Early Postnatal and Preschool-Age Infection by *Fasciola* spp.: Report of Five Cases from Vietnam and Worldwide Review

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Abstract. Fascioliasis is reported in five Vietnamese children aged 4 years or younger. A 10-month-old girl child and a 12-month-old boy child are the youngest patients ever diagnosed. Eggs in stools suggested an infection occurred at 5–6 months and 7–8 months of age, respectively. DNA sequencing and egg size indicated this to be the first report of a verified *Fasciola gigantica* infection in so small children. No specific diagnosis could be obtained in two 3-year-old children detected in the acute phase. A big and gravid ectopic *F. gigantica*-like worm was surgically found in a 4-year-old boy presenting with peritonitis. A worldwide review showed only 38 past cases in preschool children. They included 3, 7, 12, and 16 cases of 1, 2, 3, and 4 years, respectively, with a faster infection increase in males from 2 years onward. Reports were from all continents, except Oceania, including severe complications and death. The causal agent, when specifically diagnosed, was always *Fasciola hepatica*. Analyses include detection in hospital, surveys, and family outbreaks; infection sources; disease phases; parasite burden; ectopic cases; symptom onset; eosinophilia; biochemical markers; and clinical complications. C-reactive protein, creatinine, and γ -glutamyl transferase are the most useful biomarkers. A serological test and a coprological analysis are recommended for so small children, in which typical symptoms may be overlooked. Treatment problems were described with many drugs, except triclabendazole. Triclabendazole should be considered the drug of choice for such small children. The possibility of a very early infection by *Fasciola* spp. should be henceforth considered.

INTRODUCTION

Fluke diseases are included in the groups of schistosomiasis and food-borne trematodiasis among the list of neglected tropical diseases by the WHO.¹ At present, these snail-borne helminthiasis are in the spotlight because of the climate and global change impacts on these diseases, from geographical spread,² outbreaks, and transmission seasonality modifications³ up to prevalence and intensity alterations.⁴

Fascioliasis is highlighted because of its almost worldwide distribution⁵ and potential extreme pathogenicity.⁶ This zoonotic disease is caused by two liver fluke species: *Fasciola hepatica* in Europe, Africa, Asia, the Americas, and Oceania, and *Fasciola gigantica* restricted to Africa and Asia.⁵ *Fasciola* species are transmitted by specific freshwater lymnaeid snails with a marked amphibious behavior which underlies its pronounced susceptibility to climate changes.⁷ Clinically, it presents two disease phases: 1) an initial 3- to 4-month-long invasive or acute phase corresponding to the migration of the excysted, 250- μ m-long infective juveniles through the intestine wall, abdominal cavity, and liver parenchyma until finally penetrating into the bile ducts, a highly symptomatic phase during which the infection may be detected serologically but not coprologically; and 2) a biliary or chronic phase corresponding to the parasitization by fluke adults in the biliary ducts and gallbladder, which may last up to 13.5 years and is characterized by fluke egg detection in stools.⁸

Of veterinary importance due to the big losses it causes in livestock husbandry,⁹ the global scenario of fascioliasis has changed in the last three decades,¹⁰ with the description of numerous human-endemic areas and an increase of human

reports in many countries.⁸ Human fascioliasis shows large transmission pattern complexity and epidemiological heterogeneity throughout, in great part linked to differences in human behavior, traditions, and diet underlying a marked diversity of human infection sources and incidence factors.¹¹ New knowledge about involved foods, freshwater, and combinations of both carrying infective metacercariae allows us to understand the frequency of infection and reinfection by *Fasciola* spp. in rural areas.^{11,12}

In human-endemic areas of developing countries, children appear to be the most affected, with prevalences and intensities showing a peak in the 9- to 11-year-old group.¹³ Information on rural child infection mainly relies on school surveys in which children are almost always detected in the chronic phase. Age-groups analyzed comprise from 5-year-old onward. The short knowledge about infection in preschool children aged 4 years or younger relies on sporadic reports.

