

## EVALUATION OF FAS2-ELISA FOR THE SEROLOGICAL DETECTION OF *FASCIOLA HEPATICA* INFECTION IN HUMANS

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**Abstract.** The performance of Fas2-ELISA for the diagnosis of *Fasciola hepatica* infection in children living in areas of high endemicity for fascioliasis in the Peruvian Andes is analyzed. Fas2-ELISA is based on the detection of circulating IgG antibodies elicited in infected individuals against a *F. hepatica* antigen termed Fas2. The study was conducted in three Andean localities, Huertas-Julcan in Junin, Asillo in Puno, and Cajamarca, with a total population of 634 children in an age range 1 to 16 years old. Child fascioliasis prevalence was 21.1% in Huertas-Julcan, 25.4% in Asillo, and 24% in Cajamarca, estimated by coprological inspection. The seroprevalence of *F. hepatica* infection, determined by Fas2-ELISA, was 27.8% in Huertas-Julcan, 44.6% in Asillo, and 29.1% in Cajamarca. The overall sensitivity of Fas2-ELISA was 92.4%, the specificity 83.6%, and the negative predictive value 97.2%. No association between OD<sub>450</sub> Fas2-ELISA and infection intensity measured by egg counting was observed. 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.

### INTRODUCTION

Human fascioliasis was regarded as a secondary disease until the mid 1990s.<sup>1,2</sup> However, this inappropriate status that underestimated its importance changed recently by the identification of human endemic areas<sup>3</sup> and the increasing number of reports of human infection in 51 countries of the five continents.<sup>4</sup> Studies carried out in recent years have shown that this infection is a serious public health problem worldwide,<sup>5</sup> with a rate of human fascioliasis ranging from 2.4 million to 17 million people.<sup>6,7</sup> The major health problems are known in Andean countries as Bolivia and Peru.<sup>8,9</sup> Fascioliasis, especially *Fasciola hepatica*, has a high spreading power thanks to a large colonization capacity of the causal agents and vector species and is at present emerging or re-emerging in many countries.<sup>10</sup>

The disease includes four clinical periods: (1) incubation phase (from the ingestion of metacercariae to the appearance of the first symptoms), (2) invasive or acute phase (fluke migration up to the bile ducts) with fever, abdominal pain, gastrointestinal disturbances, urticaria, respiratory symptoms, hepatomegaly and splenomegaly, ascites, anemia, chest signs and jaundice, (3) latent phase (maturation of the parasites and starting of oviposition) with eosinophilia, gastrointestinal complaints, and relapses of the acute symptoms, and (4) obstructive or chronic phase.<sup>4</sup> In the latter, the disease manifests a range of complications such as bile duct obstruction, cholecystitis, cholangitis, liver abscess, hemorrhage, lithiasis, and cirrhosis.<sup>1,11-14</sup> Moreover, the chronic stage may be asymptomatic and extended for years if the disease remains untreated, as is usual in human endemic areas. The evidence points out that this parasite might cause a severe liver impairment in infected humans.

This situation is aggravated by the limitations of the current diagnosis methods.<sup>15</sup> The microscopic detection of *F. hepatica*

eggs in feces is considered as the definitive diagnosis of human fascioliasis.<sup>4,15</sup> However, the parasitological diagnosis is not always sensitive because erratic and acute infections pass undetected, situations of eggs in transit may give erroneous results, the egg output dynamics in humans is unknown, and infections by immature flukes in non-human endemic areas are not detected.<sup>4</sup> Due to the limitations of the parasitological diagnosis, standardized indirect tests are urgently needed for both individual patient diagnosis and epidemiologic surveys in human endemic areas.

Antibody response detection is clearly the method of choice for the immunodiagnosis of fascioliasis.<sup>16</sup> Serological tests have proven their usefulness for human fascioliasis since long ago,<sup>17-19</sup> although there are still disadvantages as the lack of commercially available defined, specific antigens, test systems, and no consensus concerning the optimal test system for the serologic analysis of human fascioliasis. Today, a good test should be useful for a rapid screening of people living in human endemic areas, as well as to diagnose occasional liver fluke infections in patients from non-human endemic areas.

