Hyperendemic human fascioliasis in Andean valleys: An altitudinal transect analysis in children of Cajamarca province, Peru

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ABSTRACT

A coprological survey including 476 2–18 year old school children from six rural localities between 2627 and 3061 m altitude was performed in Cajamarca province, Peru. Prevalences of fascioliasis ranging from 6.7 to 47.7% (mean 24.4%) proved to be the highest so far recorded in that human hyperendemic area. Higher prevalences in females and in the 2–5 year old group were not significant. Intensities ranged from 24 to 864 eggs per gram (arithmetic mean: 113; geometric mean: 68), the majority shedding less than 100, and without significant differences according to gender or age group. *Fasciola hepatica* was the most common helminth within a spectrum of 11–12 protozoan and 9–11 helminth species, 97.3% of the children showing infection with at least one parasite. The highest levels corresponded to coinfection with seven different species in females and subjects older than 5 years. Fascioliasis prevalence correlation with altitude appeared significant. An epidemiological characterisation of the valley transmission pattern of fascioliasis in Cajamarca is made by comparison with other better known hyperendemic areas. Results suggest that human fascioliasis may be widespread throughout different parts of Cajamarca province, even far away from the city, and that long-term fascioliasis chronicity and superimposed repetitive infections may be probably frequent.

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1. Introduction

Fascioliasis was only considered a secondary disease in humans due to the relatively small number of only around 2500 reported cases before the 90s (Chen and Mott, 1990), despite its high pathogenicity well known in livestock since very long ago (Torgerson and Claxton, 1999). The human fascioliasis scenario began to change from that decade, due to the description of large endemic areas including even human hyperendemic situations in different continents. The recent fascioliasis emergence has been related to climate change, at least in part and in given countries (Mas-Coma et al., 2008, 2009b), given the dependence of fascioliasis transmission on climate and environmental characteristics (Ollerenshaw and Smith, 1969; Fuentes et al., 1999, 2001).

Additionally, pathogenicity and immunity studies have shown this disease to be pronouncedly more complicated and with a greater impact in long-term infection than what was believed until the 90s (Valero et al., 2003, 2006, 2008; Girones et al., 2007). Emergence, long-term pathogenicity and immunological interactions are in the background of the decision taken by WHO to include this disease within the so-called neglected tropical diseases (NTDs). Their control and elimination is now recognized as a priority for achieving United Nations Millennium Development Goals and targets for sustainable poverty reduction (Hotez et al., 2007, 2008).

In the Americas, animal fascioliasis is caused by only *Fasciola hepatica* (Mas-Coma et al., 2009a) mainly transmitted by lymnaeid snail vectors of the *Galba/Fossaria* group (Bargues et al., 2007). Areas of high human impact seem to focus on Andean countries, above all in high altitude areas where fascioliasis transmission is increased as a consequence of the adaptation of both liver fluke and lymnaeid vectors to the extreme environmental conditions (Mas-Coma et al., 2001). Among Andean countries, Peru appears as the country presenting a larger human fascioliasis health problem. Human infection has been diagnosed in inhabitants from almost all Andean areas, including from the Altiplano (Esteban et al., 2002) up to inter-Andean valleys (e.g., Storck et al., 1973; Ortiz et al., 2000), and even urban areas surrounding the capital of Lima (Blancas et al., 2004)

and low altitude areas closer to the Pacific coast (Picoaga et al., 1980). Many of these areas have proved to be human endemic. A rural population of almost 8 million people is estimated at risk in this country (WHO, 1995).

Within the human fascioliasis high altitude transmission pattern related to *F. hepatica* transmitted by lymnaeid vectors of the *Galba/Fossaria* group, two different subpatterns have been distinguished in Peru according to physiographic and seasonal characteristics (Mas-Coma, 2005; Mas-Coma et al., 2009a): (a) the altiplanic pattern, with endemicity distributed throughout an area of homogeneous altitude and transmission throughout the whole year due to high evapotranspiration rates leading lymnaeid vectors to concentrate in permanent water bodies (Mas-Coma et al., 1999); examples are the Northern Bolivian Altiplano and the Peruvian Altiplano of Puno; (b) the valley pattern, with endemicity distributed throughout an area of heterogeneous altitude and seasonal transmission related to climate (Claxton et al., 1997, 1999); Peruvian examples are the valleys of Cajamarca and Mantaro.

In recent years, WHO has launched a worldwide initiative against human fascioliasis (WHO, 2007, 2008), including action in different epidemiological situations and transmission patterns (Mas-Coma, 2005). The Cajamarca province was selected as representative of the valley pattern of human hyperendemic. The aim of the present study is to analyse the results obtained from the human surveys performed in different rural areas of Cajamarca province, to establish a baseline on which to carry out the pilot activities. The article focuses on the main epidemiological characteristics of prevalences, intensities and geographic distribution of human infection, as well as of coinfections of fascioliasis with other protozoan and helminth infections, in the way to characterize human fascioliasis by comparison with human hyperendemic areas in other Andean regions which have been more widely and deeply studied.

2. Materials and methods

2.1. Study area and population

Coprological studies were made in the Departmento de Cajamarca, which covers an area of around 35,400 km^2 in the northern Andean part of Peru and is inhabited by 1,416,000 people. This department comprises 13 provinces and the province of Cajamarca in its turn includes 12 districts. Surveys were performed in the schools of six communities (population data from 2005 census): La Colpa (07°13′24.9″S; 78°26′44.0″W) and Yanamango (07°13′35.7″S; 78°25′47.2″W) (Jesus district, with a total of 14,075 inhabitants and a density of 52.6/km^2); Llimbe (07°12′33.9″S; 78°24′03.5″W) and Shaullo Grande (07°10′31.2″S; 78°25′04.3″W) (Llacanora district, with 4651 inhabitants and a density of 94.1/km^2); Huayrapongo Grande (07°10′58.7″S; 78°26′55.6″W) (Baños del Inca district, with 31,764 inhabitants and a density of 114.9/km^2); and Santa Rosa de Chaquil (07°07′48.2″S; 78°21′06.8″W) (Encañada district, with 22,397 inhabitants and a density of 35.3/km^2) (Figs. 1 and 2).