Among the many human-endemic regions throughout, Southeast Asia has recently emerged. In Vietnam, an epidemic started in the mid-1990s.¹⁴ At present, many thousands of patients, mostly adult subjects, are yearly diagnosed in hospitals throughout the country. Both fasciolid species appear sympatrically, and hybrid forms have been described.¹⁵ This large public health problem in Vietnam may have expanded to neighboring Laos,¹⁶ and southern China.¹⁷

We here report five very young infected Vietnamese children, including early postnatal infection in two of them and an alarming picture leading to surgery in another. Focus is given to clinical features, useful diagnostic tools, molecular confirmation, treatment, and epidemiological aspects of interest. These cases may be interpreted as a worrying jump from an epidemic to an endemic situation. The long life span of *Fasciola* spp. in humans may give rise to chronic children if infection remains undiagnosed, as usual in rural areas. It should be considered that sequelae in this disease are known to increase with infection length.^{6,18}

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A comparison is made with similar cases reported worldwide. Reports in small children are analyzed, with special emphasis on the problems posed by diagnosis and treatment. Results highlight the need for physicians to consider potential infection by *Fasciola* spp. in not only very small children, even lactant ones, mainly when living in or visiting rural areas but also in urban children when suspicious foods and drinks made from sylvatic vegetables, fruits, tubercles, natural water, and/or infection risky behavior are involved.¹¹

MATERIALS AND METHODS

Ultrasonography (US) and serum biochemical parameters.

An ultrasound-based imaging technique by means of a sonographic scanner was used because of its noninvasive technology appropriate for small children. Ultrasonography findings are nonspecific (diffuse liver echogenicity increase) during the invasive phase, but useful in the biliary period because of its ability to detect worm movements. Serum biochemical parameters useful in liver fluke-infected patients¹⁹ were measured within 2 hours after blood extraction by means of a standard enzymatic colorimetric method.

Coprological analysis. The Kato–Katz technique was applied to stool samples. Slides were microscopically examined at $\times 400$ within 1 hour of preparation to avoid egg over-clarification. Egg counts (eggs per gram of feces [epg]) were used as indicator of liver fluke burden to assess whether special precautions should be taken in the treatment of heavily infected children, according to WHO instructions.⁶ Helminth egg measurements were obtained with a calibrated microscope by placing stool samples mixed with saline solution (0.9%) onto a glass slide with a coverslip.

Serological test. A serological test is systematically applied to suspicious fascioliasis patients in Vietnam, where most patients do not shed *Fasciola* spp. eggs in stools, similarly as in other regions, for example, Europe.⁸ This ELISA to detect *Fasciola* spp. antibodies is based on a 27-kDa component protein from excretory/secretory products of Vietnamese *F. gigantica*. This ELISA test showed a high sensitivity (100%) and specificity (97.67%).²⁰ The cutoff applied was an optical density (OD) of 0.3.

Molecular techniques. For DNA extraction, patients' eggs were isolated and embryonated up to inner miracidium development in 20 days under laboratory conditions. A 435-bp fragment of the mtDNA gene NADH dehydrogenase subunit I (*nad1*) could be obtained, using primers FND1F (forward) (5'TGGGGTCTGTTGCAGAGATTTGC3') and FND1R (reverse) (5'ATCCAATGGAGTACGGTTACA3') following methods previously described.¹⁵ Nucleotide and corresponding amino acid sequences were analyzed by alignments with *nad1* sequences available in GenBank. Homologies were performed using the BLASTN program from the National Center for Biotechnology information website (<http://www.ncbi.nlm.nih.gov/BLAST>).

Literature review and quality assessment. A wide literature review to find reports of children aged 4 years or younger was performed by including databases, web entries, web platforms, multi-title packages, libraries, free collections, and personal e-mail requests when appropriate. The key criteria used to select the articles were the contents on 1) infection by *F. hepatica* or *F. gigantica*, and 2) children aged 4 years or younger. No restriction was applied to the period of time taken

