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Asymptomatic *Fasciola hepatica* Infection Presenting with Hypereosinophilia

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Abstract

Background: Fascioliasis is a worldwide zoonosis caused by trematodes *F. hepatica* and *F. gigantica*. The clinical signs, symptoms and laboratory tests are related to acute, latent or chronic phase of infection. Usually it is characterized by fever, abdominal pain, hepatosplenomegaly, high leukocyte count, vague gastrointestinal disturbances, biliary obstruction with intermittent jaundice, and eosinophilia.

Methods and Findings: A 7-year-old girl from a rural Andean area of Ecuador presented with a persistent hypereosinophilia. Leukocyte count was 35,200 mm³, with 60% eosinophilia. Anamnesis and physical examination did not reveal any signs or symptoms of infection. Ova, characteristic of *Fasciola* spp. were observed in the patient's stool by direct microscopic examination and confirmed to be *F. hepática* by the sequence analysis of the PCR amplicons of the second internal transcribed spacer (ITS2) gene. Abdominal ultrasonography showed a liver of normal shape, size and location with no dilation of the intra or extra-hepatic biliary tract. The patient was successfully treated with triclabendazole, 10 mg/kg for two consecutive days.

Conclusions: Clinicians and lab technicians working in endemic and non-endemic areas for fascioliasis should consider the possibility of this parasitic infection in clinically asymptomatic patients with persistent eosinophilia in order to avoid erroneous diagnosis and therapeutic interventions.

Keywords: Fascioliasis; *Fasciola hepática*; Andes; Ecuador

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Introduction

Eosinophils are white blood cells that play a vital role in fighting parasitic infections. Eosinophilia, is a term that defines blood eosinophil counts exceeding 5% ($>0.5 \times 10^9/L$) of the total leukocytes count. A recent consensus on terminology defined hypereosinophilia (HE) as eosinophilia $>1.5 \times 10^9/L$ in the peripheral blood on two occasions >1 month apart with or without tissue hypereosinophilia [1].

HE due to helminths infection has been reported with trichinellosis, strongyloidiasis, hookworm infections, ascariasis, visceral larva migrans, and fascioliasis [2,3]. *F. hepática* infection usually is characterized by an elevated eosinophil count, between 5% to 83%, with a leukocytosis ranging from normal up to 43,000/mm³ [2,4-7]. In the acute and latent-chronic phases of fascioliasis, diagnosis can be made based on eosinophilia [8,9].

Fascioliasis is a zoonosis caused by trematodes *F. hepatica* and *F.*

gigantica. Human infection occurs mainly due to the ingestion of uncooked aquatic vegetables containing encysted metacercariae, including watercress (*Nasturtium officinale*), particularly in communities where infected cattle and/or sheep are present. *F. hepatica* is widespread throughout the world, including the highlands of Bolivia, Ecuador and Peru, with children the most likely to become infected [8]. Human infections by *F. gigantica*

are rare and only had been recorded in Africa and Asia continents, however in some countries like Japan both species overlap, causing identification problems [4]. In Ecuador, the incidence of human infections is quite rare, although it is highly prevalent in cattle and sheep [10]. Foodborne trematodiasis, including fascioliasis, is neglected in international public health programs in comparison to other helminthic diseases [8].

The acute phase of fascioliasis is characterized by fever, abdominal pain, hepatosplenomegaly, gastrointestinal disturbances, skin rashes, high leukocyte count, eosinophilia, anemia, and elevated erythrocyte sedimentation rate (ESR); abnormal liver function tests have also been reported [4]. The latent phase is characterized by nonspecific symptoms, including vague gastrointestinal disturbances, and intermittent eosinophilia. The chronic phase is characterized by regular and constant biliary obstruction; symptoms include biliary colic pain, intermittent jaundice, eosinophilia, and right upper-quadrant abdominal tenderness. However, the percentage of asymptomatic infections is high [8].

Classical coprology (stool examination by microscopy) and serology (using enzyme-linked immunosorbent assay, ELISA) are the most commonly used diagnostic tests [8]. Neither of these methods can differentiate *F. hepatica* from *F. gigantica*. Detection of fasciolid ova in the stools of infected patients is the most definitive evidence for diagnosis. Ultrasonography and computed tomography are useful for complementary image diagnostics [11]. Molecular diagnoses by DNA isolation, real-time PCR analysis and gene sequencing have been developed with higher sensitivity, and the added benefit of species differentiation [12].

