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High fascioliasis infection in children linked to a man-made irrigation zone in Peru

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Summary We detected 10 protozoan and nine helminth species in surveys of 338 5–15 year-old Quechua schoolchildren in three communities of the Asillo zone of the Puno region, located at a very high altitude of 3910 m in the Peruvian Altiplano. The area proved to be hyperendemic for human fascioliasis with a 24.3% overall mean prevalence of *Fasciola hepatica*, local prevalences ranging between 18.8 and 31.3%, and infection intensities of up to 2496 eggs per gram of faeces (epg), with 196–350 epg (mean: 279 epg) and 96–152 epg (123 epg) as arithmetic and geometric means, respectively. Prevalences did not significantly vary between schools and in relation to sex. No statistical differences were found in intensities between schools, nor according to sex or age groups between and within schools, although the highest overall egg counts were detected in girls and in the youngest age group. Asillo zone is a man-made irrigation area built only recently to which both liver fluke and lymnaeid snails have quickly adapted. The region appears to be isolated from the Northern Bolivian Altiplano natural endemic area. Such man-made water resources in high altitude areas of Andean countries pose a high fascioliasis risk. Significant positive association of *F. hepatica* with protozooses following a one host life cycle, such as *Giardia intestinalis*, suggests that human infection mainly occurs through drinking water. This is supported by additional evidence such as the absence of typical aquatic vegetation in the drainage channels inhabited by lymnaeid snails, the absence of aquatic vegetables in the traditional nutrition habits of the Quechua inhabitants, and the lack of potable water systems inside dwellings, which requires inhabitants to obtain water from irrigation canals and drainage channels.

keywords *Fasciola hepatica*, prevalence, intensity, associations, schoolchildren, Peru

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Introduction

Fascioliasis due to *Fasciola hepatica* is a great health problem in many countries. The conception of human fascioliasis has changed in recent years. Reports of humans infected by *F. hepatica* have been significantly increasing since 1980 worldwide (Esteban *et al.* 1998a). Several areas are truly endemic, with singular epidemiological characteristics (Mas-Coma *et al.* 1999a,b). High prevalences in humans are not necessarily related to areas where fascioliasis is an important veterinary problem. Globally, several million people are estimated to be infected. Although *F. hepatica* infection occurs across all ages, children appear to be the most affected in areas of high human endemicity. Fascioliasis causes severe symptomatology and pathology (Chen & Mott 1990; Mas-Coma *et al.* 1999a, 2000) and increasing evidence suggests that exposure to fascioliasis compromises an individual's ability to resist other endemic infectious diseases (Brady *et al.* 1999; Mas-Coma *et al.* 1999a). Hence in human fascioliasis-endemic areas, studies on other parasitic diseases, mainly those involving recognized pathogenicity, need to be undertaken to investigate their coexistence and possible relationships with human fascioliasis (Esteban *et al.* 1997a, 1998b,c; Flores *et al.* 2001). Human fascioliasis should no longer be considered merely as a secondary zoonosis, but as an important human parasitic disease (Mas-Coma *et al.* 1999b).

One of the singular epidemiological characteristics of human fascioliasis is the link between hyperendemic areas and very high altitude regions (2000–4200 m), at least in South America (Mas-Coma *et al.* 1999a). This concerns the Andean countries, mainly Bolivia and Peru (WHO 1995). High altitude climatic characteristics markedly differ from those of fascioliasis-endemic lowland areas in northern latitudes (Fuentes *et al.* 1999). Endemic highlands with their extreme and severe conditions show remarkable stability. Transmission foci are marked by the presence of lymnaeid populations in water bodies both during the year and throughout pluriannual periods. The parasite distribution appears irregular in these areas, the transmission foci being patchily distributed and linked to bodies of water. Human prevalences in schoolchildren appear to be related to the distance to water bodies with lymnaeids and to the snail population's size and extent (Mas-Coma *et al.* 1999c).