into consideration, that is, from the beginning of the oldest publication on human infection up to the present. Key words for literature searches included combinations of the following terms: fasciol, fluke, trematode, digenean, liver, hepatic, child, lactant, boy, girl, school, pre-school, diagnos, serol, coprol, stool, ultrasound, age, years, clinic, symptom, patho, eosinophil, anamnesis, epidemi, survey, famil, hospital, treatment, and fasciolicide. These terms were used in English, Spanish, French, German, Portuguese, Italian, and Vietnamese. Articles in other languages such as Polish, Turkish, or Russian were translated by using Internet translation tools. Old reviews and books were in need to be read in full because the appropriate information did not appear in titles, abstracts, summaries, or key words. Literature noted as references in published reports was useful. International library networks were used for the obtaining of publications not appearing in Internet or in cases in which only the title and/or the abstract could be found in Internet databases such as, for instance, Centre for Agriculture and Bioscience International, Pubmed, and others. Main criteria used to select the articles included verification that infection by *Fasciola* spp. was not misdiagnosed, and the information provided was sufficiently detailed. No restriction was applied to sources, that is, searches were not restricted to medical sources. Among gray literature analyzed, different types of reports, government documents, and working papers of agencies, universities, and research centers were screened by using Google open search through Internet. In countries presenting human fascioliasis-endemic areas, a big amount of such literature was available in paper form, thanks to the network of scientists and centers collaborating in the Worldwide Initiative of WHO against Human Fascioliasis coordinated by the WHO Collaborating Centre on Fascioliasis and its Snail Vectors of Valencia, Spain.

Many reports were in publications not available in electronic databases. Special efforts were dedicated to obtain copies of the original publications or when impossible to at least get the possibility of direct or indirect consultation access.

In one case, fluke ascription to the genus *Fasciola* proved later to be a misdiagnosis. Four immature trematodes were found in the eye of a 5-month-old child in Germany 2 centuries ago.²¹ The causal agent was ascribed to *F. hepatica* several years after.^{22,23} This was a fatal case which generated a wide body of literature during the 19th and 20th centuries. However, the last recent review concluded that it should not be considered a fascioliasis case because present evidences indicate these four ocular worms to have been mesocercariae of a trematode of the genus *Alaria* most probably after eventual transmammary transmission.⁶

A few surveys or case series referring to infection in the 0- to 4-year age-group in several countries included no detailed data and are consequently not considered in the present review. However, these data and related references may be of interest and are summarized in the following.

In France, at the occasion of a fascioliasis epidemic occurred in an area close to the city of Nantes in the years 1965–1967, a report refers to 50 patients infected by *F. hepatica* and diagnosed at an hospital, including ages from 3 to 74 years.²⁴ There is no other reference in the text as to even assess whether there was only a 3-year-old child or perhaps more than one aged 4 years or younger.

In Portugal, among a total of 561 subjects infected by *F. hepatica* in the northern part of the country close to Porto, a total of 21 children aged 0–6 years were noted to be

coprologically positive by egg detection, with a mean of 124 epg/child.²⁵ No other information was furnished.

In Argentina, a 3-year-old child was noted to be infected by *F. hepatica*, supposedly in the area of Cordoba. Unfortunately, no additional information could be obtained despite the efforts made to have access to the unpublished PhD thesis where this case was reported (Siciliano C. Distomatosis hepática, perfil clínico. Tesis Doctoral, Universidad Nacional de Córdoba, Argentina. 1982; 1–118).

In Peru, in a retrospective study of 101 coprologically positive subjects in the northern province of Cajamarca, nine boy children and 19 girl children were noted to be infected in the 0- to 9-year-old group, without any additional clarification. Anyway, reference to children in the school-age period suggests that all these children should probably have been 5 years or older.²⁶ In a review of the clinical pictures of 277 3- to 86-year-old patients diagnosed in an hospital in Lima during the 1970–2002 period, no detail is unfortunately noted about the smallest children infected.²⁷ In a survey of schoolchildren in the Peruvian Altiplano, the 4- to 7-year-old group was considered among 61 positive cases examined, although no further details about the infection in 4-year-old children were mentioned.²⁸

In Nigeria, a coprological survey of 6,430 children aged between 2 and 20 years was carried out in Benin City. Eggs of *F. gigantica* were noted to be found in the stools of two boy children and four girl children in the 2- to 5-year-old group of nursery pupils, without any additional information. It was noted, however, that false positives due to spurious infection by eggs in transit linked to local cattle infection were not disregarded.²⁹

