

## REVIEW/ARTÍCULO DE REVISIÓN

### UPDATE ON HUMAN FASCIOLIASIS IN PERU: DIAGNOSIS, TREATMENT AND CLINICAL CLASSIFICATION PROPOSAL

### ACTUALIZACIÓN EN FASCIOSIS HUMANA EN EL PERÚ: DIAGNÓSTICO, TRATAMIENTO Y PROPUESTA DE CLASIFICACIÓN CLÍNICA

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Suggested citation: Marcos, LA & Terashima, A. 2007. Update on human fascioliasis in Peru: diagnosis, treatment and clinical classification proposal. Neotropical Helminthology, vol. 1, no. 2, pp. 85-103.

#### Abstract

Human *Fasciola hepatica* infection in Peru is an emerging infectious disease. In this review we describe the fascioliasis situation in Peru, based on the most recent studies about epidemiology, diagnostic tools and treatment. We propose a new clinical classification according to the stage of the disease. Recent reports have highlighted the clinical variability ranging from an indolent to a severe life-threatening infection. Increasing numbers of human cases have been reported worldwide, especially from the Andean Region in South America. Most common clinical manifestations in the acute phase are: hepatomegaly, fever, weight loss, and eosinophilia. In the chronic phase, it can be mild and unspecific, including: biliary obstruction, bacterobilia, liver cystic calcifications, gallstones, and liver fibrosis. The Rapid Sedimentation Technique described by Lumberas should be applied to diagnose the chronic phase as well as for epidemiological studies in endemic areas. The direct smear only detects 2% of cases. The novel diagnostic test Fas2 (cathepsin L1) ELISA has a sensitivity of 92.4% and specificity of 83.6% in 634 Peruvian subjects in endemic areas and it is helpful for both the acute and chronic phases. The most common radiological abnormalities in the acute phase are: track-like hypodense hepatic lesions, liver "abscesses" and/or subcapsular hematomas. Abdominal ultrasound has a low sensitivity in chronic cases and is not recommended for screening. Triclabendazole is the treatment of choice even with a single dose (cure rate  $\geq 90\%$ ) for both phases, but resistance is now a concern in animals. The new arsenal of available information can be applied to prevention and control programs in Peru.

**Key words:** *Fasciola hepatica* - Fascioliasis/diagnosis - Fascioliasis/epidemiology - Zoonosis - Review - Peru

#### Resumen

La infección humana por *Fasciola hepatica* en el Perú es una enfermedad infecciosa emergente. En esta revisión describimos la situación de la fasciolosis en el Perú, en base a los estudios más recientes sobre epidemiología, métodos de diagnóstico y tratamiento. Proponemos una nueva clasificación clínica en base al estado de la enfermedad. Estudios recientes han destacado la variabilidad clínica de esta infección que puede comprender desde una infección indolente a una severa que puede comprometer la vida del paciente. Un incremento en el número de casos humanos ha sido reportado en todo el mundo, y en especial en la Región Andina de Latinoamérica. Las manifestaciones clínicas más comunes en la fase aguda son: hepatomegalia, fiebre, pérdida de peso y eosinofilia. En la fase crónica, esta puede ser leve e inespecífica, pero también severa como obstrucción biliar, colangitis, quistes calcificados hepáticos, cálculos vesiculares y fibrosis hepática. La Técnica de Sedimentación Rápida descrita por Lumberas debe ser aplicada para el diagnóstico de la fase crónica y para estudios epidemiológicos en zonas endémicas. El examen directo solo detecta 2 % de los casos. El nuevo examen diagnóstico ELISA Fas2 (catepsina L1) tiene una sensibilidad del 92,4 % y especificidad del 83,6 %, y esto fue observado en 634 sujetos peruanos en áreas endémicas y es útil tanto para la fase aguda como la crónica. Las anomalías radiológicas más comunes en la fase aguda son: lesiones hipodensas hepáticas en forma de "camino", abscesos hepáticos y hematoma subcapsular. La ecografía abdominal tiene una baja sensibilidad en casos crónicos y no es recomendada para tamizaje. El triclabendazol es el tratamiento de elección con una dosis única (tasa de curación  $\geq 90\%$ ) para ambas fases, pero casos de resistencia a la droga es ahora una preocupación aunque solo en animales. El nuevo arsenal de información disponible puede ser aplicado a los programas de prevención y control en el Perú.

**Palabras claves:** *Fasciola hepatica* - Fascioliasis/diagnóstico - Fascioliasis/epidemiología - Zoonosis - Revisión - Perú.

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## INTRODUCTION

The liver flukes *Fasciola hepatica* and *F. gigantica* infection cause the zoonotic disease fascioliasis. In Peru, *F. hepatica* is endemic, while *F. gigantica* has never been detected. Recent advances around the world have elucidated better the geographic distribution (including paleoparasitology), clinical manifestations, novel diagnostic tools and treatment regimens in this parasitic infection. Likewise, the increased recognition of human fascioliasis has led to the World Health Organization (WHO) to include fascioliasis into the list of important human parasitic diseases (Mas-Coma, 2005). We need to emphasize that in spite of the fact we have searched extensively about fascioliasis in the Peruvian literature, there were many difficulties and limitations to find more valuable information.

This article highlights recent progress in human fascioliasis in Peru, its impact on clinical practice, from epidemiology to recent vaccines, and emphasizes persistent gaps in our knowledge that merit further study (Summarized in conclusions & Figure1).

The aim of this review is to update the knowledge in fascioliasis in Peru, based on the most recent studies about epidemiology, diagnostic tools, treatment and we propose a novel clinical classification according to the stage of the disease.



Figure 1. Lesions are more central than subcapsular. Case with CT scan after endovenous contrast showing nodular images (arrows), perivascular, some serpiginous (arrowhead), track-like and subcapsular-peripheral lesions = Pathognomonic of fascioliasis. Most lesions are central, scattered throughout the liver parenchyma. Splenomegaly is present. \*With permission of Marcos *et al.*, 2008.

## EPIDEMIOLOGY

### A) Life cycle

The adult *F. hepatica* flukes are large, flat, brown and leaf-shaped, measuring approximately 2.5 to 3 by 1 to 1.5 cm. The broad, anterior portion is covered with scale-like spines. The adult fluke lives in the common and hepatic bile ducts of the human or animal host. The eggs are oval, yellow-brown, and measure approximately 130 to 150 by 60 to 90  $\mu\text{m}$ .