In Bolivia, human fascioliasis as a health problem appears restricted to an isolated endemic area in the northern Bolivian Altiplano, between Lake Titicaca and the valley of the city of La Paz (Mas-Coma *et al.* 1999c). This area is located at an altitude of 3800–4100 m and has the highest known human fascioliasis prevalences: up to 70% in surveys with coprological methods and up to 100% by serology (Hillyer *et al.* 1992; Bjorland *et al.* 1995; Mas-Coma *et al.* 1995, 1999c; Esteban *et al.* 1997a, 1999; O'Neill *et al.* 1998). Infection intensities > 100 eggs per gram of faeces (epg) are very common, including up to > 5000 epg (Esteban *et al.* 1997a,b, 1999).

In Peru, a rural population of almost 8 million people is estimated to be at risk (WHO 1995). Human cases have been detected in the whole country, with mesoendemic and hyperendemic zones in the Andes. Results of surveys carried out in Arequipa, Mantaro valley and Cajamarca valley determined prevalences (Esteban *et al.* 1998a).

To identify the most appropriate control measures, additional multidisciplinary studies are needed to ascertain whether the high altitude regions of Peru have similar epidemiological characteristics and transmission patterns as the northern Bolivian Altiplano. Our study is part of a large international cooperation project undertaken for this purpose. We report the results of the surveys in the Asillo zone of the Puno region, located at an altitude of 3910 m in the Northern Peruvian Altiplano, and its fascioliasis epidemiological characteristics.

Materials and methods

Study area

We conducted a coprological survey in the Asillo irrigation area (Province of Azángaro) in May 1999. This area is about 140 km north-west of Puno, the capital of the Puno Department, Peru, which is located on the northern shore of Lake Titicaca (Fig. 1). The Asillo irrigation area covers 5000 ha, and is delimited to the north by the Cotarsaya Lagoon, to the north-east by the San Antón river, to the north-west by the Nuñoa river and to the south-east by the Azángaro river. The irrigation infrastructure of this area, built between 1956 and 1974, comprises primary and secondary irrigation canals as well as drainage channels (Fig. 2) that conduct water from the Cotarsaya Lagoon to a cultivable area of 1559 ha, of which 90% are natural pastures or used for crops and forage to feed livestock. The remaining 10% are used for subsistence agriculture. Various problems prevented the construction of tertiary irrigation canals and of some drainage channels, resulting in deterioration of the secondary canals, poor use of the water resources and flooding or oversaturation of the plots designated for crops.

The 2000 families that inhabit this zone are very poor and live in unhealthy conditions. The main economic activity of the rural Quechua communities we surveyed is livestock breeding (mainly sheep and cattle, but also llamas, pigs and horses). There are no basic services such as piped potable water, sewage and rubbish disposal. Indiscriminate defecation is common and people and animals share the same faeces-contaminated water sources. The surveys were conducted at random on a given day among all participating students, and the sample size in each school was representative of both the student enrolment in the school and the number of children present on the day of the survey (at least 95%). During the collection of faecal samples, school directors, teachers and some parents were interviewed, and all stated that in this zone they do not eat raw liver.

Stool collection and laboratory methods

The coprological survey involved 338 Quechua schoolchildren (196 boys, 142 girls) aged 5–15 years (mean ± SD = 10.1 ± 2.9), from the schools of three communities. 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), and one aliquot was preserved in 10% formalin solution. Slides were initially examined within 1 h of preparation to avoid overclarification of some helminth eggs. Samples fixed in 10% formalin were processed by a formol-ether