In Egypt, 134 subjects proven to have chronic fascioliasis after stool examination were enrolled in a study to evaluate the treatment with different triclabendazole regimes. A total of 87 subjects were females whose ages ranged from 2 to 60 years, but no further details about the smallest female(s) appear noted in the publication.³⁰

In Iran, a survey of 452 blood samples was implemented after the human fascioliasis outbreak occurred in 1988 in Guilan Province, in the north of the country. The 0- to 9-year-old age-group was distinguished, although no further information was provided as to know whether children aged 4 years or younger were infected.³¹

Indirect access was obtained to consult an unpublished Medicine Speciality Thesis (Gomez CG. Fasciolosis hepática en humanos, hallazgos en ocho casos. Tesis de Especialidad en Anatomía Patológica, Facultad de Medicina, Universidad Nacional Autónoma de México, México. 1992; 1–40), in which two preschool child cases reported from Mexico were considered of interest and their data consequently included in the review.

Ethical statement. This study was approved by the Institutional Review Board for Ethics in Biomedical Research, Hanoi Medical University, Ministry of Health (Institutional Review Board No. 4018/HMUJRB). Patients originated from provinces of Vietnam and were diagnosed at the National Hospital of Pediatrics in Hanoi and the Hanoi Medical University. Informed consent from parents of the children was obtained in each case.

RESULTS

Infection in very young Vietnamese children. Results of the biochemical analyses of the five patients are shown in

Table 1. C-reactive protein (CRP) followed by creatinine (Cre), and from 3 years onward, also γ -glutamyl transferase (GGT) were the markers showing alterations.

Case 1. A 10-month-old girl, from the rural area of Phu Tho Province, presented with fever (39°C), upper abdominal pain, plodding, weight loss (0.5 kg), and allergy. The parents referred to symptom onset 3 days before. Eosinophilia (20%) and a visible lesion (2.45 × 2.07 cm) in the right liver lobe by ultrasound (Figure 1A) were found. The serological analysis was positive (titer 1/6,400; OD = 2.2). Coprology showed an intensity of 48 epg, fluke eggs measuring 160 × 90 μ m (Figure 2A). After treatment with 10 mg/kg single dose of triclabendazole, clinical symptoms and eggs in feces disappeared in 1 month, with evident gain in weight (0.6 kg in 1 month). The parents had no symptoms and were serologically and coprologically negative. According to the parents, at home the baby was able to crawl to the yard and may have eaten (suck or chew) some freshwater plants collected from the field for their pig. Alternatively, the infection could have occurred by the consumption of freshwater plants added to the rice powdered soup given to the baby because of its 5 month age and the mother's insufficient lactating capacity.

Case 2. A 12-month-old boy, from the rural area of Bac Ninh Province, presented with fever (39–40°C), upper abdominal pain, and weight loss (0.7 kg). Eosinophilia (25%) and an evident damage (3.50 × 2.82 cm) in the right liver lobe by ultrasound were found (Figure 1B). Positive serological test showed a titer of 1/3,200 (OD = 1.6). Intensity was 120 epg, eggs measuring 162 × 92 μ m (Figure 2B). A similar successful recovery was obtained after a 10-mg/kg triclabendazole single dose treatment. Affected liver area damage progressively reduced up to total disappearance in 6 months. The parents were asymptomatic and negative serologically and coprologically.

Case 3. A 3-year-old girl, from the urban area of Dien Bien Province, presented with abdominal pain, chest pains, and fever (38.5°C) as first symptoms. A weight loss of 1.5 kg/month had been observed. A 20% eosinophilia was found. A liver damage of 2.78 × 2.16 cm in the right liver lobe was seen at ultrasound (Figure 1C). The serological positivity showed a titer of 1/3,200 (OD = 1.5). No eggs were detected in stool analyses, suggesting infection in the acute phase. After a month hospitalization, a fluke moving toward the pericardium and pericardial effusion were observed at US. The child fully recovered after a 20-mg/kg (1 tablet for 16 kg) triclabendazole treatment. No other family member proved to be infected after serological and coprological examinations. The baby used to eat freshwater vegetables together with her parents.