When parasite eggs in mammalian stool are deposited in tepid water (22-26°C) miracidia appear, develop, and hatch in 9-14 days. These miracidia then invade many species of freshwater snails, in which they multiply as sporocyst and redia for 4-7 weeks. They leave as free-swimming cercaria that subsequently attach to watercress, water lettuce, mint, parsley, or khat. Free-swimming cercaria may remain suspended in the water and encyst over a few hours.

When humans consume contaminated plants or water, the larvae excyst in the duodenum, migrate through the bowel wall and peritoneal cavity, and penetrate the Glisson capsule, actions that initiate the acute larval, hepatic, and invasive stages of human infection. Larvae sometimes also travel to ectopic body sites. This stage may last 3-5 months, during which the larvae mature and migrate through the liver into the large hepatic and common bile ducts. Mature flukes consume hepatocytes and duct epithelium and reside for years in the hepatic and common bile ducts and occasionally in the gall bladder; this is the chronic adult biliary stage of infection. Adult fluke worms produce eggs about 4 months (with a range of 3-18 mo) after infection; these eggs traverse the sphincter of Oddi and intestine and then continue the cycle of infection. Acute and chronic stages can overlap, particularly in a high-level infection.

### B) Intermediate hosts in Peru

Snails of the family Lymnaeidae are of great parasitological importance, because of their capacity to act as intermediate hosts for numerous trematode parasites, including those of medical and veterinary impact such as *F. hepatica* (Tantalean *et al.*, 1974). In Peru, *F. viatrix* (= *F. viator*) and *L. diaphana* (Cordova *et al.*, 1961) have been recognized to have

the ability to be intermediate hosts for fascioliasis. They are distributed throughout Peru but in special in the highlands valleys of the Andean Region.

### **C) Geographic distribution of *F. hepatica***

#### **C.1. In the world: A cosmopolitan parasite?**

New evidence has showed that *F. hepatica* was first documented in the Gallo-Roman period (Da Rocha *et al.*, 2006). Currently, an estimated of 91 million people are at risk of this infection (Keiser & Utzinger, 2005). Globally, the total estimated number of people infected is 2.4 million in 61 countries (In Haseeb *et al.*, 2002). In the world, the Andean Region of South America is the most affected by this parasitic infection. For example, up to 67% prevalence in the Bolivian Altiplano (Esteban *et al.*, 1999; Parkinson *et al.*, 2006) and 72% in the Peruvian Altiplano (Marcos *et al.*, 2005a). The number of people infected in some countries has been estimated as well. For example, 830,000 in Egypt, 742,000 in Peru, 360,000 in Bolivia, 37,000 in Yemen, 20,000 in Ecuador and 10,000 in Iran. Likewise, a higher number of human cases have been reported in the last years around the world: Argentina (Kleiman *et al.*, 2007), Venezuela (Incani *et al.*, 2003), Chile (Llanos *et al.*, 2006), Ecuador (Trueba *et al.*, 2000), Mexico (Cruz Lopez *et al.*, 2006), Turkey (Turhan *et al.*, 2006; Kaya *et al.*, 2006), Thailand (Aroonroch *et al.*, 2006), Japan (Inoue *et al.*, 2007), Korea (Lee & Kim, 2006), USA (Graham *et al.*, 2001; Fullerton *et al.*, 2006), Tunisia (Khelifi *et al.*, 2006) and Lebanon (Birjawi *et al.*, 2002).

Most reported cases are clinical complications of the infection; the real number of subjects with fascioliasis is undoubtedly underestimated. For example, in Peru, recent epidemiological studies were initiated from the complicated cases seen in the clinical setting in Lima hospitals, after that, multiple studies were carried out throughout Peru and we will present the results in the next paragraphs which will guide clinicians in endemic areas to recognize the infection with the only objective to treat it before developing complications.

#### **C.2. Situation of *F. hepatica* in Peru**

The current situation of human fascioliasis in Peru is dramatic, though unknown for most clinicians. The highest prevalence rates by coprological tests range from 8% in Cajamarca (Knobloch *et al.*, 1985); 34.2% in Santa Ana, Junín (Stork *et al.*, 1973); 28.3% in the same region 31 years later (Marcos *et al.*, 2004); 15.7% in Asillo, Puno (Mas-Coma *et al.*, 1999a); and 35% in the same area 9 years later (Esteban *et al.*, 2002); and by serological tests up to 36.3% in Junín (Marcos *et al.*, 2004). Most cases are school-age children 5-15 years old. A number of cases has been reported in many Peruvian hospitals; in Arequipa, 220 cases were reported until 1977 (Picoaga *et al.*, 1980); in Cuzco, 18 cases were diagnosed in patients undergoing cholecystectomy (Vilca, 1982); in Lima, where fascioliasis is not endemic, 16 cases were reported in the 90's in the Arzobispo Loayza Hospital (Jimenez *et al.*, 2001) and 277 cases in a period of 32 years in Cayetano Heredia National Hospital (Blancas *et al.*, 2004); and in Cajamarca, 101 cases were observed from 1996 to 2001 (Alban *et al.*, 2002).

A recent study done by our group confirms the high number of infected humans in our country. Almost 1701 cases were reported from 1963 to 2005 throughout Peru with 70% of the Peruvian territory likely infected (Marcos *et al.*, 2007e). This number of cases was increased to 1877 by the observations carried out by Mayta-Tristan & Caro (2008). The number of cases may still be underestimated in these reports because only complicated chronic severe infections were included. Furthermore, new endemic areas (e.g Huarochiri and Canta in Lima, Peru) continue to be identified by studying family members of index cases diagnosed in urban areas (Marcos *et al.*, 2007a). This might be a strategy for Public Health to uncover endemic areas in future studies.