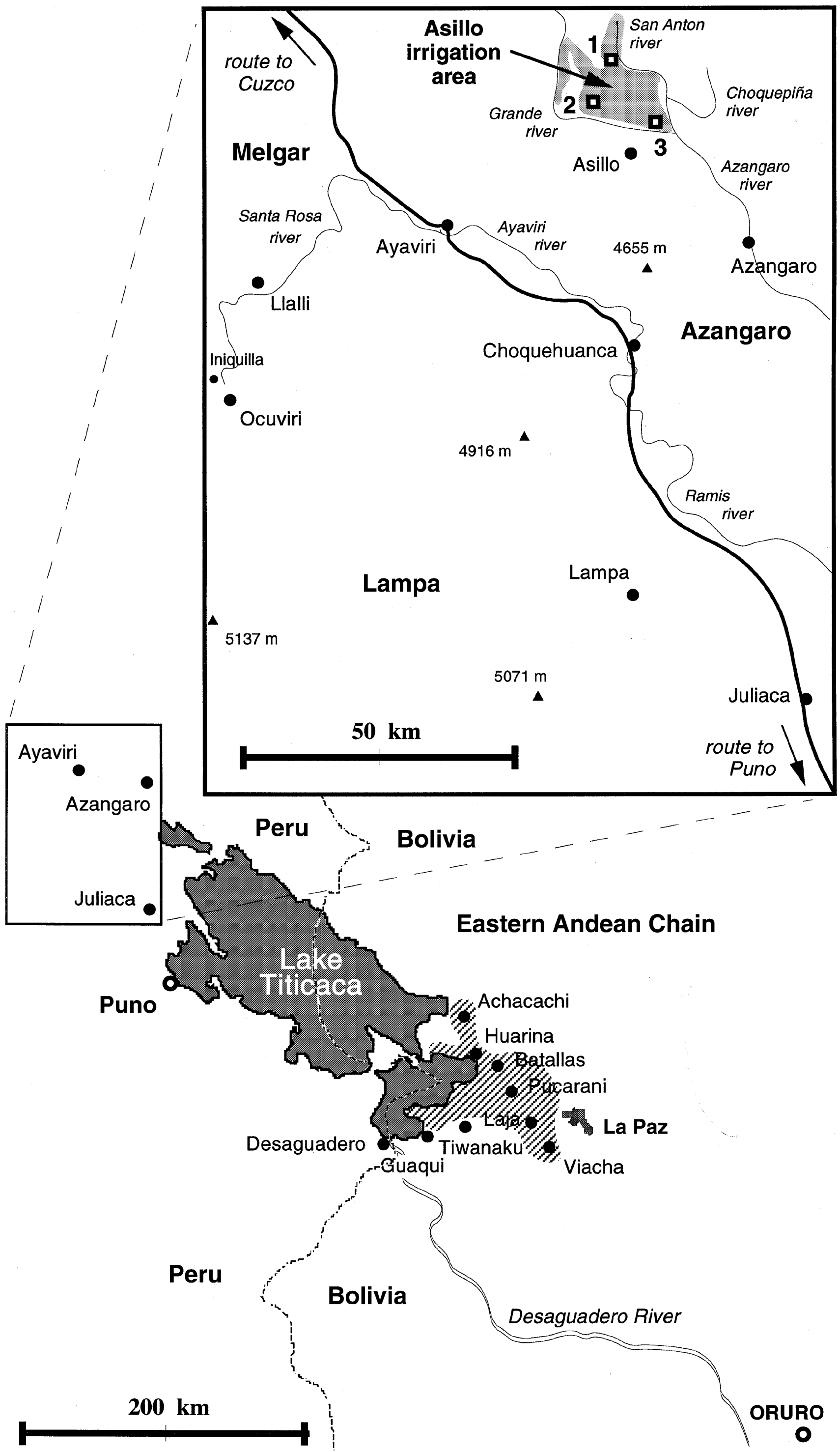

Figure 1 Map of the Peruvian and Bolivian zones of the Northern Altiplano showing the Asillo and the Bolivian human fascioliasis endemic areas near the Lake Titicaca. Localities studied: 1 = Jila; 2 = Accopata; 3 = Ñaupapampa.