In the present work we present the evaluation of Fas2-ELISA as a serological technique to detect *F. hepatica* infection in humans from human endemic areas. Fas2-ELISA is based on the IgG antibody capture by a purified protein Fas2, which is an immunodominant antigen in the human infection.<sup>18</sup>

### MATERIALS AND METHODS

**Study sites.** The Andean valleys of Cajamarca and Jauja are located at an altitude of 2670 m and 3420 m in the North and Center of Peru respectively, and the village of Asillo in Puno is located in the South of Peru at an altitude of 3909 m. These areas are cold and have at least two well-defined seasons: rainy from December to May and dry from June to December. The sites were selected because of demonstrated endemicity for fascioliasis reported previously.<sup>9,20,21</sup> An expedition to the villages of Huertas and Julcan, Province of Jauja, Junin, was organized by the team from Universidad Cayetano Heredia and samples collected in December 2000. A second

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expedition was organized by the same team to Asillo, Province of Azangaro, Puno, and samples collected in March 2001. The third expedition organized by the team of Universidad de Valencia, Spain, collected samples in the districts of Santa Rosa de Chaquil, El Rescate-La Collpa, and Yanamango-Jesus in Cajamarca in September 2001. The houses in these villages are adobe with no sanitation facilities; a limited supply of potable water is available but no sewage and waste disposal. The economic activity of the population is predominantly agriculture. Parents were invited to enroll their children in the study after being informed about the project. *F. hepatica*-infected children in Junin and Puno, positive to fluke eggs in feces, received a 10 mg/kg single dose of triclabendazole under medical observation. Children under flukicide treatment were clinically supervised until becoming copro-negatives for *F. hepatica* eggs. In Cajamarca, diagnostic results were furnished to the Cajamarca Regional Health Office for appropriate individual treatments.

**Serum and stool samples.** The census form for villagers included identification (last name, first name, age, and sex). The study participants were mostly schoolchildren from three villages in the Peruvian Andes. A total of 161 stool samples and 144 serum samples were collected in Huertas-Julcan, 236 stool and 232 serum samples from children of Asillo, and 237 stool and blood samples in Cajamarca. All fecal samples were preserved in 10% formalin solution and those from Huertas-Julcan and Asillo transported to the Institute of Tropical Medicine Alexander von Humboldt in Lima, whereas stool samples from Cajamarca were evaluated at the Department of Parasitology, Universidad de Valencia. Serum samples were transported to the laboratory and kept at  $-20^{\circ}\text{C}$  until analysis.

**Parasitological analysis.** The rapid sedimentation procedure was used to process fecal samples from Huertas-Julcan and Asillo.<sup>22</sup> Samples from Cajamarca were processed by a formol-ether concentration technique.<sup>23</sup> The parasitological inspection was done under microscope by a trained parasitologist. Egg count was performed by the Kato-Katz methodology.<sup>24</sup>

**Fas2 preparation.** Fas2 was purified from the regurgitated material produced by *F. hepatica* adult worms when placed in cold water for 2 hours. The suspension was then centrifuged at  $7000 \times g$  for 10 min. The supernatant was lyophilized and stored at  $-20^{\circ}\text{C}$ . A total of 250 mg of this material was resuspended in 0.2 M sodium citrate buffer, pH 4.9, containing 0.1 mM  $\text{HgCl}_2$  at  $4^{\circ}\text{C}$ . Chilled ethanol (98%) was added to a final concentration of 60% and then incubated for 15 min on ice.

The suspension was centrifuged at  $4^{\circ}\text{C}$ ,  $12,000 \times g$  for 10 min. The supernatant was dialyzed in 10 mM of sodium citrate buffer containing 0.1 mM  $\text{HgCl}_2$  at  $4^{\circ}\text{C}$ , in dialysis membranes (cut-off 12 kDa), then fractionated in carboxymethyl sephadex C-50 column (1.8 cm  $\times$  35 cm) previously equilibrated in 10 mM sodium citrate buffer, pH 4.9, containing 0.1 mM  $\text{HgCl}_2$ . Proteins were eluted with a gradient of NaCl (0–1 M) in citrate buffer. Fractions with proteolytic activity were lyophilized and stored at  $-20^{\circ}\text{C}$ . Fas2 eluted as a single protein peak with 0.6 M NaCl.<sup>25</sup>