The Departmento de Cajamarca includes many inter-Andean valleys irrigated by more or less wide and fast-flowing rivers. The mean yearly temperature is 15.3 °C, with pronounced daily variation (July: −5.0 to 23.8 °C; January: 0.2–24.2 °C) and wide monthly differences (extreme mean temperatures of 3 °C in July and 22 °C in November). The relative humidity is 64%, and the total yearly precipitation is 769 mm, with a monthly minimum of 6 mm in July and a maximum of 107 mm in October. The rainy season includes from October to April (mainly December to March), and the dry season from May to October. Mean annual evapotranspiration is 99 mm,

with monthly variation between 84 mm in April–May and 109 mm in December.

Main economic activities are agriculture and mining, the latter above all in the western mountainous area. Around 75.3% of the population is rural, with agriculture focusing mainly on potato, barley, wheat, and corn. Dwellings are constructed with traditional materials and there is insufficient sanitary availability. About 33% of people does not have access to a treated water system and hence water is directly obtained from rivers and other natural water collections. A 76% of the population lacks access to a sewer system and rubbish removal service, and 80% lacks electricity (Instituto Nacional de Estadística e Informática, Peru: http://www.inei.gob.pe).

2.2. Stool collection and laboratory methods

The coprological survey involved 476 subjects (246 males and 230 females) of 2–18 years of age (mean ± S.D. = 9.1 ± 2.6). The surveys were made on randomly selected subjects on a given day among all participating students. The beginning of September was the period selected for the surveys, given the information on disease seasonality which indicates that livestock shows highest faecal egg shedding in August–September (Claxton et al., 1997, 1998). A clean, plastic, wide-mouth, numbered container with a snap-on lid was given to every participant. All subjects were then asked to try to fill the container with their own faeces and to return it immediately. One stool sample per subject was collected and personal data (name, sex, and age) were noted on delivery of the container. Faecal specimens were transported to a laboratory of the Universidad Nacional de Cajamarca within 1–3 h of collection. In this laboratory, a Kato-Katz slide was made from each stool sample following WHO recommendations, using a template delivering about 41.7 mg of faeces (Ash et al., 1994). These slides were initially examined within 1 h of preparation to avoid overclarification of some helminth eggs. If sufficient material of each stool sample was present, one aliquot was preserved in 10% formalin solution (1:3).

Coproparasitological studies were carried out at the Departamento de Parasitología (Valencia, Spain). Samples fixed in 10% formalin were processed by a formol-ether concentration technique (Knight et al., 1976) and one aliquot of sediment obtained with this technique was stained using a modified Ziehl–Neelsen technique (Henriksen and Pohlenz, 1981). Two slides per specimen were fully examined by one of the authors (LCG) and finally by the second author (JGE). Microscopic slides and materials from the human parasite collection of the Parasitology Department of the University of Valencia were used for quality control when needed. Recent corrections introduced into liver fluke egg characteristics for the coprological diagnosis in both human and animals were taken into account (Valero et al., 2009).

The sediments of the concentration technique and the Kato-Katz slides were used for prevalence data. According to the techniques used, prevalence results of the pinworm, *Enterobius vermicularis*, may not be considered definitive because anal swabs would be the adequate technique for the detection of the eggs of this nematode species. The Kato-Katz slides were analysed for egg counts. Intensity of infection, measured as eggs per gram (epg) as an indicator of *F. hepatica* burden in infected subjects, was described by range, arithmetic mean (AM), geometric mean (GM) and intensity classes.