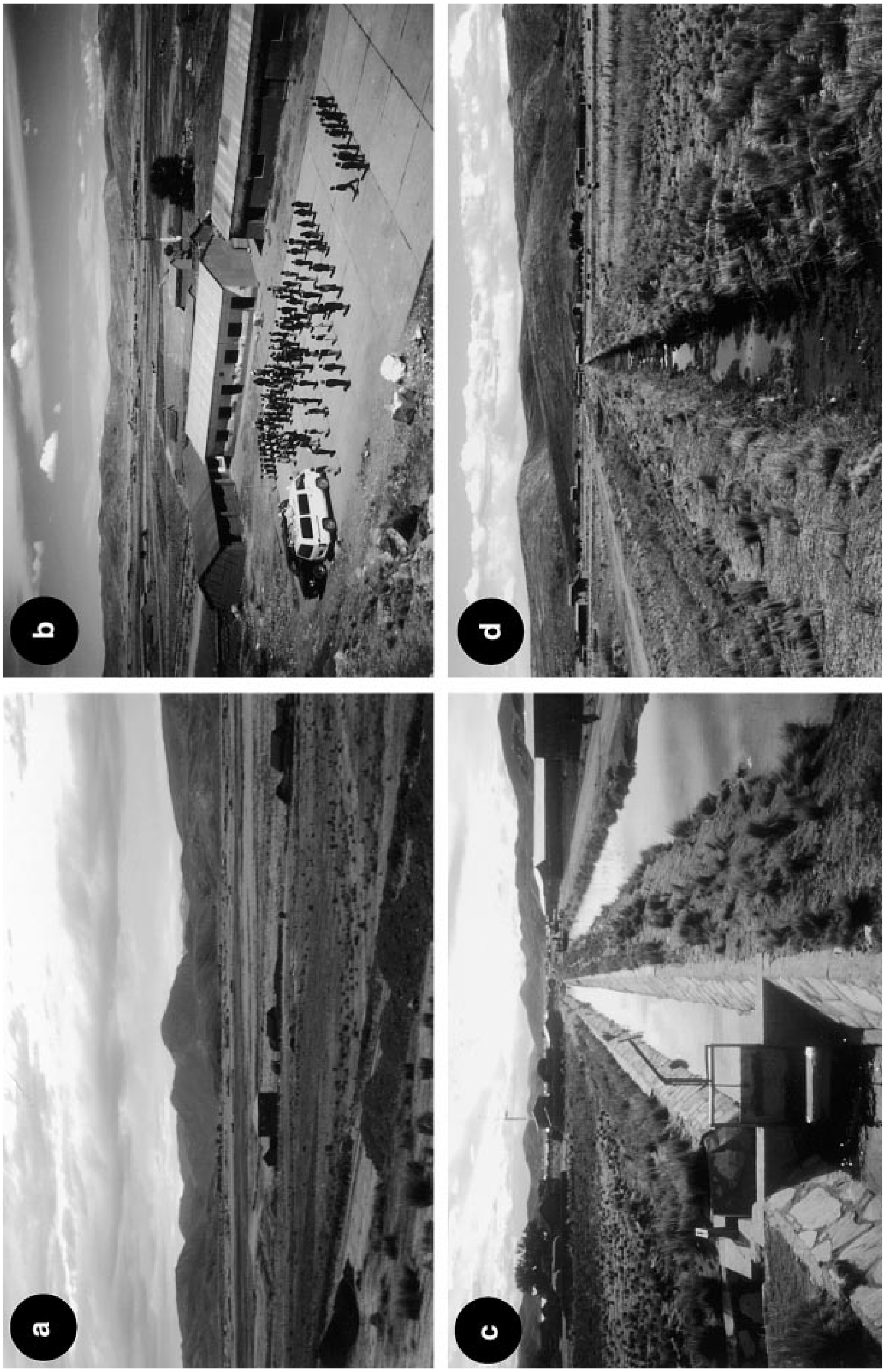

Figure 2 The man-made irrigation zone of Asillo: (a) a general view of the flat irrigation area; (b) school of Accopata with human fascioliasis endemic area in the background; (c) a main irrigation canal besides the school of Accopata showing the flood-gate which opens to a drainage channel; (d) near-by view of a drainage channel inhabited by lymnaeids and lacking aquatic plants.

concentration technique (Knight *et al.* 1976) and one aliquot of sediment obtained with this technique was stained using a modified Ziehl–Neelsen technique (Henriksen & Pohlenz 1981). The Kato-Katz slides were analysed for egg counts. Intensity of infection, measured as epg as an indicator of *F. hepatica* burden in infected subjects, was described by range, arithmetic mean and geometric mean. These three techniques are not comparable but complementary.

According to the techniques used, prevalence results of given helminth species may not be considered definitive, as in the cases of *Enterobius vermicularis* (anal swabs would be the adequate technique for the detection of the eggs of this nematode species) and ancylostomids, *Strongyloides stercoralis* and *Trichostrongylus* (the agar plate method would be better for the detection of the larvae of these geohelminths). But these techniques could not be used because of their methodological difficulties in field work, the lack of necessary infrastructure nearby and the peculiarities of the Altiplano inhabitants.