**Fas2-ELISA.** Fas2-ELISA was done as previously described,<sup>16</sup> but with modifications. Fas2 (300 ng/well) was bound to microtiter plates IMMULON®4HBX (Dynex Technologies, Inc., Chantilly, VA) by incubation for 16 hours at  $4^{\circ}\text{C}$ . Plates were washed five times in PBS with 0.05% Tween 20 (PBST) and then incubated in 2% BSA in PBST (PBSTB) for 1 hour at  $37^{\circ}\text{C}$ . One hundred  $\mu\text{L}$  of serum that was previously diluted 1/500 in PBSTB was added to the well and incubated for 1 hour at  $37^{\circ}\text{C}$ . Plates were thoroughly washed five times in PBST. An amount of 100  $\mu\text{L}$  of goat anti-human IgG conjugated to horseradish peroxidase, previously diluted to 1/2000 in PBSTB, was added to each well and incubated for 1 hour at  $37^{\circ}\text{C}$ . A color reaction developed when TMB (3, 3', 5, 5'-tetramethyl-benzidine) was incorporated into the reaction, which was stopped by adding 50  $\mu\text{L}$  of 2M  $\text{H}_2\text{SO}_4$ . The optical density was measured at 450 nm ( $\text{OD}_{450}$ ) in a microplate reader (Benchmark Bio-Rad).

**Statistical analysis.** The statistical analysis was done using SPSS 9.0 for Windows (SPSS Inc., Chicago, IL). Logistic analysis was performed to determine risk factors associated to *F. hepatica* infection. The sensitivity, specificity, and predictive values for Fas2-ELISA were estimated in a  $2 \times 2$  contingency table.<sup>26</sup> A *P* value  $< 0.01$  was taken as statistically significant. The  $\text{OD}_{450}$  cut-off value was set as the mean  $\text{OD}_{450}$  for Fas2-ELISA of serum samples from 46 healthy volunteers plus two times the value of the standard deviation.

**Ethical aspects.** The Ethical Committee of the Universidad Peruana Cayetano Heredia approved the present project. Children were enrolled in the study after acceptance of the parents by a signed consent.

## RESULTS

Fascioliasis is the most prevalent helminth infection in children in the Andean villages studied here. Coprological examination of the children population resulted in prevalences of 21.1% for Huertas-Julcan, 25.4% for Asillo in Puno, and 24.1% for Cajamarca (Table 1). Other high prevalent hel-

TABLE 1  
Prevalence of helminth infection and intestinal protozoa at study sites

Locality (n)	Helminth infection							
	<i>A. duodenale</i>	<i>A. lumbricoides</i>	<i>E. vermicularis</i>	<i>F. hepatica</i>	<i>H. nana</i>	<i>S. stercolaris</i>	<i>Taenia</i> spp.	<i>T. trichiura</i>
Huertas-Julcan (161)	0.6 (1)	9.9 (16)	3.1 (5)	21.1 (34)	18.6 (30)	1.2 (2)	0.6 (1)	11.8 (19)
Asillo (236)	0 (0)	11 (26)	2.5 (6)	25.4 (60)	7.6 (18)	2.1 (5)	0 (0)	4.7 (11)
Cajamarca (237)	0 (0)	18.1 (43)	3.4 (8)	24.1 (57)	13.1 (31)	0 (0)	1.3 (3)	6.8 (16)
Locality (n)	Intestinal protozoa							
	<i>B. hominis</i>	<i>C. mesnili</i>	<i>E. coli</i>	<i>E. nana</i>	<i>E. histolytica/dispar</i>	<i>G. intestinalis</i>	<i>I. bütschlii</i>	
Huertas-Julcan (161)	62.7 (101)	13.7 (22)	93.2 (150)	71.4 (115)	3.7 (6)	34.8 (56)	42.9 (69)	
Asillo (236)	31.8 (75)	8.9 (21)	85.6 (202)	66.9 (158)	11.4 (27)	22.5 (53)	35.6 (84)	
Cajamarca (237)	72.2 (171)	5.5 (13)	76.4 (181)	52.3 (124)	35.9 (85)	25.3 (60)	39.7 (94)	

TABLE 2

Logistic analysis of risk factors associated to *Fasciola hepatica* infection

Factor	Variable	OR	CI 95%	P value
Age	Range in years*			
	6-8	2.7143	0.89-8.22	0.0776
	9-11	5.1025	1.73-15.02	0.0031
	≥ 12	4.3419	1.36-13.80	0.0129
Infection	<i>A. lumbricoides</i> *	3.6869	1.50-9.02	0.0043
	+ 1 helminth†	1.3795	0.56-3.40	0.4846
	+ 2-3 helminths†	5.0579	1.66-15.34	0.0042

\* In Asillo.