2.3. Statistical analysis

Statistical analyses were done using SPSS 15 software package (SPSS Institute, Chicago, IL) for Windows. For the evaluation of categorical variables, the chi-square test or Fisher's exact test was used. The Mann–Whitney *U* test and Kruskal–Wallis (*H*) test were used for non-normally distributed data. Associations between liver
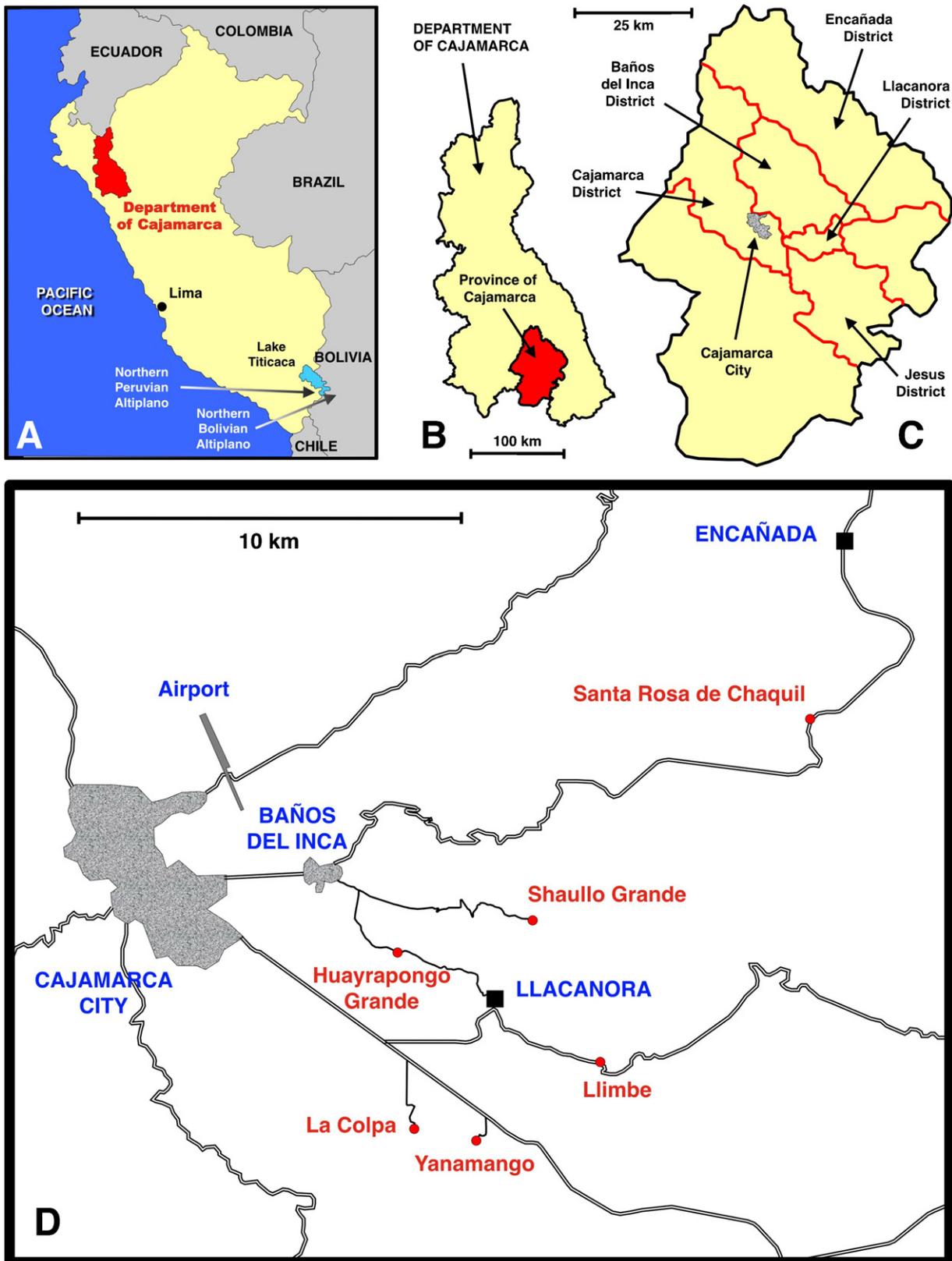
Fig. 1. Maps showing location of the Department of Cajamarca within Peru (A), the province of Cajamarca within the department (B), the districts of Jesus, Llacanora, Baños del Inca and Encañada within Cajamarca province (C), and the schools surveyed and respective accession roads from Cajamarca and Baños del Inca cities (D).

fluke infection and other parasite species were investigated by 2×2 contingency tables, from which the chi-square statistical was calculated. By processing with PASW statistics 17, bivariant correlations (Pearson's correlation) were calculated to assess the relationship (i) between locality altitude and prevalence of *F. hepatica* or any other parasite species found, as well as (ii) between prevalence of *F. hepatica* and prevalence of any other parasite species. A *P* value less than 0.05 was considered significant.

Fig. 2. Views of schools surveyed showing environmental characteristics: (A) La Colpa; (B) Yanamango; (C) Shaullo Grande; and (D) Santa Rosa de Chaquil.

2.4. Institutional ethical review procedure

Surveys and diagnostic analyses were carried out after informed consent was obtained from the local authorities of the communities, as well as from child parents and directors and teachers of each school, following the principles expressed in the Declaration of Helsinki. Each community surveyed was informed in previous meetings about the public health relevance and impact of the intestinal and hepatic parasitic infections, and on the need for the development of control programmes at national, regional, district and village levels. The initiative was performed with the Dirección Regional de Salud de Cajamarca, and approved by the Dirección General de Zoonosis of the Ministerio de Salud (MINSA) of Lima and by the Centro de Investigación y Control de Enfermedades Transmisibles of the Universidad Nacional de Cajamarca, Cajamarca.

3. Results

The overall prevalences of fascioliasis by communities, and according to sex and age groups are shown in Table 1. The highest prevalences of *F. hepatica* were recorded in Santa Rosa de Chaquil (47.7%) and Huayrapongo Grande (25.0%), both significantly different from those found in the other communities ($X^2 = 61.2$, $P = 0.0001$).

Prevalence difference between males and females was not significant, although females (26.5%) showed a higher prevalence than males (22.4%). Age groups were established according to the following criterion: infants (1–5 years old); school children (6–11 years old); and adolescents (12–18 years old). The highest prevalences were detected in the infant group (32.0%), but no statistically significant differences between the prevalence rates by age groups were detected. All the ages appeared to be susceptible to infection.

The overall intensities of fascioliasis by communities, and according to sex and age groups, are shown in Table 2. Absolute egg counts in the infected subjects ranged from 24 to 864 epg, with AM and GM of 113 and 68 epg, respectively. No statistical differences were found in intensities between communities, nor according to

sex and age groups within communities, although the highest overall egg counts were detected in the community of La Colpa, in males and in the adolescent group, although the range was of higher level in the 6–11 year old group.