Statistical analysis

For the statistical analyses we used the SPSS 6.1 package for Windows, for statistical comparison of categorical variables, chi-square or Fisher's exact test. The Mann–Whitney *U*-test and Kruskal–Wallis (H) test were used for non-normally distributed data. Associations between *F. hepatica* infection and other parasite species were investigated by 2×2 contingency tables, from which the chi-square statistic was calculated. Values of $P < 0.05$ were taken as significant.

Institutional ethical review procedure

This study was conducted with the agreements of the authorities of the Asillo zone, the Universidad Nacional del Altiplano de Puno, Puno, and the Instituto de Medicina Tropical 'Daniel A. Carrión' of the Universidad Nacional Mayor de San Marcos, Lima, and was conducted in collaboration with the Parasitology Laboratory of the Faculty of Veterinary Medicine of Puno. All investigations were conducted with the consent of the local authorities of the communities, as well as of each school's director and teachers. Diagnostic results were sent to the Peruvian authorities responsible for child health; these health workers were charged with treatments.

Results

This coprological study revealed 10–11 protozoan and 9–11 helminth species. Prevalences in the surveys carried out in the three schools are shown in Table 1. All tested children were infected with at least one protozoan species and 54.4% with at least one helminth species. *Entamoeba coli* (91.4%) and *E. hartmanni* (90.8%) were the most common protozoans, while the liver fluke was the most prevalent helminth with an overall mean prevalence of 24.3%.

The overall prevalences and intensities of fascioliasis by schools, and according to sex and age groups are shown in Table 2. The highest prevalence of *F. hepatica* (31.3%) was recorded in Accopata, almost reaching statistical significance (chi-square = 5.8, $P = 0.055$). While prevalence rates did not significantly vary in relation to sex between and within schools, a statistically significant difference was found among the schools in 5–9-year-old children from Accopata compared with the same age group in the other schools (chi square = 6.4, $P = 0.041$). No statistical differences were found in intensities between schools, nor according to sex and age groups within schools, although the highest overall egg counts were detected in girls and in the youngest age group.

The results on *F. hepatica* infection intensity levels according to schools surveyed and to sex and age groups are shown in Table 2. Overall, males (chi square = 12.9; $P = 0.0003$) and the 10–15 years-old group (chi square = 8.7; $P = 0.003$) shed up to 100 epg, while females (chi-square = 16.0; $P = 0.001$) and 5–9 years-olds (chi-square = 31.1; $P = 0.001$) were shedding up to 400 epg. In Accopata, we found three 9 year olds excreting between 1800 and 2496 epg.

Fasciola hepatica always occurred as a coinfection with another parasite (Table 3). The highest number of coinfecting parasites, whether pathogenic or not, was detected in the 5–9 years-old group. The only significant positive association found was that between *F. hepatica* and *Giardia intestinalis* (chi-square = 9.7, $P = 0.0019$).

We detected numerous multiparasitism cases associated with *F. hepatica* infection. Up to 10 species per child, *F. hepatica* included, were found in the schools surveyed. Sixty-one percentage of the 82 *F. hepatica*-positive cases were infected with six or more species, for example a child with *F. hepatica*, *Entamoeba histolytica*/*E. dispar*, *G. intestinalis*, *Taenia* spp., *Trichuris trichiura* and *Ancylostoma duodenale*/*Necator americanus* in Jila, a child with *F. hepatica*, *G. intestinalis*, *Hymenolepis nana* and *T. trichiura* in Accopata, and child with *F. hepatica*, *G. intestinalis* and *Ascaris lumbricoides* in Ñaupapampa.