† In relation to *F. hepatica* infected with no other helminth infection in Huertas-Julcan.

minthiasis included *Ascaris lumbricoides* (9.9%–18.1%) and *Hymenolepis nana* (7.6%–18.6%), *Trichuris trichiura* (4.7%–11.8%), and *Enterobius vermicularis* (2.5%–3.4%). The prevalence of mixed helminth infections was 14.5%, 17.3%, and 15.3% for Huertas-Julcan, Asillo, and Cajamarca, respectively. Concurrent infection with three different helminth species was detected in eight children in Huertas-Julcan, in three children in Asillo, and in six children in Cajamarca, being *F. hepatica* with either *A. lumbricoides* or with *H. nana*, a common co-infection observed (data not shown).

A high prevalence of pathogenic protozoan infection was also observed in the children (Table 1). In Asillo, age and *A. lumbricoides* infection resulted in risk factors to *F. hepatica* infection. Children in the age range 9 to 11 years old are at higher risk (5 times) than children in the age range 1 to 6 years old. In addition, fascioliasis was shown to be associated with *A. lumbricoides* infection in Asillo and co-infection with ≥ 2 helminths associated with higher risk to *F. hepatica* infection in Huertas-Julcan (Table 2). No significant association was observed of *F. hepatica* infection to protozoan species.

The infection is acquired early in life in these endemic localities, 16.15% of the children in the age range 1 to 5 years old being copropositives for *F. hepatica*. A steady increase of the prevalence in relation to age is observed in the whole population and in males (Table 3). No similar pattern is observed with the seroprevalence estimated by Fas2-ELISA in relation to age or gender (Table 3). The seroprevalence reaches a plateau in the population earlier than the prevalence estimated by coprology.

Overall seroprevalence by Fas2-ELISA resulted in 34.5% of the total population of 626 children (Table 4). Asillo had

TABLE 3

Prevalence of fascioliasis by age and gender

Age range	Sex (n)	Coprology + (n)	Fas2 + (n)
1-5	♂ (31)	9.7% (3)	30% (9/30)
	♀ (31)	22.6% (7)	48.4% (15)
6-8	Grouped (61)	16.1% (10)	39.3% (24)
	♂ (84)	20.2% (17)	33.7% (28/83)
9-11	♀ (86)	22.1% (19)	30.2% (26)
	Grouped (169)	21.2% (36)	32% (54)
≥ 12	♂ (81)	25.9% (21)	31.6% (25/79)
	♀ (76)	31.6% (24)	43.4% (33)
Grouped (155)	♂ (54)	28.7% (45)	37.4% (58)
	♀ (30)	37% (20)	47.2% (25/53)
Grouped (83)	♂ (54)	20% (6)	36.7% (11)
	♀ (30)	31% (26)	43.4% (36)

PPV = positive predictive value; NPV = negative predictive value.

TABLE 4

Seroprevalence of *Fasciola hepatica* infection and performance characteristics of Fas2-ELISA by study site

Locality (n)	Prevalence % (n)	Sensitivity %	Specificity %	PPV %	NPV %
Huertas-Julcan (158)	27.8 (44)	92.6	89.6	69.4	97.9
Asillo (231)	44.6 (103)	88.3	70.8	51.5	94.5
Cajamarca (237)	29.1 (69)	96.5	92.2	79.7	98.8
Total (626)	34.5 (216)	92.4	83.6	63.9	97.2

PPV = positive predictive value; NPV = negative predictive value.

the highest rate of seroprevalence, 44.6%, whereas it was lower in Huertas-Julcan and Cajamarca (27.8% and 29.1%, respectively). Fas2-ELISA estimation of fascioliasis in children resulted in higher value of prevalence than those estimated by coprological inspection (Table 3). Fas2-ELISA had the highest strength of agreement with coprology in Cajamarca and Huertas-Julcan ( $\kappa = 0.828$  and  $\kappa = 0.731$ , respectively). The strength of agreement between techniques was good in Asillo ( $\kappa = 0.659$ ).

Diagnostic parameters of Fas2-ELISA were estimated by choosing coprology as the “gold standard” assay to detect *F. hepatica* infection in humans. Overall sensitivity of Fas2-ELISA was 92.4%, but the sensitivity values were higher in Cajamarca and Huertas-Julcan than in Asillo. The sensitivity of Fas2-ELISA in Cajamarca was 96.5%, in Huertas-Julcan it was 92.6%, and in Asillo 88.3%. Fas2-ELISA specificity for Cajamarca was 92.2%, in Huertas-Julcan 89.6%, and in Asillo 70.8% (Table 4).