Results on fascioliasis intensity levels in the total of the communities surveyed according to sex and age groups (Fig. 3) revealed no statistical differences, although females and mainly the school children group included a greater number of subjects shedding more epg than subject number in males and other age groups, respectively. The highest percentage (68.9%) of students infected was shedding not more than 100 epg. Students excreting more than 400 epg were only detected in La Colpa (11.1%), Santa Rosa de Chaquil (4.8%) and Shaullo Grande (4.2%).

F. hepatica, with an overall mean prevalence of 24.4%, proved to be the most common helminth within the total spectrum of parasites found. This coprological study revealed 11–12 protozoan and 9–11 helminth species. Parasite prevalences obtained in each community surveyed and in the total study are shown in Table 3. *Blastocystis hominis* (76.1%) and *Entamoeba coli* (74.6%) were the most prevalent within protozoans, and among helminths *Hymenolepis nana* and *Ascaris lumbricoides* followed *F. hepatica*, although with pronouncedly lower prevalences (15.1% and 14.7%, respectively). It is worth mentioning that the 97.3% of the students analysed showed infection with at least one parasite species.

Numerous multiparasitism cases associated with *Fasciola* infection were detected. The highest levels corresponded to coinfection with seven different species and were detected in females and in subjects older than 5 years. The extreme was the finding of up to 10–11 species in a 7 year old girl from Santa Rosa de Chaquil, namely *E. coli*, *Entamoeba histolytica/Entamoeba dispar/Entamoeba moshkowskii*, *Entamoeba hartmanni*, *Endolimax nana*, *Chilomastix mesnili*, *B. hominis*, *F. hepatica*, *Trichuris trichiura*, *A. lumbricoides* and *E. vermicularis*. A detailed analysis of *Fasciola* coinfection with other parasites showed only a significant positive association with *E. hartmanni* ($X^2 = 5.4$, $P = 0.002$).

The bivariant correlation analysis showed that the relationship between fascioliasis prevalence and altitude was significant

Table 1
Prevalence of fascioliasis in the communities studied by sex and age-group.

Communities surveyed	Total prevalence		Sex		Age-group (years)[a]		
	No. inf./no. tes.[b]	% (CI)[c]	Male No. inf./no. tes. (%)[d]	Female No. inf./no. tes. (%)	2–5 No. inf./no. tes. (%)	6–11 No. inf./no. tes. (%)	12–18 No. inf./no. tes. (%)
La Colpa	18/101	17.8 (10.3–25.3)	11/41 (26.8)	7/60 (13.3)	1/7 (14.3)	12/76 (15.8)	5/18 (27.8)
Yanamango	6/90	6.7 (1.5–11.9)	3/51 (5.9)	3/39 (7.7)	0/0 (0.0)	4/76 (5.3)	2/14 (14.3)
Llimbe	4/30	13.3 (1.1–25.5)	2/15 (13.3)	2/15 (13.3)	0/0 (0.0)	3/24 (12.5)	1/6 (16.7)
Shaullo Grande	24/117	20.5 (1.3–39.7)	11/68 (16.2)	13/49 (26.5)	0/3 (0.0)	20/92 (21.7)	4/22 (18.2)
Huayrapongo Grande	2/8	25.0 (0.0–55.0)	1/4 (25.0)	1/4 (25.0)	0/0 (0.0)	2/6 (33.3)	0/2 (0.0)
Santa Rosa de Chaquil	62/130	47.7 (39.1–56.3)	27/67 (40.3)	35/63 (55.6)	7/15 (46.7)	40/86 (46.5)	15/29 (51.7)
Total	116/476	24.4 (20.5–28.3)	55/246 (22.4)	61/230 (26.5)	8/25 (32.0)	81/360 (22.5%)	27/91 (29.7%)

[a] According to infants (2–5), school children (6–11) and adolescents (12–18) criteria.
[b] Number of *F. hepatica* infected subjects/number tested.
[c] Percentage (95% confidence interval).
[d] Number of *F. hepatica* infected subjects/number tested (percentage with respect to the number tested).

Table 2
Intensity of fascioliasis in the communities studied by sex and age-group.

Communities surveyed	Total intensity (epg)[b]	Sex		Age-group (years)[a]		
	Range (AM/GM)	Male Range (AM/GM)	Female Range (AM/GM)	2–5 Range (AM/GM)	6–11 Range (AM/GM)	12–18 Range (AM/GM)
La Colpa (n = 18)[c]	24–864 (156/77)	24–864 (177/77)	24–228 (123/79)	24 (–)	24–864 (188/88)	24–216 (106/73)
Yanamango (n = 6)[c]	24–384 (124/66)	24–384 (208/126)	24–72 (40/35)	–(–)	24–216 (84/35)	24–384 (204/96)
Llimbe (n = 4)[c]	24–72 (36/32)	24 (24/24)	24–72 (48/42)	–(–)	24–72 (40/35)	24 (–)
Shaullo Grande (n = 24)[c]	24–456 (138/100)	24–456 (164/117)	24–240 (116/88)	–(–)	24–456 (128/92)	72–360 (186/156)
Huayrapongo Grande (n = 2)[c]	48–96 (72/68)	48 (–)	96 (–)	–(–)	48–96 (72/68)	–(–)
Santa Rosa de Chaquil (n = 62)[c]	24–504 (96/59)	24–408 (91/65)	24–504 (100/55)	24–264 (93/68)	24–504 (92/54)	24–408 (109/70)
Total (n = 116)[c]	24–864 (113/68)	24–864 (126/75)	24–504 (102/62)	24–264 (84/60)	24–864 (112/66)	24–408 (124/78)