Discussion

Taking into account that only one coprological sample per child was analysed, the overall and particular fascioliasis prevalences detected in this study are among the highest

Table 1 Prevalences (%) of parasite species detected in the schools surveyed

Parasite species	Jila (n§ = 138)	Accopata (n = 131)	Ñaupapampa (n = 69)	Total (n = 338)
Protozoa	100.0	100.0	100.0	100.0
Entamoeba coli	92.8	93.9	84.1	91.4
*E. histolytica**	43.5	40.5	21.7	37.9
E. hartmanni	92.8	93.1	82.6	90.8
Endolimax nana	44.9	51.9	43.5	47.3
Iodamoeba buetschlii	59.4	54.2	46.4	54.7
Chilomastix mesnili	3.6	9.2	8.7	6.8
Giardia intestinalis	29.0	33.6	26.1	30.2
Enteromonas hominis	0.7	0.0	1.5	0.6
Blastocystis hominis	79.0	87.0	88.4	84.0
Balantidium coli	0.7	1.5	0.0	0.9
Helminths	55.1	58.8	44.9	54.4
Fasciola hepatica	20.3	31.3	18.8	24.3
Hymenolepis nana	13.8	23.7	10.1	16.9
Taenia spp.	0.7	0.0	0.0	0.3
Trichuris trichiura	20.3	21.4	8.7	18.3
Ascaris lumbricoides	14.5	1.5	7.3	8.0
Enterobius vermicularis†	0.7	2.3	1.5	1.5
Ancylostoma duodenale‡	1.5	0.0	0.0	0.6
Strongyloides stercoralis	1.5	0.0	0.0	0.6
Trichostrongylus sp.	2.2	2.3	1.5	2.1
Total	100.0	100.0	100.0	100.0

* And/or *Entamoeba dispar*.
† Detected in stool samples.
‡ And/or *Necator americanus*.
§ Number of school children studied.

known to date and characterize this area as hyperendemic (Mas-Coma *et al.* 1999b). Interestingly, in Asillo there were no significant differences between prevalences in males and females, which disagrees with results of other reports (Mas-Coma *et al.* 1999a). In high prevalence areas children under 15 usually present the highest rates (Esteban *et al.* 1999; Mas-Coma *et al.* 1999b), which is consistent with our results.

The very high egg counts, up to 2496 epg, are exceeded only by those found in people from the northern Bolivian Altiplano (up to 5064 epg) (Esteban *et al.* 1999). As in the Bolivian endemic zone, the higher *F. hepatica* egg counts in girls are surprising, an aspect which evidently requires further study, as do the relationships between such high epg levels and the symptoms and pathology of the infected children.

The Peruvian fascioliasis-hyperendemic zone studied is both geographically and epidemiologically isolated from the northern Bolivian Altiplano endemic area because of the aridity and absence of appropriate water bodies in the areas surrounding the Asillo zone and up to Lake Titicaca. The isolation of the Peruvian area is moreover confirmed by results obtained in the specific and molecular characterization of the intermediate snail hosts. Despite initial reports of two lymnaeid transmitting species (*Lymnaea viatrix* d'Orbigny 1835 and *L. cubensis* Pfeiffer 1839 variety) in the Northern Bolivian Altiplano (Ueno *et al.* 1975), only one transmitting species appears to be present. Shell morphology and visceral mass anatomy (Oviedo *et al.* 1995; Samadi *et al.* 2000), molecular (Bargues & Mas-Coma 1997; Bargues *et al.* 1997) and isoenzyme (Jabbour-Zahab *et al.* 1997) studies have proved that this species is the European *L. truncatula* (Müller 1774). In the Puno region, two lymnaeid species (*L. viatrix* and *L. diaphana* King 1830) have been described (Larrea *et al.* 1990, 1994; Oviedo *et al.* 1993), although only *L. viatrix* has been reported in the Asillo endemic zone (Hurtado *et al.* 1994). Similar to the Bolivian area, ribosomal DNA (rDNA) sequencing recently demonstrated that this specific classification was erroneous and that the lymnaeid species involved in fascioliasis transmission in the Asillo zone was in fact also *L. truncatula*. Interestingly, however, rDNA sequences of the Peruvian lymnaeid presented several mutations compared with the Bolivian snail (M.D. Bargues *et al.* unpublished data), which also corroborates the isolation of both endemic areas from one another.