The performance diagnostic characteristics of Fas2-ELISA in relation to age and gender are shown in Table 5. Sensitivity shows a pattern of steadily decrease in relation to age, the highest value (100%) being observed in the age range 1 to 5 years old and the lowest in the age range ≥ 12 years old (84.6%); a similar trend in relation to sensitivity and age-sex is observed between groups (data not shown). No similar pattern was observed with other performance characteristics in relation to age or sex, with the exception of the negative predictive value that decreases in relation to age.

The population of a fascioliasis endemic area can be grouped as result of the parasitological and Fas2-ELISA serological evaluation as follows: copronegatives/seronegatives; copronegatives/seropositives; copropositives/seropositives; and copropositives/seronegatives. Population distribution in Junin, Asillo, and Cajamarca was statistically different ( $P < 0.01$ ), with more copronegatives/seropositives in Asillo than in Huertas-Julcan and Cajamarca (Figures 1, 2, and 3). No statistical difference was observed in Fas2-ELISA OD<sub>450</sub> val-

TABLE 5

Performance characteristics for Fas2-ELISA by age and gender

	Sensitivity	Specificity	PPV	NPV
Age range (n)				
1-5 (61)	100%	72.5	41.7	100
6-8 (169)	94.4%	85	63	98.3
9-11 (155)	93.3%	85.5	72.4	96.9
≥ 12 (83)	84.6%	75.4	61.1	91.5
Gender (n)				
Female (223)	98.2	82	64.7	99.3
Male (245)	86.9	81.5	60.9	94.9

PPV = positive predictive value; NPV = negative predictive value.

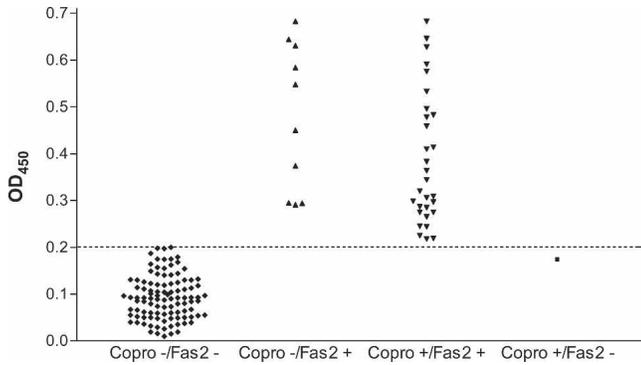


FIGURE 1. Fas2-ELISA with sera from children that provided a least one stool and blood sample ( $N = 144$ ) from Huertas-Julcan. Data points represent the mean absorbance at 450 nm obtained from three replicates of each serum tested. The dotted line represents the cut-off value 0.2 units of OD at 450 nm.

ues between copronegative/seropositives and copropositives/seropositives (data not shown). No correlation of egg output, which is a measure of intensity of the infection, to OD<sub>450</sub> value of Fas2-ELISA was observed at the two study sites (Figure 4).

DISCUSSION

To explore the feasibility of the application of Fas2-ELISA in localities previously reported as having high rates of human and animal infection,<sup>9,20,21</sup> we evaluated the performance characteristics of Fas2-ELISA in a population of 634 schoolchildren, sampled at random, in the age range 1 to 16 years old in Huertas-Julcan, Asillo, and Cajamarca. The results of the coprology and serology surveys confirmed the character of hyper-endemicity for *F. hepatica* infection in these Peruvian villages. The children population is ridden infected with pathogenic intestinal parasites, helminths, and protozoa, as a result of the poor sanitary condition prevailing in these localities. All tested children were at least infected with one protozoon and 46.4% with at least one helminth species. *Fasciola hepatica* infection was the most prevalent helminth infection in the children population present as single or mixed