In the Institute of Tropical Medicine *Alexander von Humboldt* of the Universidad Peruana Cayetano Heredia, Lima, Peru; under this strategy, we have discovered the areas of where patients with fascioliasis originated; some reported others only seen in the clinical setting (Terashima, personal communication). The division was made by

departments and towns and the following are the most likely endemic areas in Peru. In Lima: Canta, La Chacra, Huarochirí, Sangallaya, San Pedro de Casta, Marcahuasi, San Damián, Yauyos, Huaral, La Florida, Acos, Aucayama, Oyón, Caujul, Huancahuasi, Churin, Chancay (Huiza, 1973; Raymondí, 1986; Marcos *et al.*, 2007a; Maco *et al.*, 2003). In Ancash: Huari, Pallasca, Rahuapampa, Aija, Huacllan. Huachón, La Merced, Huaraz, Recuay, Cotaparaco, Ocos, Huayllas, Caraz (Cantella *et al.*, 1964; Fabian, 2003; Lopez De Guimaraes *et al.*, 1995; 1999). In Cajamarca: Celendín, La Libertad de Pallan, Cajamarca, Baños del Inca (Shaullo), Chota, San Miguel, Llapa, Sitacocha, Mangle, La Encañada, Contumazá, Chilete, San Juan (Lumbreras, 1964; Knobloch *et al.*, 1985; Ortiz *et al.*, 2000). In Pasco: Pasco. In Huanuco: Huanuco, Tingo María (Tapia & Manrique, 1975). In Junín: Jauja, Huertas, Julcán, Yauli, Molinos, Condorsinja, Santa Ana, Pancán, Concepción, 9 de Julio, El Tambo, Huala, Huancayo, Sicaya, Chupaca, Quilcas, Tarma (Terashima, 1970; Naquira *et al.*, 1972, Ramos, 1991; Marcos *et al.*, 2004), Chupaca (Cornejo *et al.*, 2003). In Cuzco: Sicuani, Cuzco, Cusco (Abarca, 1989; Rivera, 1977), Urubamba, Paucartambo, Caycay, Pacor, Vilcabamba, Vilcanota, Calca, San Salvador, Canchis, Anta (Valdivia *et al.*, 1990), San Pedro de Cacha (Castro *et al.*, 1964; Vilca, 1982; Fernandez, 1997). In Abancay: Apurímac, Cotabambas, Ñahuinlla, Pamputa, Aymares (Tataje, 1986; Jiménez *et al.*, 2001). In Ayacucho: Huanta, Huamanga, Cora Cora, La Mar, San Miguel, Vilcashuaman (Blancas *et al.*, 2004). In Arequipa: Arequipa (Cerpa, 1989, Perez, 1995), La Campiña, Uchumayo, Camaná, Ocoña, Sachaca (Perez, 1998), Caraveli (Ayaqui, 2000). In Ica: Chíncha Alta (Blancas *et al.*, 2004; Marcos *et al.*, 2005b). In Moquegua: Puquina. In Tacna: Candarave. In Amazonas: Chachapoyas (Ibañez *et al.*, 2004). In Puno: Azángaro, Asillo, Progreso, Ilave, Cabanillas, Yunguyo, Desaguadero (Esteban *et al.*, 2002; Marcos *et al.*, 2002, 2005a,c; 2006). In Huancavelica: Acobamba, Tayacaja, Ñahuimpuquio, Antajaja (Chinchihuasi), Huancavelica (Valencia *et al.*, 2005), Churcampa, El Carmen, Paucarbambilla (Jiménez *et al.*, 2001). Some of these areas have been explored and reported several human cases, others not yet; we strongly recommend to students and academicians involved in the medical field to

look for human cases in these areas and report them. A geographical system has been described recently in order to localize endemic areas in the Andean Region using climatic data to calculate forecast indices and other parameters (Fuentes *et al.*, 2005) but not yet widely available.

In summary, these results suggest that Peru is one of the countries with the widest regional distribution of human fascioliasis brought on by *F. hepatica* in the world. A rural population of almost 8 million people is estimated at risk in this country (WHO, 1995). Some regions still have the same – or an even higher – prevalence rate than years before. This fact is explained by the lack of control and prevention measures in those very regions. Human fascioliasis should no longer be considered a secondary zoonosis especially in Peru; but rather, an important human parasitic disease.

#### **D) Risk Factors for *F. hepatica* infection**

One of the singular epidemiological features of human fascioliasis in Peru is the route of infection how some people are infected by *F. hepatica*, since the classical watercress, is not as common here as in other countries. In a series of 277 patients with fascioliasis diagnosed in Lima, only 45.6 % mentioned having eaten watercress, the rest have acquired it from eating other plants such as lettuce (31.6 %), alfalfa (10.5 %), or spinach (5.3 %), drinking water from *puquiales* (10.5 %) (Natural water from small streams), or emollients (5.3 %) (emollients are warm beverage made from various plants, chiefly alfalfa and watercress, and supposed to be good for liver diseases), among others (Blancas *et al.*, 2004). Interestingly enough, the emollients may be a risk factor in the Mantaro Valley, Junín (Marcos *et al.*, 2004).

Others mention that the vehicle of contamination varies, depending on the region such as in France, *Taraxacum dens leonis* (dandelion leaves), *Valerianella olitoria* (lamb's lettuce), and *Mentha viridis* (spearmint); in the Islamic Republic of Iran, other green leafy *Nasturtium* spp. and *Mentha* spp.; and in the Bolivian Altiplano, *Juncus andicola* (Juncaceae), *Juncus ebracteatus* (Juncaceae), *Mimulus glabratus* (Scrophulariaceae), *Nostoc* sp. (Cyanofitas), among others (Bjorland *et al.*, 1995;

Mas-Coma *et al.*, 1999b). Water also has been described as a possible risk factor (Mas-Coma *et al.*, 1995). According to Esteban *et al.* (2002), there are two main vehicles of infection in Peru: water from streams and watercress; however, there are at least 40% of patients who deny exposure to them (Blancas *et al.*, 2004). There is a need of disclosing additional factors involved in fascioliasis transmission, to improve control and prevention programs in the near future.

In the searching of risk factors in Peru, a number of epidemiological studies have been carried out in endemic areas (Table 1). Housing characteristics (material, water and sewage), relation to water supply (channels, river, streams), water supply itself, consumption of certain plants or vegetables such as lettuce, onion, spinach, salads, watercress juice, drinking water from streams, breeding of llamas or alpacas, history of having taken antihelminthic drugs, past coprological diagnosis of intestinal parasitosis and history of surgery, were not significantly associated with *F. hepatica* infection.

Alfalfa juice, emollients and water from the irrigation channels which carry the metacercariae play a key role in the transmission of fascioliasis in endemic areas. Hypothetically, exportation of plants or other products could lead to transmission in non-endemic areas, as some

patients have been reported in Lima (Marcos *et al.*, 2007c). Treatment of contaminated plants with high doses of potassium permanganate decreases metacercaria viability and could be used to prevent infection (Ashrafi *et al.*, 2006) but not evaluated in endemic areas yet. The control and prevention programs under this epidemiological evidence have a support and enthusiastic programs will likely appear in the following years.

### E) Role of Gender in fascioliasis

There is a tendency for women to have higher prevalences and to be more insensitively infected. In a 60 children case-control study in Puno, the intensity of the infection was mild to moderate (101 and 400 eggs per gram -epg), two cases had more than 401 epg (up to 528 epg); and only one had less than 100 epg.

