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Fasciola hepatica phenotypic characterization in Andean human endemic areas: Valley versus altiplanic patterns analysed in liver flukes from sheep from Cajamarca and Mantaro, Peru

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ABSTRACT

Fascioliasis is a zoonotic parasitic disease caused by *Fasciola hepatica* and *Fasciola gigantica*. Of both species, *F. hepatica* is the only one described in the Americas, mainly transmitted by lymnaeid snail vectors of the *Galba/Fossaria* group. Human fascioliasis endemic areas are mainly located in high altitude areas of Andean countries. Given the necessity to characterize *F. hepatica* populations involved, the phenotypic features of fasciolid adults infecting sheep present in human fascioliasis endemic areas were analysed in the Cajamarca Valley and Mantaro Valley (valley transmission patterns) and the northern Bolivian Altiplano (altiplanic transmission pattern). A computer image analysis system (CIAS) was applied on the basis of standardized measurements. The aforementioned highland populations were compared to standard lowland natural and experimental populations of European origin. Liver fluke size was studied by multivariate analyses. Two phenotypic patterns could be distinguished in *F. hepatica* adult size: the valley pattern (Cajamarca and Mantaro, Peru) and the altiplanic pattern (northern Altiplano, Bolivia). Results showed that the Andean valley population and European standard populations presented a phenotypic homogeneity. The Altiplano population showed a large size range with a pronouncedly lower minimum size indicating that uterus gravidity is reached at a smaller size than in valley populations. The results of this study demonstrate that there is no apparent relationship between the shape of fasciolid adults with regard to altitudinal difference or geographical origin and that allometry-free shape appears as a more stable trait than size in fasciolid species. Results are analysed in terms of intensity/crowding effect aspects and permanent/seasonal transmission characteristics.

1. Introduction

Fascioliasis is a parasitic disease of different epidemiological, pathological and control characteristics depending on the causal agents, *Fasciola hepatica* and *Fasciola gigantica*. Classically it has been accepted that *F. hepatica* is present worldwide, while the distribution of the two species overlaps in many areas of Africa and Asia (Mas-Coma et al., 2005, 2009). Their specific identification poses problems in the aforementioned overlap areas. It has been demonstrated that differentiation of both species cannot be made by traditional diagnostic methods (Marcilla et al., 2002; Valero et al., 2009) but only by adult morphometric markers (Periago et al., 2006, 2008; Ashrafi et al., 2006), molecular tools (Marcilla et al., 2002) and DNA sequences (Mas-Coma et al., 2009).

Of both species, *F. hepatica* is the only one described in the Americas (Mas-Coma et al., 2009), mainly transmitted by lymnaeid snail vectors of the *Galba/Fossaria* group (Bargues et al., 2007). Human fascioliasis cases have been reported from many Latin American countries, including high impact areas, described in the last two decades, focusing on Andean countries, above all on high and very high altitude areas where fascioliasis transmission appears to be enhanced as a consequence of the adaptation of both the liver fluke and its lymnaeid vectors to the extreme altitudinal environmental conditions (Mas-Coma et al., 2001).

In Andean countries, fascioliasis is a serious public health problem. Human fascioliasis hyperendemic areas have been described in the northern Altiplano of Bolivia (Esteban et al., 1997a,b, 1999; Mas-Coma et al., 1999) and also Peru (Esteban et al., 2002), as well as in Andean valleys such as the Peruvian valleys of Cajamarca (Ortiz et al., 2000; Hillyer et al., 2001; Espinoza et al., 2007; Gonzalez et al., 2011) and Mantaro (Stork et al., 1973; Marcos et al., 2004).

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Within the human fascioliasis high altitude transmission pattern related to F. *hepatica*, two different subpatterns of the general Andean pattern have been distinguished in South America according to physiographic and seasonal characteristics (Mas-Coma, 2005; Mas-Coma et al., 2009):

(a) The altiplanic subpattern, with very high prevalences and intensities in humans, endemicity distributed throughout an area of homogeneous altitude and transmission along the entire year due to high evapotranspiration rates leading lymnaeid vectors to concentrate in permanent water bodies (Mas-Coma et al., 1999), e.g. the northern Bolivian Altiplano (Esteban et al., 1997a,b, 1999) and the Peruvian Altiplano of Puno (Esteban et al., 2002).
(b) The valley subpattern, with very high prevalences but somewhat lower intensities in humans, endemicity distributed throughout an area of heterogeneous altitude, including prevalences correlating with altitude (Gonzalez et al., 2011), and seasonal transmission related to climate (Claxton et al., 1997, 1999),e.g. the valleys of Cajamarca and Mantaro, both in Peru.

In recent years, the availability of a very effective drug against fascioliasis, triclabendazole (Savioli et al., 1999), prompted WHO to launch a worldwide initiative against human fascioliasis (WHO, 2007, 2008). This initiative focuses on human fascioliasis endemic areas presenting different epidemiological situations and transmission patterns (Mas-Coma, 2005). Bolivia and Peru were selected for priority intervention due to the very large public health problem posed by this disease. Various pilot schemes were designed to assess the best control strategies according to the different epidemiological situations and transmission patterns. The northern Bolivian Altiplano and Cajamarca province were selected as models of the altiplanic and valley patterns of human hyperendemic areas, respectively.