The recommended drug for treatment in all cases is triclabendazole (TCZ), 10 mg/kg of body weight, taken as a single dose. A double dose (20 mg/kg of body weight) can be administered in case of treatment failure or if the physician judges it necessary in the management of individual cases [13].

Here, we report a parasitological and molecular confirmed human case of fascioliasis due to *F. hepatica*, presenting only with HE, and treated successfully with two doses of TCZ.

Case Report

A 7-year-old indigenous girl was referred for investigation of persistent eosinophilia which was found incidentally during a routine health control at her primary school. She was born and resides in a community of the Chimborazo province. It is located in the Andean mountains range of central Ecuador, at an altitude of 3200 m, approximately 310 km from the capital of Quito. This is a temperate region with temperatures ranging from 5°C to 12°C. The area, characterized by the Andean high plateau ecosystem, is fragmented into small parcels for cattle ranching, with scattered houses mainly inhabited by Kichwa indigenous people. Cows, sheep and pigs are the animals raised in this region.

The girl was admitted to the Children Hospital in Quito, with a 5-months history of persistent hypereosinophilia. Past medical history revealed her not having any allergies or taking any medications. Eosinophilia of 42% of the total leucocyte count

was detected for the first time (July 2014) during a routine health control for school children. The second and third time, eosinophilia was 60% and 44%, respectively. On admission (December 2014), the patient was non-febrile, with no abdominal pain or diarrhea. According to her mother, she has a history of anorexia. Upon questioning of her food consumption and eating habits, her mother admitted of the family eating watercress (*Nasturtium officinale*) prepared in salads, either grown in their backyard or collected elsewhere in the community. The family raises sheep, cows and chickens. In the community, animals are allowed to roam freely throughout the area.

During the physical exam, the vital signs were all within the normal limits for her age; her axillar temperature was 36.7°C, the respiratory rate was 20 breaths/min, and her heart rate was 88 beats/min. She was at the 3th percentile for weight (17.4 kg) and > 3th percentile for height (110 cm). There was no pain upon abdominal palpation. No hepatosplenomegaly, ascites, or masses were found. There was no evidence of lymphadenopathy or skin rash. The rest of the physical examination was unremarkable. Blood tests showed a white blood cell count of $17.1 \times 10^9/L$, with 8% neutrophils, 28% lymphocytes, and 64% eosinophils. The ESR was 18 mm/h, with normal hemoglobin (13.1 g/dL), a hematocrit of 38.4%, and a platelet count of 571,000/ μL . Serology, using *Fasciola* excretory-secretory antigens (FES-Ag) for detection of anti-*Fasciola* antibodies in serum, was positive with a titration of 1/2560. All results of the basic metabolic panel were within normal limits. Aspartate aminotransferase (AST) level was at 41 IU, serum alanine transferase (ALT) at 44 IU, gama glutamyl transferase at 50 U/L, lactodehydrogenase at 410 U/L, and procalcitonine at 0.09 ng/mL; all within normal limits for her age.



Figure 1 An ovoid, yellow-green, operculated ova observed in the stool of the 7-year-old girl. The ova measured $137 \times 70 \mu m$. (400x magnification).

Urine test results were normal. Stool examination performed for three consecutive days showed numerous operculated ova, yellow-green in color, and measuring approximately 138 x 70 µm, characteristic of *F. hepatica* (Figure 1). All stool specimens were negative for any other intestinal parasites. The ultrasound image revealed a liver with normal shape, size and location. There was heterogeneous echogenicity especially in the left lobe. No dilation of intra or extra-hepatic biliary tract was found. The gallbladder was thin walled and internally showed a motile hyperechogenic image with irregular borders.