The intensity of infection is measured by means of Kato-Katz Technique quantifying the eggs per gram of feces (Katz *et al.*, 1972). The intensity was higher in girls than boys, as was reported in a previous study in the same region (Esteban *et al.* 2002) and in the Northern Bolivian Altiplano (Esteban *et al.*, 1999). This latter finding deserves special attention: women show more complication rates than do men in adulthood, as has been reported in a total of approximately

Table 1. Risk factors associated to *Fasciola hepatica* infection in humans in Peru.

| Risk factor                         | Odds Ratio =<br>OR(Confident Interval<br>CI 95%) | p value     | Reference                   |
|-------------------------------------|--|-------------|-----------------------------|
| Multivariate Analysis               |  |             |                             |
| Drinking [u1]                       | 5.2 (1.7-15.6)                                   | $p < 0.05$  | Marcos <i>et al.</i> , 2004 |
| Living close to irrigation channels | 7.2 (2.8-106.7)                                  | $p < 0.05$  |                             |
| Multivariate Analysis               |  |             |                             |
| Eating salads                       | 3.3 (1.2 - 9.0)                                  | $p < 0.001$ | Marcos <i>et al.</i> , 2005 |
| Multivariate Analysis               |  |             |                             |
| Drinking alfalfa juice              | 4.5 (1.7—11.1)                                   | $p < 0.001$ | Marcos <i>et al.</i> , 2006 |
| Familiarity with aquatic plants     | 4.3 (1.7—10.5)                                   | $p = 0.028$ |                             |
| Univariate Analysis                 |  |             |                             |
| Water supply from channels          | 2.4 (1.1-5.3)                                    | $p = 0.01$  | Marcos <i>et al.</i> , 2006 |
| Consumption of aquatic plants       | 2.5 (1.1-5.6)                                    | $p = 0.01$  |                             |
| Breeding 5 or more cattle           | 2.5 (1.1-5.6)                                    | $p = 0.01$  |                             |
| Owning dogs                         | 3.2 (1.3-8.1)                                    | $p = 0.000$ |                             |
| Defecation site in fields           | 2.6 (1.3-5.6)                                    | $p = 0.003$ |                             |
| Familiarity with aquatic plants     | 3.9 (1.8-8.3)                                    |             |                             |
| Breeding more than 5 sheep          | 0.3 (0.1-0.7)                                    |             |                             |

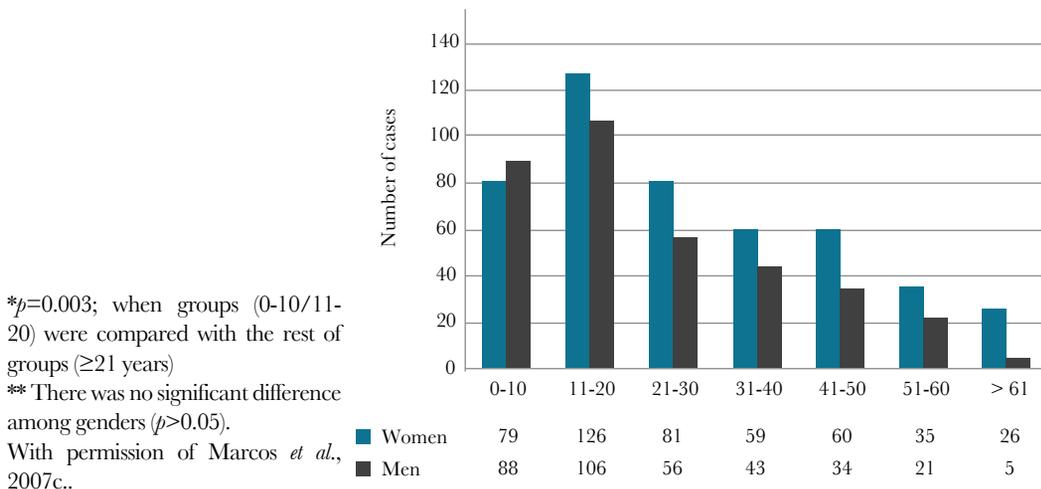


Figure 2. Distribution by age and gender of fascioliasis in Peru between 1963-2004.

622 cases in Peruvian hospitals since 1970 (Fig. 2); around 55% are women, in which there is an increase of abdominal surgeries. The percentage is more evident as age increases (Picoaga *et al.*, 1980; Vilca, 1982; Jimenez *et al.*, 2001; Alban *et al.*, 2002; Blancas *et al.*, 2004). In conclusion, more women are infected with *Fasciola* and have more complications from the infection.

## CLINICAL AND LABORATORY MANIFESTATIONS IN *F. HEPATICA* INFECTION: NEW EVIDENCE (SUMMARIZED IN TABLE 2)

### A) Acute phase of *F. hepatica* infection: a classic triad?

The acute phase, up to 4 months in duration, is characterized by the migration of larvae from the duodenum through the intestinal wall, peritoneal cavity, across Glisson's capsule entering the liver parenchyma and reaching the biliary ducts.

While there have been few series of acute cases reported in the literature, the clinical picture, laboratory data and radiological findings of acute fascioliasis have nevertheless been clearly described (Marcos *et al.*, 2007c). The classic triad consists of hypereosinophilia, right upper quadrant (RUQ) pain ("Murphy sign") and fever. A series of 10 carefully described Peruvian cases of acute massive fascioliasis found that RUQ pain was present in 80%, fever  $\geq 38^\circ\text{C}$  (70%), malaise (60%), anorexia (50

%), weight loss  $>10$  kg (50%), nausea and vomiting (30%) (Marcos *et al.*, 2007c). The RUQ pain and positive "Murphy sign" was misdiagnosed with "acute cholecystitis" and the CT abdominal findings were very similar to metastases (see Imaging Section). Interestingly, hyperbilirubinemia was absent (Table 3) (Marcos *et al.*, 2008). In some patients the eosinophil counts were slightly elevated, being a few days later  $>1500$  cells per  $\text{mm}^3$ , this finding was also found in large series of patients recently (Marcos *et al.*, 2005b; Gil-Gil *et al.*, 2006). This laboratory abnormalities are explained because of the parasite larva migration through the liver. Histologically, it produces subcapsular hemorrhages, hepatic degeneration, eosinophils infiltration, lymphocytes and macrophages, fibrosis, venous thrombosis, appearance of necrotic cords with giant cells and granulation tissue and granuloma-containing parasite eggs (Dalton, 1999; Hamir *et al.*, 2002) and even eggs in the blood vessels (Marcos *et al.*, 2006). In humans, subcapsular hemorrhages (Loja *et al.*, 2003; Marcos *et al.*, 2007c), hepatic rupture (Montesinos *et al.*, 1971), multiple hepatic abscesses (Marcos *et al.*, 2007c), hepatic necrosis (Kim *et al.*, 1999; Marcos *et al.*, 2007c), liver calcifications (Marcos *et al.*, 2007c) and severe anemia (Vilchez *et al.*, 1983); have been reported. Fascioliasis must be ruled out in any patient in an endemic area with hepatic dysfunction or liver failure without known cause.

