional status	Hemoglobin (g/dL)	Anemia	Other parasites	Hemoglobin (g/dL)	Anemia	Other parasites
1	A	Male	11	Normal	11.1	Yes	None	10.8	Yes	<i>Ascaris lumbricoides</i> <i>Trichuris trichura</i> Hookworm
2	A	Female	8	Normal	11.5	No	<i>T. trichura</i>	11.9	No	<i>A. lumbricoides</i> <i>T. trichura</i> Hookworm
3	B	Female	23	—	12.9	No	<i>T. trichura</i>	12.3	No	<i>A. lumbricoides</i> Hookworm
4	C	Female	20	—	13.2	No	<i>Giardia intestinalis</i> <i>Blastocystis hominis</i>	NA	NA	NA
5	D	Female	13	Normal	13.5	No	Hookworm <i>B. hominis</i>	No blood	No blood	<i>T. trichura</i> Hookworm

NA = not available.

FIGURE 2. Phylogenetic tree obtained from multiple alignment of the cytochrome oxidase subunit 1 (*COI*) gene sequence comparing different trematodes and the subjects' samples by ClustalW. The tree is drawn to scale with branch lengths measured in the number of substitutions per site.

National Park and are in contact with Yomibato population. These include ungulate species like deer (*Mazama americana*), pecari (*Tayassu pecari* and *Tayassu tajacu*), and tapir (*Tapirus terrestris*).¹⁰ Also, wild rodents like agouti (*Dasyprocta variegata*, *Agouti paca*), acouchy (*Myoprocta pratti*), and capybara (*Hydrochoerus hydrochaeris*) are present in proximity with the community.¹⁰ Wild rodents like guinea pig (*Cavia aperea*) and rats (*Ratus ratus*) have been reported as natural reservoirs for *F. hepatica* in Europe and South America.^{14,16,17}

The presence of a suitable snail host is crucial to maintain *F. hepatica* transmission.^{18,19} *Fasciola hepatica* infects snails of the *Lymnaea* genus, and *Galba truncatula* is the most common and widely distributed secondary host. *Galba truncatula* has not been reported in the jungle of Peru and limited data exist on the distribution of *Lymnaea* sp. snails in the Peruvian Amazon.²⁰ However, snail species considered competent *Fasciola* hosts have been described in lowland or jungle areas. Snails from the *Galba/Fossaria* group, a potential *Lymnaea* host, have been described in areas east of the Andes in Peru.²¹ *Lymnaea columella*, a competent *Fasciola* host, has been described throughout Brazil including areas in the rain forest neighboring Peru.²² The non-*Lymnaea* snail *Biomphalaria* sp. was found to be a competent host for *F. gigantica* in Egypt.¹⁵ *Biomphalaria* sp. have been reported in Madre de Dios river that runs across the Manu National Park.¹⁹ Further epidemiological studies are required to confirm the presence of *F. hepatica* autochthonous transmission and its secondary hosts in the Manu National Park.

Our study was limited by the retrospective collection of data and lack of active follow-up of subjects. The remoteness of the area limited our ability to perform a formal epidemiologic evaluation of the cases and the community. Stool specimen's amount, transportation, and preservation in these adverse environmental conditions may have played a role in our inability to obtain sequencing information on two of the samples tested. Also, the condition of the stool samples could have affected the sensitivity of the sedimentation and Kato-Katz tests leading us to underestimate the *Fasciola* prevalence in the community.

In conclusion, we report five cases of *F. hepatica* infection in an indigenous population living in a remote area of the jungle of Peru. The morphological characterization and the molecular data from patients support the finding of *F. hepatica*. Further investigations are needed to evaluate the epidemiology of the infection in the Manu area and establish the primary and secondary hosts maintaining the life cycle of *Fasciola* in the jungle.

Received October 20, 2015. Accepted for publication December 21, 2015.

Published online March 14, 2016.

Acknowledgments: We thank Nancy Santullo for allowing us to access her nongovernmental organization database.

Financial support: This study was supported, in part, by the National Institute of Allergy and Infectious Diseases Grant 1R01AI104820-01.

Disclaimer: The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases.

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