[a] According to infants (2–5), school children (6–11) and adolescents (12–18) criteria.
[b] epg, eggs per gram of faeces; AM, arithmetic mean; GM, geometric mean.
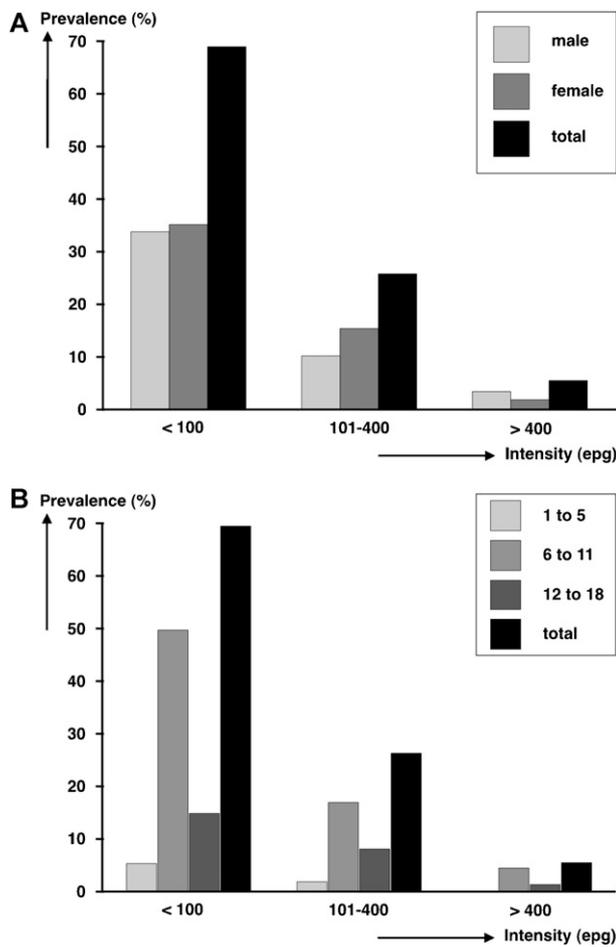[c] Number of *F. hepatica* infected subjects.

Fig. 3. Fascioliasis intensity levels in children from Cajamarca province: (A) according to gender and (B) according to age groups.

($R = 0.949$, $P = 0.014$) (Fig. 4), after excluding Huayropongo Grande due to the insufficient number of subjects studied in this locality (Table 4). A similar correlation with altitude was detected in the prevalence of *Iodamoeba buetschlii* ($R = 0.950$; $P = 0.013$) (Fig. 4), and that of *B. hominis* was almost significant too ($P = 0.064$). The prevalences of both protozoans showed, moreover, an interesting correlation with *F. hepatica* prevalence ($R = 0.908$, $P = 0.033$ and
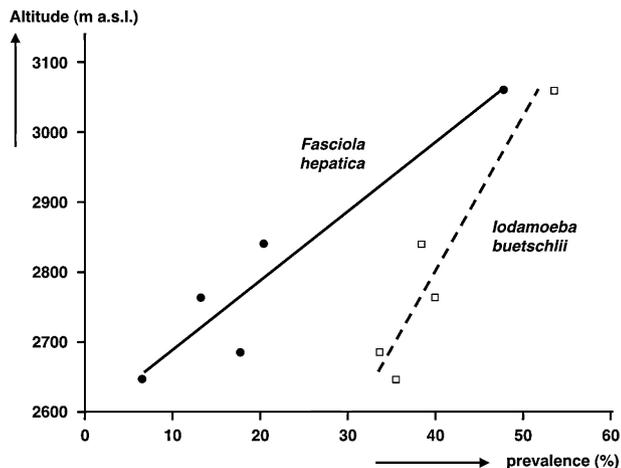

Fig. 4. Significant bivariant correlation lines of infection by *Fasciola hepatica* and *Iodamoeba buetschlii* in school children from Cajamarca province with regard to altitude of the localities surveyed.

$R = 0.884$, $P = 0.047$, respectively). Additionally, the appearance of *B. coli* and *Taenia* sp. only in the highest altitude locality is worth mentioning (Table 4).

4. Discussion

The fascioliasis problem in Cajamarca appears early up in the literature (Chavez, 1961; Lumbreras, 1964; Cosme-Contreras et al., 1971) and attracted many multidisciplinary studies from that moment. Research initiatives focused on livestock infection and its epidemiology and control (Claxton et al., 1997, 1998, 1999), fascioliasis in other local domestic animals as guinea-pigs (Gamarra, 1996; Dittmar, 2002), diagnostic methods and drug assays in humans (Knobloch, 1985; Knobloch et al., 1985; Jave et al., 1999; Ortiz et al., 2000; Hillyer et al., 2001; Favennec et al., 2003; Espinoza et al., 2007), clinical aspects (Alban Olaya et al., 2002), and even also related knowledge and behaviour of the inhabitants (Rivera-Jacinto et al., 2010). Thus, an appropriate study concentrating on main epidemiological aspects of human fascioliasis in the rural areas was still lacking.

4.1. Prevalences

Prevalences found in the present study indicate that they are pronouncedly higher, both in average and local maximum, than what was mentioned before: 3.5% in Cajamarca city (Ortiz et al., 2000); 6% in Asuncion district (Sanchez, 1992 in Ortiz et al., 2000); 8% in Cajamarca (Knobloch et al., 1985); 13% in Shaullo Grande, Shaullo Chico and Baños del Inca (Caceda, 1991 in Ortiz et al., 2000); 15% in San Juan rural community at 2800 m altitude (Ortiz et al., 2000). In a retrospective analysis of 101 fascioliasis patients coprologically diagnosed when attending the Regional Hospital of Cajamarca, the geographical origins of the patients included the districts of Cajamarca (52.5% of the cases), Namora (8.9%), Jesus (6.9%) and Baños del Inca (6.9%) (Alban Olaya et al., 2002). This, together with our finding in Santa Rosa de Chaquil (Encañada district), relatively far away from Cajamarca city, suggests that human infection by *F. hepatica* is widespread throughout different parts of the province of Cajamarca, most probably even far away from the capital given the widespread presence of livestock raising. Unfortunately, studies on livestock infection have also concentrated on particular farms (Claxton et al., 1997, 1998, 1999), so that information on livestock infection in different parts of the province is lacking, similarly as with human infection. Establishing geographical extent, prevalences and intensities in domestic animals should be one of the future priorities, in the way to assess where fascioliasis transmission occurs and consequently where human infection risk is present.