The present study is a further step in the characterization of fascioliasis in human hyperendemic areas of the valley pattern, furnishing the baseline on which to design appropriate control measures for each South American disease transmission pattern. Although metacercarial infectivity does not appear to differ in isolates from different livestock species (Valero and Mas-Coma, 2000), the animal species affected pronouncedly influences the phenotype of both adult stage and egg of the liver fluke, mainly due to the different size of the liver duct microhabitat (Valero et al., 2001a, b, 2002, 2009). Therefore, a computer image analysis system (CIAS) method (Valero et al., 2005) was applied to flukes from sheep only, considering the typical host species for *F. hepatica* worldwide. Studies were performed on the basis of standardized measurements known to be useful for the differentiation of both fasciolid species (Periago et al., 2006). To complete the characterization, all these South American highland populations were compared to standard lowland populations of (i) *F. hepatica* natural infection from Valencia, Spain, and (ii) *F. hepatica* experimental adults from Bialowieza National Park, Poland.

2. Materials and methods

2.1. Parasites

Post-mortem examinations were carried out on all sheep (*Ovis aries*) immediately after death. The liver fluke in the definitive host presents parasite migration from the liver parenchyma to the adult location in the bile duct. Valero et al. (2006) modelled through a logistic model the liver fluke body growth, characterized by two phases: the 'exponential' part of logistic growth corresponding to body development during migration in the abdominal cavity and liver parenchyma as well as to development and sexual maturation in the biliary duct system up to the onset of egg production. From this moment, development follows the 'saturated' part of logistic growth, i.e. entry into the bile duct induces maturation and egg production. In this sense, bile ducts were examined and only flukes inside were analysed. In this study only fasciolids with eggs in the uteri were included ($n = 542$). The samples analysed in each of the five geographical areas included the largest possible worm variability (different stages of maturity, body size and gravid uteri). Adult worms were fixed in Bouińs solution between slide and coverglass but without coverglass pressure to avoid distortion. The specimens were stained with Grenachefs borax, differentiated, dehydrated and finally mounted in Canada balsam.

2.1.1. Natural F. hepatica infections

All the sheep analysed were adults originating from areas close to the slaughterhouses where the parasite material was obtained:

(A) Material from Cajamarca Valley (Peru): *F. hepatica* adults from 8 Merino sheep (130 worms; 2,2,12,15,18,21,28,32 worms per sheep, respectively), slaughterhouse of Rodicio.
(B) Material from Mantaro Valley (Peru): 47 *F. hepatica* adults from the 5 autochthonous Junin breed (47 worms; range: 3,5,8,10,21), slaughterhouses of Huayucachi, Pachacayo and Huancayo.
(C) Material from the northern Altiplano (Bolivia): *F. hepatica* adults from 12 Merino sheep (201 worms; 6,8,9,12,15,18, 19, 22,22,26,30), slaughterhouse of Batallas (altitude around 4000 m).
(D) Material from the Mediterranean coast (Spain): *F. hepatica* adults from 5 Merino sheep (37 worms, range: 2,7,8,10,10), slaughterhouse of Massamagrell (close to sea level).

2.1.2. Experimental infections with F. hepatica

Fasciola hepatica metacercariae were experimentally obtained as previously described (Mas-Coma et al., 2001). *Fasciola hepatica* and its snail host *Galba truncatula* originated from Bialowieza, Poland and Valencia Albufera, Spain, respectively. The experimental group included four four-to-five-week-old sheep (Guirra autochthonous breed) bred and kept close to sea level. The animals were infected *per os* with 200 *F. hepatica* metacercariae, respectively. Food and water were provided *ad libitum*. Permission for animal research was obtained from the Ethics Animal Research Committee of Valencia University. Animal ethics guidelines were strictly adhered to in the care of the animals. Necropsy was carried out 40 weeks post-infection. A total of 127 (20, 28, 34 and 45 worms per sheep, respectively) *F. hepatica* adults was recovered.

2.2. Measurement techniques

All standardised measurements of adults were made according to methods proposed for Fasciolidae by Valero et al. (2005) and Periago et al. (2006, 2008) (Fig. 1):

(A) Lineal biometric characters: body length (BL), maximum body width (BW), body width at ovary level (BWOv), body perimeter (BP), body roundness (BR), cone length (CL), cone width (CW), maximum diameter of oral sucker (OS max), minimum diameter of oral sucker (OS min), maximum diameter of ventral sucker (VS max), minimum diameter of ventral sucker (VS min), distance between the anterior end of the body and the ventral sucker (A-VS), distance between the oral sucker and the ventral sucker (OS-VS), distance between the ventral sucker and the union of the vitelline glands (VS-Vit), distance between the union of the vitelline