The large spectrum of protozooses and helminthiases detected, several of recognized pathogenicity, forms a

Table 2 Prevalences and intensities of fascioliasis by sex and age groups in the schools surveyed

School	Prevalence % (CI)	Intensity (epg) Range	AM/G	< 100	101–400	401–1000	> 1000
Jila (n = 138)							
Sex							
Boys	23.4 (9.5)	24–1272	195/86	66.7	16.7	11.0	5.6
Girls	16.4 (9.3)	24–624	199/115	40.0	50.0	10.0	–
Age group (years)							
5–9	17.6 (8.7)	24–600	164/100	46.2	46.2	7.6	–
10–15	23.4 (10.4)	24–1272	224/92	66.7	13.3	13.3	6.7
Total	20.3 (6.7)	24–1272	196/96	57.1	28.6	10.7	3.6
Accopata (n = 131)							
Sex							
Boys	28.6 (10.1)	24–1008	196/96	54.6	31.8	9.1	4.5
Girls	35.2 (12.7)	24–2496	528/256	21.1	52.6	10.5	15.8
Age group							
5–9	34.0 (9.6)	24–2496	384/160	37.5	43.8	6.2	12.5
10–15	24.3 (13.8)	24–600	227/123	44.4	33.3	22.3	–
Total	31.3 (7.9)	24–2496	350/152	39.0	41.6	9.8	9.8
Ñaupapampa (n = 69)							
Sex							
Boys	14.3 (10.6)	48–624	180/111	66.6	16.7	16.7	–
Girls	25.9 (16.5)	24–1008	281/108	57.1	14.3	14.3	14.3
Age group							
5–9	21.1 (13.0)	24–1008	261/107	62.5	12.5	12.5	12.5
10–15	16.1 (12.9)	48–600	192/114	60.0	20.0	20.0	–
Total	18.8 (9.2)	24–1008	235/110	61.5	15.4	15.4	7.7
Total	24.3 (4.6)	24–2496	279/123	48.9	32.9	10.9	7.3

n = number of school children examined;% (CI) = percentage of infected children (95% confidence interval); epg = eggs per gram of faeces; AM/GM = arithmetic mean/geometric mean; <100 to >1000 = distribution of the intensity ranges in epg in the children infected, expressed as percentage of the total number of *Fasciola*-infected children in each school surveyed.

parasitic framework similar to that known in the northern Bolivian Altiplano (Esteban *et al.* 1997a, 1998b,c; Flores *et al.* 2001). However, the absence of *Cryptosporidium* compared with Bolivia and of *Cyclospora*, a well-known parasite in Peru, plus the detection of a very few *Strongyloides* and *Trichostrongylus* cases, both absent in the northern Bolivian Altiplano, are worth noting. The numerous multiparasitism cases reflect the poor hygienic conditions of the local population.

Both high altitude endemic areas share several physiographical and climatic characteristics because of being located in the northern Altiplano. But the natural conditions in the Bolivian zone are conducive to liver fluke development: meltwater from snow in the high mountains, subsoil effluences from shallow phreatic layers, small watercourses and rivers, flooding areas (the so-called *bofedales*), shallow wells, pools, etc. (Mas-Coma *et al.* 1999c), whereas the Peruvian zone is the result of a man-made irrigation area. Although it was built relatively recently, between 1956 and 1974, both liver fluke and lymnaeid snails have quickly colonized it (Mas-Coma 1998). Such man-made water resources carry a high risk to human health, as has been demonstrated around the world regarding the introduction, spread and increased transmission of schistosomiasis (Hunter *et al.* 1993; WHO 1993). Our results question the rationality of the irrigation projects of Iniquilla (from the Santa Rosa river, between Ocuviri and Llalli, near Ayaviri, in Melgar and Lampa provinces), already under construction in the Puno region, Peru, and of Huarina-Batallas in the Bolivian northern Altiplano (Libermann *et al.* 1987).