Both overall prevalence (24.4%) and local maximum (47.7%) demonstrate that Cajamarca province is a high human hyperendemic area, according to the WHO epidemiological classification (Mas-Coma et al., 1999, 2009a; Mas-Coma, 2005). These data are similar to those described in school children in the Peruvian Altiplano of Puno by coprology (mean: 24.3%; local maximum: 31.3%) (Esteban et al., 2002), and in Junin at the Mantaro valley (Storck et al., 1973: 34.2% by coprology; Marcos et al., 2004: 28.3% by coprology and 36.3% by serology), and also to those found in the Northern Altiplano of Bolivia, at the other side of the Lake Titicaca, by coprology (mean: 14.8%; local maximum: 72.0%) (Esteban et al., 1997a,b, 1999; Mas-Coma et al., 1999) and also serology (27.0%) (Hillyer et al., 1992).

The very high level of fascioliasis prevalences in Cajamarca is highlighted when comparing with human prevalences known in other regions of the world: 0.64–0.75% (mean 0.70%) in Chile (Apt et al., 1993), 2.7–7.3% (mean 3.2%) in Portugal (Sampaio Silva et al.

Table 3
Prevalences of parasite species detected in the communities surveyed in the Cajamarca province.

District	Jesús		Llacanora		Baños del Inca	La Encañada	Total survey
Community Parasite species	La Colpa (n^d = 101)	Yanamango (n = 90)	Llimbe (n = 30)	Shaullo Grande (n = 117)	Huayrapongo Grande (n = 8)	Santa Rosa de Chaquil (n = 130)	(n = 476)
Protozoa	92.1	93.3	93.3	98.3	100.0	99.2	96.0
Entamoeba coli	65.3	71.1	70.0	81.2	75.0	79.2	74.6
Entamoeba histolytica[a]	42.6	18.9	40.0	33.3	75.0	60.8	41.2
Entamoeba hartmanni	57.4	24.4	46.7	38.5	87.5	72.3	50.4
Endolimax nana	38.6	45.6	36.7	52.1	12.5	56.9	47.7
Iodamoeba buetschlii	33.7	35.6	40.0	38.5	25.0	53.8	41.0
Chilomastix mesnili	10.9	2.2	10.0	9.4	0.0	10.0	8.4
Giardia intestinalis	27.7	28.9	13.3	29.1	25.0	28.5	27.5
Enteromonas hominis	0.0	1.1	0.0	0.0	0.0	1.5	0.6
Retortamonas intestinalis	1.0	0.0	0.0	0.9	0.0	0.0	0.4
Blastocystis hominis	68.3	66.7	60.0	78.6	87.5	89.2	76.1
Balantidium coli	0.0	0.0	0.0	0.0	0.0	2.3	0.6
Helminths	41.6	46.7	30.0	47.0	100.0	59.2	48.9
Fasciola hepatica	17.8	6.7	13.3	20.5	25.0	47.7	24.4
Hymenolepis nana	7.9	22.2	13.3	22.2	50.0	7.7	15.1
Taenia spp.	0.0	0.0	0.0	0.0	0.0	3.1	0.8
Trichuris trichiura	7.9	11.1	0.0	12.0	25.0	4.6	8.4
Ascaris lumbricoides	17.8	22.2	3.3	6.8	37.5	16.2	14.7
Enterobius vermicularis[b]	3.0	5.6	3.3	0.9	12.5	3.1	3.2
Strongyloides stercoralis	0.0	0.0	0.0	1.7	0.0	0.0	0.4
Ancylostomatidae[c]	1.0	0.0	0.0	0.0	0.0	1.5	0.6
Trichostrongylus sp.	1.0	1.1	0.0	0.0	0.0	1.5	0.8
Total	95.0	95.6	96.7	98.3	100.0	99.2	97.3

[a] And/or *Entamoeba dispar/E. moshkowskii*.
[b] Detected in stool samples.
[c] *Ancylostoma duodenale* and/or *Necator americanus*.
[d] Number of school children studied.

in WHO, 1990), 0.61/100,000 year in France (Vaillant et al., 2003), 5.2–19.0% (mean: 12.8%) in Egypt (Esteban et al., 2003), 0.9–6.1% in Turkey (Demirci et al., 2003), undetermined low prevalences but with large epidemics in Iran (Moghaddam et al., 2004; Ashrafi et al., 2006; Rokni, 2008), up to 8% seropositivity in Vietnam (Hien et al., 2008).