Ectopic migration and other clinical manifestations:

- In the acute stage (Migratory nodule under

Table 2. Proposed clinical classification according to the stage of fascioliasis from a review of 1700 cases in Peru

| Stage of Disease                        | Oligo-symptomatic  | Clinical Picture  |
|---|--|---|
| <b>Acute</b>                            | <ul style="list-style-type: none"> <li>- Mild diffuse abdominal pain</li> <li>- Eosinophilia</li> <li>- Hyperglobulinemia</li> </ul> | <ul style="list-style-type: none"> <li>- Prolonged fever (weeks or months)</li> <li>- Abdominal pain with hepatomegaly.</li> <li>- Eosinophilia (any cell count level)</li> <li>- Biliary hemorrhage</li> <li>- Hepatic Rupture (seen in CT scan)</li> <li>- Anemia- Lost weight - Urticaria</li> <li>- Hepatic Abscesses</li> </ul>  |
| <b>Chronic most common presentation</b> | <ul style="list-style-type: none"> <li>- Mild abdominal pain in RUQ or epigastrium or asymptomatic.</li> <li>- Dizziness.</li> </ul> | <ul style="list-style-type: none"> <li>- Abdominal pain in RUQ</li> <li>- Biliary colicky (not associated with food)</li> <li>- Nausea, Vomiting</li> <li>- Recurrent or intermittent jaundice</li> <li>- Urticaria</li> </ul>  |
| <b>a) Complicated</b>                   |  | <p><b>Liver:</b></p> <ul style="list-style-type: none"> <li>- Hepatic Abscesses</li> <li>- Liver fibrosis and ultimately cirrhosis</li> <li>- Necrotic granuloma (increase ALT and AST)</li> <li>- Tumors</li> </ul> <p><b>Biliary:</b></p> <ul style="list-style-type: none"> <li>- Cholangitis choledocolithiasis</li> <li>- Cholecystitis</li> <li>- Tumors (s)</li> </ul> |
| <b>b) Uncomplicated</b>                 |  | <p><b>Liver:</b></p> <ul style="list-style-type: none"> <li>- Cysts</li> <li>- Nodules</li> <li>- Tumor (s)</li> </ul> <p><b>Biliary:</b></p> <ul style="list-style-type: none"> <li>- Colicky</li> <li>- Chronic cholecystitis</li> </ul>  |

\*Asymptomatic cases are occasionally seen in native people in endemic areas (acute stage) or detected by routine coprological studies in endemic areas (chronic stage).

the skin or peritoneal cavity, Arthralgias, Lymphadenopathies, Hemolytic anemia, Seizures, Pleural effusion).

- In the chronic stage (Subcutaneous nodules and Gastric nodule).

A slightly elevated eosinophil count does not exclude a diagnosis of acute fascioliasis, and a repeated cell blood count a few days later will show a dramatic elevation. Despite the typical abdominal pain in RUQ, this might be not specific in subjects with multiple parasitic infections, as occurs in endemic rural areas. However, acute fascioliasis should be ruled out in any patient from endemic area who presents with abdominal pain with eosinophilia. A

liver function test might be the next step (depending on the clinical context) and if altered, a serological test needs to be ordered to confirm diagnosis though a trial with triclabendazole may be initiated if serology is not available. A rapid clinical improvement in the next day and decrease levels of eosinophils will be seen in the next 3-5 days.

Sometimes, the juvenile larvae may reach other anatomic locations such as the subcutaneous tissue, pancreas, eye, brain, stomach wall, etc. This is called ectopic fascioliasis and it might be not unusual to see them in endemic areas if clinicians carefully examine the patient's skin (Fernan Zegarra *et al.*, 1961; Bejar *et al.*, 1996; Beltran *et al.*, 2004).

Table 3. Time of infection and laboratory analysis results on presentation in acute massive fascioliasis

|  | Range (Mean $\pm$ SD)      |
|--|----------------------------|
| Time of disease (weeks)                    |                            |
| <b>LABORATORY ANALYSIS</b>                 |                            |
| Hemoglobin (g/dl)                          | 0.5 – 22 (7.7 $\pm$ 6.7)   |
| Liver span (cm) x CT                       | 10.3-14.7 (12.3 $\pm$ 1.5) |
| Leukocyte count (x10 <sup>9</sup> /liter)  | 12-16 (14.1 $\pm$ 1.4)     |
| Eosinophil count (x10 <sup>9</sup> /liter) | 10.0-26.5 (17.3 $\pm$ 5.8) |
| ALT (U/liter)                              | 3.2-16.8 (10.5 $\pm$ 4.8)  |
| AST (U/liter)                              | 28-202 (88.3 $\pm$ 57.6)   |
| Alkaline phosphatase (U/liter)             | 21-74 (52.6 $\pm$ 19.3)    |
|  | 55-1800 (529 $\pm$ 552)    |

- Normal values are as follows: for haemoglobin, 13 to 18 g per deciliter; for the leukocyte count, 4 x 10<sup>9</sup> to 11 x 10<sup>9</sup> per liter; for the eosinophil count, less than 0.5 x 10<sup>9</sup> per liter; for alanine aminotransferase (ALT), 6 to 53 U per liter; for aspartate aminotransferase (AST), 13 to 33 U per liter. Alkaline phosphatase, 5 to 216 U per liter. \*With Permission of Marcos *et al.*, 2008.