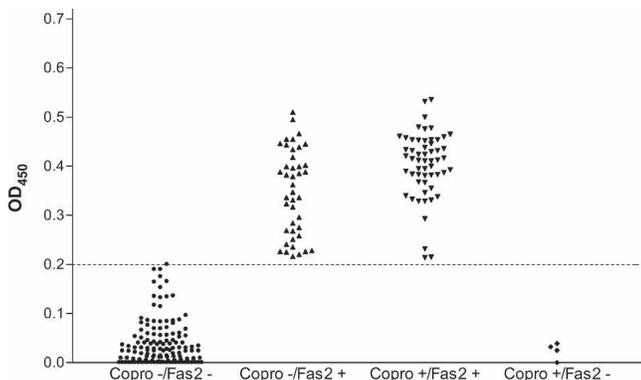


FIGURE 2. Fas2-ELISA with sera from children that provided a least one stool and blood sample ( $N = 232$ ) from Asillo. Data points represent the mean absorbance at 450 nm obtained from three replicates of each serum tested. The dotted line represents the cut-off value 0.2 units of OD at 450 nm.

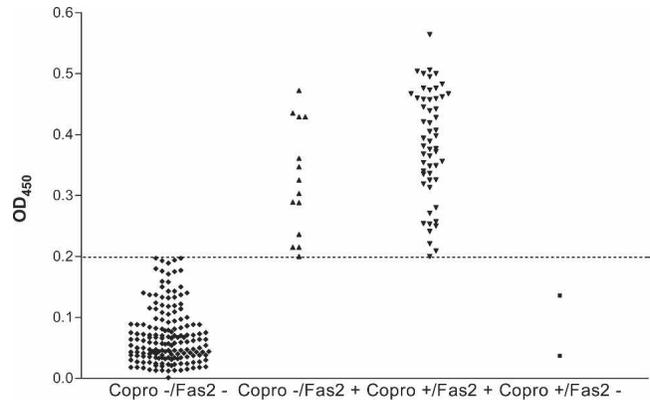


FIGURE 3. Fas2-ELISA with sera from children that provided a least one stool and blood sample ( $N = 237$ ) from Cajamarca. Data points represent the mean absorbance at 450 nm obtained from three replicates of each serum tested. The dotted line represents the cut-off value 0.2 units of OD at 450 nm.

infections. *F. hepatica* infection appeared to be associated to *A. lumbricoides* infection in Asillo and children bearing more than two co-infecting helminth were at higher risk for *F. hepatica* infection in Huertas-Julcan. The cause of this association is not clear but it may reflect the particular transmission pattern of the infection at each locality. In Asillo, drinking contaminated water from water channels infested with floating infective forms is incriminated as the route of transmission of *F. hepatica* infection.<sup>9</sup>

A high proportion of schoolchildren were positive to both serology and coprology for fascioliasis, the prevalence for *F. hepatica* infection ranged from 21.1%–25.4%, as estimated by parasitologic inspection of a single stool specimen. On the other hand, seroprevalence for *F. hepatica* determined by Fas2-ELISA ranged 27.8%–44.6%. Coprological detection appears to underscore real prevalence of *F. hepatica* infection assessed by serological procedures, as reported by others.<sup>17,27</sup> The largest difference is observed in the children of either sex in the age range 1 to 5 years old. Besides the much discussed factors that may account for the discordant results between

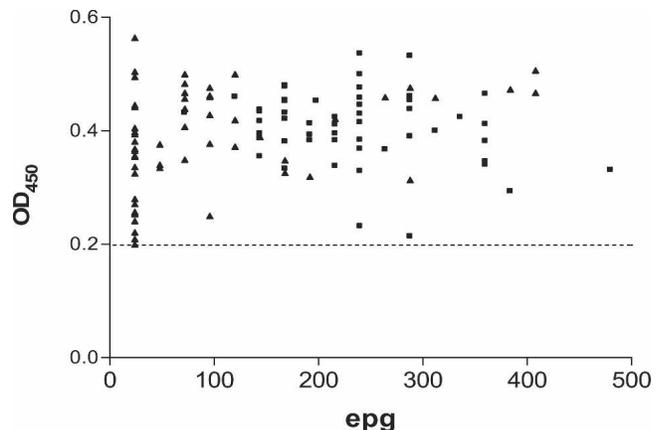


FIGURE 4. Fas2-ELISA and intensity of *Fasciola hepatica* infection. Data points represent the mean absorbance at 450 nm from copropositive children from Asillo and Cajamarca. epg represents the egg count per gram of stool. The dotted line represents the cut-off value 0.2 units of OD at 450 nm for Fas2-ELISA.