The non-significant difference in prevalence by gender although with a trend to somewhat higher prevalences in females, agrees with the findings in 101 fascioliasis patients passively detected in the Regional Hospital in Cajamarca (66.3% in females) (Alban Olaya et al., 2002) and also with the data from the Peruvian Altiplano of Puno (Esteban et al., 2002) and the Northern Bolivian Altiplano (Esteban et al., 1997a,b, 1999). When analysing prevalences found with regard to age groups, the peak in the 9–11 year age group detected in Bolivia (Esteban et al., 1999) does not appear evident, and the non-significant but higher prevalences in the infant group agree with the results found in Puno (Esteban et al., 2002), but not with the age spectrum of Cajamarca hospital patients, in whom there were more patients among the 10–19 year age group (34.7%) than in the 0–9 year age group (27.7%) (Alban Olaya et al., 2002). Anyway, studies in Bolivia have already shown that rural young children do not usually attend hospitals for diagnosis. All in all, the high prevalences in the three different age groups surveyed in rural areas suggest long-term fascioliasis chronicity and superimposed repetitive infections to be probably frequent in Cajamarca province, similarly as in the Northern Bolivian Altiplano (Esteban et al., 1999).

4.2. Intensities

The intensities found in the present study (range: 24–864, AM/GM: 113/68) appear to be pronouncedly higher than those of only up to 4 epg previously noted in Cajamarca province (Knobloch et al., 1985; Ortiz et al., 2000). Anyway, these intensities are lower than those known in the Peruvian Altiplano of Puno (range: 24–2496, AM/GM: 279/123) (Esteban et al., 2002) and the North-

ern Bolivian Altiplano (range: 24–5064, AM/GM: 446/191) (Esteban et al., 1999).

Interestingly, overall intensities in Cajamarca province showed to be somewhat higher in males than in females, which is opposite to findings in Puno where females shed more eggs although with no significant difference (Esteban et al., 2002), and in Bolivia where females shed significantly more eggs (Esteban et al., 1999). With regard to age, the slightly higher mean intensities found in the adolescent group differs from results described in Puno, where children of 5–9 years were those presenting higher epg counts (Esteban et al., 2002), and in Bolivia where the epg AM was higher in the 9–12 year age group (Esteban et al., 1999). However, the intensity range in Cajamarca, of higher level in 6–11 year old children does fit with these other endemic areas.

In the analysis of intensity levels in Cajamarca, the very few cases (5.2%) in whom epg proved to be higher than 400 and the relatively low epg maximum of 864 are worth mentioning. This situation is similar to that in Puno in that the majority of children shed less than 100 epg, although in Puno a 18.2% shed more than 400 epg of whom 7.3% more than 1000 epg (Esteban et al., 2002). With regard to sex, in Cajamarca, despite arithmetic and geometric epg averages being higher in males than in females, the number of subjects shedding comparatively more epg is greater in females, a situation opposite to that detected in Puno (Esteban et al., 2002).

Summing up, intensities in both Peruvian Cajamarca and Puno appear markedly lower than in the Northern Bolivian Altiplano (Esteban et al., 1999), where children shedding up to 8000 epg have been recently found (Aguirre et al. in Mas-Coma et al., 2009a). The low intensity levels in Cajamarca province suggest that special care in treatment should be only sporadic in that endemic area, according to international recommendations (WHO, 2007).

Studies are needed in Cajamarca to assess whether epg counts differ throughout the different year seasons, as a consequence of the different seasonal infection risk. A marked seasonality has already been demonstrated in fascioliasis transmission in the Cajamarca

Table 4

Analysis of correlation (i) between locality altitude and prevalence of *F. hepatica* or of other parasite species, as well as (ii) between prevalence of *F. hepatica* and prevalence of other parasite species, according to parasite species selected as bioindicators of hygiene characteristics and different infection sources and localities ordered by increasing altitude.

School locality (District)	Altitude a.s.l. (m)	Distance by route from nearest urban locality with sewage system[b]	Prevalence of fascioliasis %/no. studied	Bioindicators of hygiene characteristics (prevalences in %)[c]							
				Indicators of mainly soil-borne infection[d]		Indicators of mainly water- and food-borne infection				Indicators of mainly zoonotic-borne infection	
				Ascaris	*Trichuris*	*Endolimax*	*Iodamoeba*	*Giardia*	*Blastocystis*	*Balantidium*	*Taenia*
Huayrapongo Grande (Baños del Inca)	2627	3.0 km from Baños del Inca by secondary route	25.0%/8	37.5	25.0	12.5	25.0	25.0	87.5	0.0	0.0
Yanamango (Jesus)	2647	11.0 km from Cajamarca by main route	6.7%/90	22.2	11.1	45.6	35.6	28.9	66.7	0.0	0.0
La Colpa (Jesus)	2685	9.4 km from Cajamarca by main route	17.8%/101	17.8	7.9	38.6	33.7	27.7	68.3	0.0	0.0
Llimbe (Llacanora)	2764	14.0 km from Cajamarca by main route	13.3%/30	3.3	0.0	36.7	40.0	13.3	60.0	0.0	0.0
Shaullo Grande (Llacanora)	2840	6.0 km from Baños del Inca by tertiary route	20.5%/117	6.8	12.0	52.1	38.5	29.1	78.6	0.0	0.0
Santa Rosa de Chaquil (Encañada)	3061	23.0 km from Cajamarca by main route	47.7%/130	16.2	4.6	56.9	53.8	28.5	89.2	2.3	3.1
P value for correlation of altitude vs prevalence[a]	–	–	0.014	0.751	0.649	0.131	0.013	0.832	0.064	–	–
P value for correlation of fascioliasis vs prevalences of other parasites[a]	–	–	–	0.959	0.698	0.172	0.033	0.659	0.047	–	–

[a] *P* values obtained when discarding Huayropongo Grande due to the insufficient number of subjects studied in this locality. A *P* value less than 0.05 was considered significant.

[b] Route distances noted for additional information only. Not considered in correlation analyses.