Molecular characterization was performed by DNA isolation from eggs, and subsequent amplification of the ITS2 region of the ribosomal RNA gene by PCR as described previously [14]. The primers used for amplification and sequencing were 3S (forward, 5'-GGTACCGGTGGATCACTCGGCTCGTG-3') [15]; and A28 (reverse, 5'-GGGATCCTGGTTAGTTTCTTTCTCCGC-3') [15]. The amplified products were sequenced using the corresponding primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) on an automated sequencer (3730xl DNA Analyzer, Thermo Fisher Scientific). Sequences were aligned and compared using GENETYX-Win software (Ver. 13, Genetyx Co., Tokyo, Japan). ITS2 products of about 590 base pairs (bp) were generated. The sequence analysis of the PCR amplicons revealed that the amplified product was 538 bp (without primer sequences). Nucleotide sequence has been deposited as the ova stage of *F. hepatica* in the DDBJ/EMBL/GenBank database, accession number LO056930.

TCZ was used for treatment as recommended by the guidelines of WHO (2011), 10 mg/kg/day for 2 days. The medication was well-tolerated. The patient was evaluated at 1, 3, 6, and 12 months post-treatment and all stool examinations were negative for *Fasciola* spp. ova. Her mother signed a consent to participate in this publication.

Discussion

The present report highlights that in asymptomatic patients, who present with only hypereosinophilia (HE), fascioliasis must be considered in the differential diagnosis, particularly when patients came from rural Andean regions. Furthermore, the history of ingestion of aquatic plants like watercress in salads and raising of cows, sheep and pigs indicate potential risks of acquiring *F. hepatica* infection. On the other hand, the DNA isolated from eggs analyzed molecularly identified them as *F. hepatica* and the sequence obtained was identical to those established from adult flukes of *F. hepatica* occurring in cattle in the same Andes region of Ecuador (DDBJ/EMBL/GenBank database, accession number LO056929).

Eosinophilia is not an uncommon finding in clinical practice

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and, when present and used in conjunction with signs and symptoms, can serve as a useful clue in the differential diagnosis. Contrarily, the etiology of only eosinophilia can be difficult to identify in asymptomatic patients [17]. With a *Fasciola* spp. infection, usually during acute and chronic phases, eosinophilia with symptoms and signs are present, but the latent phase is characterized by nonspecific symptoms [4]. In the present case, clinical symptoms and signs were not present. In contrast, leukocytosis with HE was persistent for 5 months, indicating this case was probably in the latent phase. Elevated levels of immunoglobulins against *F. hepatica* were present as detected by the ELISA method, supporting that this case was not in the acute phase. In addition, the ultrasound confirmed the patient did not have hepatomegaly. In the chronic phase, the majority of infected patients have anemia, elevated ESR, and abnormal liver function tests [4] all of which were negative in our case. The present patient corroborated with the WHO (2007) statement that the percentage of asymptomatic fascioliasis cases is apparently high [9].

In Ecuador, human infection by *F. hepatica* has rarely been diagnosed and reported, although Ecuador has been considered highly endemic for *Fasciola* infection [8]. Since the first recognized case in the year 1969 [18] only 33 cases have been documented by eggs observation, 9 cases using ELISA test for FES-antigens [10,19] and 4 cases by the FasciDIG method [20]. All were from the Andean highland communities, as is the present case. Notwithstanding, veterinary fascioliasis is quite common with prevalence of 10 to 60% in cattle from Andean localities [20]. This information should alert both physicians and researchers of the possibility that human fascioliasis can also be found in all Andean plateaus. An active search for human infection in Ecuadorian Andean communities has revealed a prevalence of infection from 1.7% to 6% [10,20] indicating the importance of performing further community-based or seroepidemiological surveys to determine the real and actual prevalence of infection.

TCZ administered postprandial at 10 mg/kg body weight for 2 consecutive days, as recommended [13] was effective in curing this case. No adverse reactions to the medication was noted. TCZ is not listed in the Ecuadorian National Guidelines for Basic Drugs [21] and is not available in the country. Health authorities have not requested TCZ from WHO, which will donate it upon request.

In conclusion, it is imperative that clinicians and lab technicians working in endemic and non-endemic areas for fascioliasis consider the possibility of this parasitic infection in clinically asymptomatic patients with persistent eosinophilia in order to avoid erroneous diagnosis and therapeutic interventions. Patient management also requires knowledge concerning the usage of both the diagnostic tests and the specific drugs needed to treat the infection.

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