### B) Chronic phase of *F. hepatica* infection: silent or severe?

The chronic phase occurs months to up to 13.5 years after infection (Chatterjee *et al.*, 1975; Dan *et al.*, 1981). It develops when adult parasites deposit eggs in the biliary ducts (MacLean, 1999). It is asymptomatic in approximately half of the cases (Marcos *et al.*, 2002) and is associated to dizziness (OR=2.5;  $p=0.016$ ) and a history of jaundice (OR=3.5;  $p=0.011$ ) (Marcos *et al.*, 2006). Symptoms usually reflect biliary obstruction with colicky pain in the right upper quadrant (RUQ) or epigastric area (Jimenez *et al.*, 2001; Maco *et al.*, 2003) or with extrahepatic cholestasis (Dobrucali *et al.*, 2004). In a large clinical-epidemiological study, increased liver enzymes were found in endemic areas (ALT elevated in 21.5 %, AST elevated 21.9 %, total bilirubin elevated 16.5 %, GGT elevated 80.6 %, and alkaline phosphatase 76.4 %), as well as imaging abnormalities including hepatomegaly, splenomegaly, periportal fibrosis, thickened gall bladder wall, dilated common bile duct, parasites in gall bladder and common bile duct, cholelithiasis, biliary duct stones, cystic liver lesions, focal lesions in the liver and ascites (El-Shazly *et al.*, 2001). Excluding viral liver infections, *F. hepatica* infection is a significant cause of cholestasis in endemic areas ( $p<0.05$ ) (El-Shazly *et al.*, 2005). These recent studies

suggest that chronic infection is severe and most of the time the hepatic damage is silent.

Chronic fascioliasis causes multiple complications. It may present as an acute eosinophilic cholecystitis (Umac *et al.*, 2006) which requires emergent surgery (Umac *et al.*, 2006), laparoscopic cholecystectomy (Bulbuloglu *et al.*, 2007), or even endoscopic retrograde cholangiopancreatography (Fullerton *et al.*, 2006). The parasites appears as intrahepatic cystic lesion(s) (Aronroch *et al.*, 2006), which can be associated with abscesses. In a rat model, a significant increased risk of bacteremia in the chronic infection has been demonstrated (Valero *et al.*, 2006) as well as gallstones (Valero *et al.*, 2003). An abscess in the liver may be secondary to fascioliasis and the treatment implies both broad-spectrum antibiotics and triclabendazole (see *treatment* section). However, even after successful treatment, the RUQ pain and weight loss may be still present in about 2-4 % of patients (Rondelaud *et al.*, 2006) but it is unclear this mechanism.

Eosinophilia is not always present in the chronic phase. Only 47 % of 277 complicated cases had eosinophilia at presentation (Blancas *et al.*, 2004), whereas 50 % of subjects with chronic fascioliasis had eosinophilia in endemic areas (Marcos *et al.*, 2002). In Turkey, only 11 % of 18 cases with fascioliasis had eosinophilia (Turhan *et al.*, 2006). However, there was a significant difference between cases and controls in a study performed in Puno, when it was compared the absolute eosinophil count (mean  $\pm$  SD = 680.5  $\pm$  850.5 cases vs. 297.4  $\pm$  392.9 controls;  $p=0.005$ ) and percentage with eosinophilia (43.5 % cases vs. 17.6 % controls;  $p=0.006$ ). Eosinophilia may be the first sign for suspicion on fascioliasis in endemic areas, but also these subjects may have multiple intestinal parasites that may increase eosinophil count especially helminths; or they may have other conditions such as asthma, among others. Clinicians need to be aware of these associations to have a comprehensive diagnostic approach. In summary, if a patient from endemic areas presents with eosinophilia and suspicion of any biliary tract abnormality, fascioliasis must be included into the differential diagnosis. Serial stool samples using the Rapid Sedimentation Technique describe by Lumbreras (Lumbreras *et al.*, 1967) must be the next step (see *Diagnosis* Section). Further studies will clarify the likely cause of eosinophilia in endemic areas.

### **B.1. Chronicity, liver fibrosis and sequelae**

*F. hepatica* infection is able to cause bile duct hyperplasia. Many years ago, a study showed that *F. hepatica* has the ability to produce proline *in vitro* (Campbell *et al.*, 1981) and is associated with bile duct hyperplasia *in vivo* (Wolf-Splenger & Isseroff, 1983). Increasing levels of type I and III collagen were finally demonstrated in an *in vivo* study in infected rats with fasciola which had significant bile duct hyperplasia (Mark & Isseroff, 1983). Biochemical and histological studies corroborate later the important role of proline in the enlargement of the bile duct in fascioliasis (Modavi & Isseroff, 1984). These changes seen in the collagen composition of the bile duct are similar to those produced in cirrhosis of the liver and other pathologic conditions including wound healing. Furthermore, the number of parasites seems to be critical in hepatic damage and liver fibrosis. In Peru, out of 30 livers of cattle 50% had liver cirrhosis (Marcos *et al.*, 2007b). In Zambia, fibrosis and calcifications in livers of cattle were associated to fasciola infection as well, being worst in the vicinity of the bile ducts (Phiri *et al.*, 2006). In two animal studies, thickened walls of bile ducts and severe cirrhosis were found microscopically (Haridy *et al.*, 1999; Shirai *et al.*, 2006). In goats, again the number of parasites was associated with the severity of the liver lesions (e.g. hepatic calcareous granuloma), including marked cirrhosis and death. These findings were observed mainly in goats given more than one infective dose (Perez *et al.*, 1999). On the other hand, liver cirrhosis has been reported in children (Almendras-Jaramillo *et al.*, 1997; Marcos *et al.*, 2005b) and adults (Heredia *et al.*, 1984; Sanchez-Sosa *et al.*, 2000) especially with massive infections.

In Peru, we developed the an experimental rodent model to study the pathogenesis of liver fibrosis associated with *F. hepatica*, concluding that the intensity of infection may be a determinant of fibrosis progression, these results provide a basis for further studies both *in vitro* and *in vivo* (Marcos, unpublished data). The process of infection by *F. hepatica* and its stimulation of fibrosis is likely to be dynamic. The parasite secretes proteins which may interact with the hepatocytes, extracellular matrix, hepatic stellate cells (the regulator cells of liver fibrosis from any injury) and other liver

components or cells. Further application of this model in future studies may uncover new insights into the clinical aspects and pathogenesis of a common parasitic disease that affects many habitants of poor countries. This can create potential experimental and therapeutic approaches. Liver fibrosis caused by *F. hepatica* infection places this parasitic infection in a new dimension into the chronic liver diseases and urge multidisciplinary control and prevention programs in endemic areas to at least stop the progression of the disease.