[c] Bioindicator parasite species selected because of presenting evident prevalence differences according to localities; *Giardia* included because of its correlation with fascioliasis verified in other Andean human hyperendemic areas; *Balantidium* included in the mainly zoonotic-borne group due to the importance of pigs as reservoirs of fascioliasis in Andean human endemic areas (Valero and Mas-Coma, 2000); see other parasite species presenting similar prevalences according to localities in Table 3).

[d] Prevalences may be distorted due to previous albendazole treatments.

inter-Andean valley, including higher infection rates of lymnaeid vectors in the January–March period, an infection peak in livestock during December–May, and a maximum of egg production in domestic animals in August–September (Claxton et al., 1997, 1998, 1999).

4.3. Coinfections

All of the localities studied show a similarly very high multiparasitism degree, suggesting similar hygienic-sanitary and socio-economic conditions. Given the immunomodulation capacity of fascioliasis during its acute phase (O'Neill et al., 2000, 2001) and its immunosuppression effect on the advanced chronic stage of the disease (Girones et al., 2007), a high morbidity by clinical synergism may be expected in children affected by fascioliasis in the Cajamarca province when taking into account the high number of both protozoans and helminths of recognized pathogenicity present in the endemic area.

When analysing the spectrum of protozoans, the absence of *Cryptosporidium*, although similar as in Puno (Esteban et al., 2002), has to be highlighted, as indeed cryptosporidiasis prevalences proved to be very high (range: 30.2–32.1%; mean: 31.6%) in the Bolivian Altiplano human fascioliasis hyperendemic area, suggesting a close contact with livestock (Esteban et al., 1998a). The absence of *B. coli* in four of the five localities studied in Cajamarca, although again similar to in Puno (Esteban et al., 2002), should also be emphasized, as this protozoan appeared in several endemic communities of the Bolivian Altiplano hyperendemic area (range: 1.0–5.3%; mean: 1.2%) suggesting a relationship with infected pigs (Esteban et al., 1998b). Among helminths, the sporadic presence of strongyloidiasis, ancylostomid infection and *Trichostrongylus* infection remembers the Puno area (Esteban et al., 2002), but differs from their absence in the Northern Bolivian Altiplano (Esteban et al., 1997a).

The lack of association between fascioliasis and giardiasis in Cajamarca differs from results found in Puno (Esteban et al., 2002) and Bolivia (Esteban et al., 1997a) and suggests that infection through drinking water may not be so usual as in both Peruvian and Bolivian Altiplanos. The unexpected fascioliasis association with *E. hartmanni* infection is difficult to interpret. Interestingly also is that prevalences of trichuriasis and ascariasis in Cajamarca do not appear to differ from those found in Puno (*T. trichiura*: 8.7–21.4%, mean 18.3%; *A. lumbricoides*: 1.5–14.5%, mean 8.0%) (Esteban et al., 2002) and in the Bolivian Altiplano (*T. trichiura*: 1.9–24.0%, mean 8.4%; *A. lumbricoides*: 1.2–28.0%, mean 11.6%) (Flores et al., 2000), despite the lower altitudes (geo- and pseudogeohelminths generally decrease in prevalence with increasing altitudes – see review in Flores et al., 2000) and official treatment initiatives with albendazole in Cajamarca.

4.4. Correlation with altitude

The significant bivariant correlation found between fascioliasis and altitude is outstanding. This is in agreement with results of previous experimental studies which proved that, at higher altitudes, infected lymnaeid vectors survive longer (Bargues et al., 1995; Mas-Coma et al., 2001), the cercarial shedding period is longer, and the number of metacercariae produced per snail is larger (Mas-Coma et al., 2001). Additionally, the appearance of *B. coli* and *Taenia* sp. only in the highest altitude locality of Santa Rosa de Chaquil suggests that the contact with livestock may be closer.

Nevertheless, an influence of remoteness cannot be ruled out, as in fact Santa Rosa de Chaquil is the locality more far away by road from an urban area with sewage system availability. Unfortunately, remoteness is difficult to be measured, because it does not only include distance but also the complex concept of accessibility (kind of roads and their status in the rainy season when non-asphalted, frequency of public transport if any, availability of electricity, water pipelines, population density, access to physicians and pharmacy, presence/absence of local health centre, livestock density and proximity, etc.). All in all, results obtained suggest that rural localities at higher altitudes, more far away from Cajamarca and Baños del Inca cities, and without accessibility by asphalted roads, may have a higher human infection risk. Unfortunately, studies carried out so far have, with the exception of San Juan rural community at 40 km southeast of Cajamarca city (Ortiz et al., 2000), only focused on localities relatively near to Cajamarca city. Consequently, appropriate surveys in high altitude remote areas become priority for future epidemiological surveys.

A patchy distribution of infection is typical within fascioliasis endemic areas given its water-borne transmission linked to freshwater lymnaeid vectors (Mas-Coma et al., 1999). Within this patchy epidemiological frame, given that the infectivity of the metacercarial stage from different livestock species isolates has shown to be similar (Valero and Mas-Coma, 2000; Valero et al., 2001), our field work did not allow us to see evident livestock reservoir population differences throughout as to explain different altitude-linked human infection risks in Cajamarca province. Thus, future priority should be given to the lymnaeid vector species as the crucial factor for the epidemiology of the disease in that area. Indeed, geographical distribution, prevalences and intensities of both human and animal infection are known to pronouncedly depend on the lymnaeid species involved in the local transmission and characteristics such as their population dynamics, anthropophylic characteristics, type of water bodies, etc. (Bargues and Mas-Coma, 2005). According to previous vector studies in Cajamarca (Claxton et al., 1999), it appears that the focus would be on the fossarine group of lymnaeid species (Bargues et al., 2007).

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