The significant association of *F. hepatica* with *G. intestinalis* agrees with that found between the same organisms in the northern Bolivian Altiplano (Esteban *et al.* 1997a). This finding in the Asillo zone suggests a similar transmission route and oral infection primarily through drinking water, and is supported by the following further evidence: typical aquatic vegetation is absent in the drainage channels inhabited by the lymnaeid snails, where children play; whereas aquatic plants are present in the

Table 3 Number of school children showing parasite species associations with and without *Fasciola hepatica* grouped by age group and sex (number of school children studied = 338; number of school children infected by *F. hepatica* = 82)

	With *F. hepatica*					Without *F. hepatica*
	5–9		10–15			
Parasite species	Male	Female	Male	Female	Total *n* (%)§	Total *n* (%)¶
Entamoeba coli	27	21	15	12	75 (91.5)	234 (91.4)
*E. histolytica**	11	5	9	2	27 (32.9)	101 (39.5)
E. hartmanni	25	21	15	8	69 (84.1)	238 (93.0)
E. nana	12	17	6	7	42 (51.2)	118 (46.1)
Iodamoeba buetschlii	19	14	12	2	47 (57.3)	138 (53.9)
Chilomastix mesnili	1	2	–	–	3 (3.7)	20 (7.8)
Giardia intestinalis	18	12	4	2	36 (43.9)	66 (25.8)
E. hominis	–	–	–	–	–	2 (0.8)
Blastocystis hominis	28	20	12	9	69 (84.1)	215 (84.0)
B. coli	–	–	–	–	–	3 (1.2)
H. nana	4	3	3	1	11 (13.4)	46 (18.0)
Taenia spp.	1	–	–	–	1 (1.2)	–
T. trichiura	4	2	5	6	17 (20.7)	45 (17.6)
A. lumbricoides	–	1	1	2	4 (4.9)	23 (9.0)
E. vermicularis†	–	–	–	–	–	5 (2.0)
A. duodenale‡	1	–	–	–	1 (1.2)	1 (0.4)
S. stercoralis	–	1	–	–	1 (1.2)	1 (0.4)
Trichostrongylus sp.	1	–	–	1	2 (2.4)	5 (2.0)

* And/or *E. dispar*.
† Detected in stool samples.
‡ And/or *N. americanus*.
§ Number of children coinfected with each species and *F. hepatica* (percentage related to the total number of positive children with *Fasciola* infection, given in parenthesis).
¶ Number of children infected with each species but not with *F. hepatica* (percentage related to the total number of children without *Fasciola* infection, given in parenthesis).

main irrigation canals which permanently conduct water. The small lateral channels are only flooded when needed, not enabling aquatic plants to grow (Fig. 2). Peruvian Quechua inhabitants of the irrigation zone mentioned that aquatic plants are not included in their diet, contrary to Bolivian Aymaras of the northern Bolivian Altiplano (Mas-Coma *et al.* 1999c). The few vegetables produced and consumed by the inhabitants of the Altiplano are tubers, leguminoses or cereals whose edible parts are not contaminated by irrigation water. Human fascioliasis cases among American communities not including aquatic vegetables in their traditional diet was noted by Hillyer and Apt (1997); floating metacercariae could cause infection (Bargues *et al.* 1996) in these circumstances. Because of the absence of piped drinking water in their houses, inhabitants obtain water from irrigation canals and drainage channels for all their needs (drinking, cooking, personal hygiene, cleaning and washing); according to responses obtained in a general survey, 97.9% of the inhabitants drink water from the irrigation resources and only 2.1% get their drinking water from wells. The small lateral drainage channels are usually located near the dwellings and frequently visited by livestock, mainly cattle and also sheep (Fig. 2). Previous surveys in the irrigation zone showed prevalences of 25 and 36% and estimated mortalities of 12 and 60% in cattle and sheep, respectively (C. Sánchez, unpublished data). Infection through drinking water would also partially agree with the intensities detected, which are relatively low considering the high prevalences compared with other highly endemic areas where infection also occurs through consumption of contaminated aquatic vegetables (Esteban *et al.* 1999; Mas-Coma *et al.* 1999c). The use of water from lymnaeid-infested drainage channels near the houses would also explain why infection intensity differences between boys and girls were less pronounced than in other endemic areas, despite the gender role differences in the family organization of these Quechua communities, where females are in charge of grazing livestock.

The most urgent control measures to be implemented in this area, which differ from those used in case of the typical contamination by eating freshwater vegetables, are basic services such as piped drinking water and sewage disposal, and prevention of livestock defecating in the canals.

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