## **IMAGING STUDIES AND ITS UTILITY IN FASCIOLIASIS**

### **A) Abdominal Ultrasound (U/S)**

Ultrasound (U/S) findings in the acute phase include focal areas of increased echogenicity, multiple nodular or irregular lesions of variable echogenicity, or a single complex mass in the liver (González-Carbajal *et al.*, 2001; Cosme *et al.*, 2001; 2003) very similar to metastases or cancer. In the chronic phase, the U/S is less specific. *F. hepatica* adult parasites may be visualized in the gallbladder (Bonniaud *et al.*, 1984) and the diagnosis confirmed by ultrasound-guided aspiration of the gallbladder (Kabaalioglu *et al.*, 1999) but it is invasive and it is not routinely used. In a study of 76 subjects with chronic fascioliasis evaluated by means of abdominal U/S, in only 11 patients (14 %) were parasites visualized and in only 2 cases (2.6 %) were parasites spontaneously moving. Thus, the detection-rate of *F. hepatica* chronic infection by U/S was disappointingly low (Ritcher *et al.*, 1999), and not specific (Turhan *et al.*, 2006). Liver U/S may be useful in the acute phase but not for chronic cases.

### **B) Computed Tomography Scan (CT)**

Multiple liver lesions, which change in position, attenuation, and shape in time; are strongly suggestive of acute fascioliasis. Interestingly, initial lesions may be strongly confused with hepatic metastases. The most common findings are: hepatomegaly, track-like hypodense lesions with subcapsular location, subcapsular hematoma and cystic calcifications (Loja *et al.*, 2003; Marcos *et al.*, 2008). The hepatic lesions correlate with time of infection. Early infection is associated with contrast enhancement of Glisson's capsule due to inflammation stimulated

as the juvenile parasite penetrates the liver capsule (Hidalgo *et al.*, 1995). This occurs in the early stage of the acute infection (first month of infection). In the intermediate stage (after the first month of infection), multiple hypodense nodular areas (abscess-like lesions) or low-density serpiginous tortuous tunnel-like branching lesions ranging from 2 to 10 mm are created by parasite migration through the liver may be visualized in the subcapsular region (Kim *et al.*, 1995; Han *et al.*, 1999; MacLean & Graeme-Cook, 2002; Gonzalo-Orden *et al.*, 2003). In the late stage ( $\geq 3$  months), necrotic granuloma are seen, which appear as a single non contrast-enhanced hypodense irregular mass in the liver parenchyma, more central than peripheral (Kim *et al.*, 1999; Noyer *et al.*, 2002).

### C) Magnetic Resonance Imagings (MRI)

Few cases showing MRI imaging have been reported. T<sub>2</sub>-weighted turbo-spin-echo image MRI showed a homogeneous hyperintense area located subcapsular containing multiple hypointense areas. T<sub>1</sub>-weighted 3D gradient-echo image displayed homogeneous contrast-enhancement (Orlent *et al.*, 2007). The hypodense lesions observed in the CT scan are of hypointense signal in T1-Weighted and hyperintense in T2 (Kabaalioglu *et al.*, 2000; Aksoy *et al.*, 2006).

### DIAGNOSES OF FASCIOLIASIS: SEROLOGICAL, COPROLOGICAL TESTS AND LIMITATIONS

Accurate identification of early fascioliasis has historically been difficult since the diagnosis of the acute phase is only confirmed by serology (Incil *et al.*, 2001) and response to therapy (Marcos *et al.*, 2007c), this last very important in endemic rural poor areas where only the treatment is available but no serology. In Peru, we have available an ELISA for fasciola with good outcomes. The Fas2 ELISA detects the antibodies against Fas2 which is a major antigen of the adult parasite. Fas2 ELISA is more specific (92 %) than Western blot (72 %) and Arc II (37 %) (Maco *et al.*, 2002; Espinoza *et al.*, 2005). In 2007, a study evaluating Fas2 ELISA in 634 children from 1-16 years old in three endemic areas in Peru, the overall sensitivity of Fas2-ELISA was 92.4 %, the specificity 83.6 %, and the negative predictive value 97.2 %. These results show that Fas2-ELISA is a highly sensitive immunodiagnostic test for the

detection of *F. hepatica* infection in children living in human fascioliasis endemic areas (Espinoza *et al.*, 2007).

However, serological tests has limited availability in endemic rural poor areas, whereas economic, simple and affective coprological tests still playing the main role in the detection of chronic cases. The Rapid Sedimentation Technique (RST) described by Dr. Hugo Lumbreras in Peru (Lumbreras *et al.*, 1967) has been used for more than 12 studies over the past 5 years (Marcos *et al.*, 2002; 2004; 2005a; 2005b; 2005c; 2006; 2007a; 2007b; 2007c; 2007d; 2007e) detecting the highest prevalence rates in this country, and it has been also compared with other techniques such as ether-formol concentration method (Maco *et al.*, 2002) and Kato-Katz technique (Canales and others, unpublished data) but the RST has better outcomes. The Kato-Katz technique may be used to measure the intensity of infection (Katz *et al.*, 1975) when indicated. Thus, we strongly recommend that the Rapid Sedimentation Technique should be applied in any future coprological epidemiological studies carry out in Peru.

### TREATMENT OF ACUTE AND CHRONIC PHASES

Many years ago, parenteral dihydroemetina at doses of 1 mg/kg for ten days was used. Then, bithionol was applied at doses of 30 to 50 mg/kg every third day with a total of 10 to 15 doses; but it is cardiotoxic and very expensive.

Triclabendazole (TCBZ) was introduced in the early 1980s for the treatment of *F. hepatica* infections in livestock. It is the treatment of choice for human fascioliasis (Keiser *et al.*, 2005) and has been placed on the WHO List of Essential Medicines (<http://www.who.int/medicines/publications/essentialmedicines/en/>). The cure rate is more than 90 % for acute stages with a single dose at 10 mg/kg (Marcos *et al.*, 2008) and similar results have been obtained for chronic infections (Apt *et al.*, 1995; Talaje *et al.*, 2004; El-Tantawy *et al.*, 2007). Despite its efficacy, triclabendazole is only registered in four countries: Egypt (registered in 1997), Ecuador (2001), Venezuela (2001) and France (2002). The importance of this treatment has been well demonstrated in Egypt where a pilot study treated 1280 school children with TCBZ, the prevalence of infection decreased from 5.2 % in 1996 to 1.2 % in 2002/2003 (Abdussalam *et al.*, 1995). However,

the intensive use of triclabendazole has resulted in the development of resistance at least in animals (Mottier *et al.*, 2006).