coprology and serology,<sup>15</sup> the passive immunity transfer of anti Fas2 IgGs by the colostrums of infected mothers can be additionally considered in this group. Anti *F. hepatica* antigens IgGs have been detected in the milk produced by infected animals.<sup>28</sup>

Antibody response methods are criticized because of not discriminating between active and resolved infections, which are cases particularly frequent in endemic areas.<sup>29</sup> A feature also observed in the serology survey by Fas2-ELISA of these children, where a high proportion of copronegative/seropositives were detected (7%–18%), whether they are individuals bearing an active or resolved infection, cases of flawed diagnosis by microscopy or serology deserves further attention. These individuals pose a serious problem in the decision to proceed with drug treatment or to ask for confirmatory diagnosis test, either by inspection of repeated stool samples or serology. *F. hepatica*-infected animals raise circulating specific anti Fas2 IgG antibodies allowing the detection of the disease as early as 10 days post infection by Fas2-ELISA,<sup>14</sup> suggesting that the assay can detect the human infection in its early acute phase. On the other hand, anti-Fas2 IgGs are short-lived with an estimated half-life of less than 6 months (Espinoza and others, unpublished results), which suggests that a proportion of copronegative/seropositive individuals, if not in acute infection, bear a recent resolved episode of fascioliasis, a clinical complication that obstructs the egg shedding or an erratic infection. Nonetheless, cases of flawed diagnosis by microscopy or serology might not be ruled out. A low proportion of the population (1.12%) was copropositive/seronegative; whether they are subjects with eggs in transit by consumption of contaminated livers or that show anti-Fas2 antibody unresponsiveness due to long-term chronic infection, or another occult cause is not known.

The overall high sensitivity of Fas2-ELISA reported here has precedents in previous reports of cysteine proteinase-based laboratory assays for the detection of fascioliasis.<sup>16,18,19,30</sup> The present study, though, tested Fas2-ELISA in a population endemically exposed to *F. hepatica* and other helminths and pathogenic protozoans. The 92.5% sensitivity found for Fas2-ELISA in the present study is consistent with a previous estimation of 95% sensitivity for the same assay obtained with a different sample population assayed in laboratory conditions,<sup>16</sup> suggesting that Fas2-ELISA is a robust assay that can detect the infection with different parasite burden.

The sensitivity of Fas2-ELISA showed a decline in relation to the age of the population. In the same context, the specificity of Fas2-ELISA depends on correctly assessing false positives, so taking coprology as “gold standard” to assess the performance diagnostic for fascioliasis is limited by the nature of *F. hepatica* infection with an acute phase that can last for months, the intermittence in egg shedding, and other clinical complications produced by the infection.<sup>15</sup> The infection is acquired early in life in these endemic localities, and the risk of infection increases with age. The high transmission index of the human infection implies that re-infection is probably not uncommon as the population grows older. The decrease in sensitivity of Fas2-ELISA in relation to age might be caused by both the natural decline in antibody response observed subjects with a long-term infection and the intermittence in egg shedding observed in patients in the chronic phase (Mas-Coma and others, unpublished).

There seems to be a wide acceptance that parasite 24–28 kDa cysteine proteinases isolated from E/S are sensitive and specific markers for the serodiagnosis of human infection by *F. hepatica*.<sup>16,18,27,31,32</sup> Among them, Fas2 is a well-characterized antigen; its immunogenicity is proven in human and animal infection, which makes it very promising for the development of a standard screen test of antibody-detection for this infection. Field screening of *F. hepatica* infection using Fas2-ELISA provides evidence of the clinical potential of this assay to diagnose fascioliasis in humans exposed to the liver fluke infection in endemic areas. This standard assay is expected to be useful as a first line test for field screening for fascioliasis in people living in endemic areas and for detecting occasionally *F. hepatica*-infected patients in clinical laboratories.

Human fascioliasis is an important public health problem in endemic areas of the Peruvian Andes and other regions in the world,<sup>33,34</sup> as demonstrated by a high proportion of individuals positive to this infection by serology and/or coprology. No major improvement in sanitation in poor rural areas is expected in the near future, so control programs are urgently needed to deal with this endemic disease. The present study provides evidence of the potential of Fas2-ELISA as a tool to screen for fascioliasis in human populations resident in human endemic areas. Fas2-ELISA might be implemented as a standard assay to both field screening for fascioliasis in people living in endemic areas, as well as for the detection of occasional *F. hepatica*-infected patients in clinical laboratories.

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