In Peru, two clinical trials haven been carried out in endemic areas for chronic *F. hepatica* infection. The first, using the veterinary triclabendazole (Fasinex® 10 %), a single dose of 10 mg/kg cure 96 %, whereas 10 mg/kg in two following days, cure 100 %; no major adverse effects were registered (Terashima and others, unpublished data). The second clinical trial, using the triclabendazole for humans (Egaten®), patients were randomly allocated in two groups to receive a single dose of triclabendazole 10mg/kg or 15 mg/kg divided in two doses (7.5 mg/kg each one) orally after fatty meals; the cure of both schemes were >95 %; and no major side effects were reported (Marcos and others, unpublished data). In summary, a dose of 7.5 mg/kg every 12 hours after meals, by having similar tolerability but more efficacy than the former dose of 10 mg/kg as a single dose, should be considered in the control programs for endemic areas to ensure the success of the cure where the follow up is difficult to achieve a sustainable control.

Besides the effect of two doses in the cure rate, both studies also focused on the side effects, being the most important the abdominal pain as biliary colic during the first week of treatment. This is caused by the passage of dead or dying parasites through the bile ducts (Ritcher *et al.*, 1999; Millan *et al.*, 2000). Antispasmodics may decrease or avoid these transitory episodes of abdominal pain and should be used in most cases. Physicians in endemic areas are encourage to evaluate a patient with hepatomegaly, eosinophilia and fever for fascioliasis, and a single dose of triclabendazole is effective and it may use as a diagnostic criterion (Marcos *et al.*, 2008)

In Peru, it is available the veterinary use though lately the Ministry of Health included the triclabendazole in the priority list of medications. Other authors have proposed 10 mg/kg per day, repeated dose in 48 hours with a cure rate of 100 % (Apt *et al.*, 1995; Tataie *et al.*, 2004). In the clinical setting, the tolerability of the drug has been excellent and there had not major side effects. (Terashima, 1997; 1999; Ortiz *et al.*, 2000). Today, the treatment of choice for fascioliasis, acute or chronic phase is triclabendazole.

Given the unlikelihood of any new drugs against *F. hepatica* being developed in the foreseeable future, the emergence of resistance represents an important threat (Alvarez-Sanchez *et al.*, 2006) but resistance in human cases have not been reported so far.

## VACCINATION MAY BE POSSIBLE

Since fascioliasis continues to cause large economic losses worldwide in veterinary, development of effective vaccines is an important advance. Preliminary studies in animals have reported significant advances (Spithill & Dalton, 1998; McManus & Dalton, 2006). Cysteine proteinases released by *F. hepatica* play a key role in parasite feeding, migration through host tissues and in immune evasion. A recombinant cysteine proteinase (CPFhW) expressed as inclusion bodies in *Escherichia coli* was used for enteral vaccination of rats against fascioliasis. In that study, oral vaccination reduced the parasite burden by 78-80 % after a challenge with metacercariae (Kesik *et al.*, 2005). The glutathione transferase superfamily (GST) from liver fluke has phase II detoxification and housekeeping roles, and has been shown to contain protective vaccine candidates (Chemale *et al.*, 2006). Promising future basic research will yield meaningful immunological targets to prevent the infection especially in the well-recognized endemic areas and in particular in children but so far the vaccines are targeted to animals but not for humans.

## CONCLUSIONS

1. Human Fascioliasis is an emerging parasitic infection disease in Peru.
2. Endemic areas are over the world being the Peruvian Andean Region in South America the most affected areas.
3. Recognized risk factors are overall consuming aquatic plants such as alfalfa juice, emollients, lettuce, atajo (a plant from the streams), watercress, drinking water from the streams, salads. Exporting aquatic plants may disseminate fascioliasis to non-endemic areas.
4. Familial Fascioliasis is a common phenomenon. Up to 76 % of relatives of the index case may have the parasitic infection. Eating salads is the most common route of dissemination of the infection to the family.
5. Clinical manifestations can vary from indolent to life-threatening. In the acute phase: abdominal pain, hepatomegaly, fever, eosinophilia, and

elevated transaminases, hypodense lesions by CT scan. In the chronic phase, patients may present with biliary obstruction (intermittent jaundice most commonly), bacterobilia, liver cystic calcifications, cholelithiasis and liver fibrosis.

6. Characteristic imaging findings in fascioliasis include track-like hypodense lesions with subcapsular location, hepatic abscesses and/or subcapsular hematomas in the acute phase. Abdominal ultrasound has a low sensitivity for detection and it is not recommended for screening for chronic infection.
7. Improved serological test (Fas2 ELISA) has been developed and when applied in endemic areas may lead to the detection of more cases but limited in poor areas.
8. Rapid Sedimentation Technique (RST) described by Lumbreras (simple, economic and effective) should be routinely used in endemic areas for diagnosis and follow up.
9. If acute phase is suspected, next step is order a serological test. If it is not available, start with triclabendazole at 10mg/kg single dose; and then reassess clinical picture next 24-48 hours and decreased eosinophils next 3-5 days. If not, it is unlikely the diagnoses of fascioliasis.
10. For suspicion on chronic cases, the RST must be performed in serial stool samples.
11. Triclabendazole is the treatment of choice (cure rate  $\geq 90\%$ ) for both phases. Resistance has been detected in animals. Abdominal pain within the first week of treatment is common. Adjuvant antispasmodic therapy should also be used.
12. Vaccine development could prove an important advance.
13. To suspect in *F. hepatica* human infection, a history and physical needs to be taken on all fronts: epidemiology, clinical picture depending on the phase, diagnosis both serology and coprology, response to treatment and to test in the relatives.

### ACKNOWLEDGEMENT

We would like to kindly thank Dr. Eduardo Gotuzzo, Director of the Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru; for his invaluable help during many years and his contributions to this review. Dr. Raúl Tello, Blgo. Marco Canales and Mrs. Carmen Quijano, Laboratorio de Parasitología,

Instituto de Medicina Tropical Alexander von Humboldt for their help in the laboratory. Dr. Charles Talakottour, Internal Medicine Department, University of Texas, Houston, Texas, USA, for his help reviewing this manuscript. There is no grant source to acknowledge and the authors have no conflicts to declare.

### Conflict of Interests: None

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**Anuncio/Anounce**

**III Congreso Latinoamericano de Zoonosis , VI Congreso Argentino de Zoonosis**  
18-20 de julio de 2008, Universidad Pontificia Católica Argentina, Buenos Aires, Argentina.

**Temas:**

Zoonosis bacterianas endémicas, zoonosis transmitidas por vectores, zoonosis parasitarias, zoonosis transmitidas por mascotas, zoonosis emergentes, zoonosis en situaciones de desastre, enseñanza de la salud pública y de la veterinaria de la salud pública y el control de las zoonosis.

Aranceles después de 30 de marzo de 2008: Socios \$ AR 150, No socios \$ AR.

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