Case 4. A 3-year-old boy, from the urban area of Nam Chai commune, Muong Khuong district, Lao Cai Province, presented with abdominal pain, cough, chest pains, and fever (39°C) as first symptoms. Initially diagnosed as bronchitis, the treatment with antibiotic drugs proved to be useless. A tumor image appeared under right costal margin 20 days later. Effusion of lung membrane, pericardium, and abdomen was observed at US and X-ray (Figure 3). Eosinophilia was 53.4% in blood, 75% in lung effusion, and 80% in abdominal effusion. IgG was 25.38 (normal is 3.3–6.2), and IgE was 3,000 (normal is 0–230). Serological positivity showed a titer of 1/3,200 (OD = 1.6). No eggs were found in stools, suggesting infection in the acute phase. The child fully recovered after a 20-mg/kg (1 tablet for 15 kg) triclabendazole treatment. No other family

TABLE 1

Results of biochemical parameters in serum obtained from two early postnatal and three preschool-age infected children in Vietnam, compared with same marker ranges for healthy children younger than 5 years in this country (*)

Biochemical markers	Units	10-month-old girl (case 1)	12-month-old boy (case 2)	3-year-old girl (case 3)	3-year-old boy (case 4)	4-year-old boy (case 5)	Healthy children*
Glutamic oxaloacetic transaminase or aspartate aminotransferase	U/L	22	25	40	41	42	15–55
Glutamate-pyruvate transaminase or alanine aminotransferase	U/L	31	28	39	40	41	5–40
γ -Glutamyl transferase or γ -glutamyl transpeptidase	U/L	28	34	41	39	40	5–32
Total bilirubin	$\mu\text{mol/L}$	9.5	11	5.5	6.6	15	2–20
Calcium	mmol/L	2.5	2.2	2.0	2.1	1.8	2.2–2.7
Chloride	mmol/L	100	103	99	98	101	98–106
Creatinine	$\mu\text{mol/L}$	60.5	66.5	76.5	63	90	27–62
Urea	mmol/L	5.6	4.7	4.6	5.3	3.5	2.5–6.4
Albumin	g/L	40	43	46	39	35	37–51
Total protein	g/L	72	62	77	66	65	57–80
Glucose	mmol/L	5.2	4.8	4.1	5.1	4.3	3.3–5.5
Amylase	U/L	160	155	100	120	150	≤ 220
Total cholesterol	mmol/L	4.5	4.0	3.9	4.1	4.6	< 4.42
Triglycerides	mmol/L	1.5	0.9	1.7	1.2	2.1	< 1.65
C-reactive protein	mg/L	8	10	12	33.95	9	< 6.0

Altered values marked in bold.

members were infected according to serology and coprology. The baby used to eat freshwater vegetables.

Case 5. A 4-year-old boy, from the urban area of Loc Vuong community in Nam Dinh Province, suffered fever of 39°C

followed by abdominal pain appearing after 2 weeks and weight loss of around 2 kg/month. A 3.23 × 2.35-cm lesion was observed in the right liver lobe by ultrasound (Figure 1D). A 17% eosinophilia was found. Two weeks later, a fluke moving

FIGURE 1. Ultrasonography images showing lesions in the right liver lobe (arrows) of four Vietnamese preschool children: (A) lesion of 2.45 × 2.07 cm in a 10-month-old girl (case 1); (B) damage sized 3.50 × 2.82 cm in a 12-month-old boy (case 2); (C) damage of 2.78 × 2.16 cm in a 3-year-old girl (case 3); (D) lesion of 3.23 × 2.35 cm in a 4-year-old boy (case 5).

FIGURE 2. Eggs of *Fasciola gigantica* fecally shed by earliest infected children ever reported: (A) from girl baby presumably infected at 5 months (case 1); (B) from male baby presumably infected at 7 months (case 2). Note the appearance of normal viable fasciolid eggs, with anterior operculum and typical roughened or irregular area at the abopercular end. This figure appears in color at www.ajtmh.org.

from the liver to the large intestine was seen at ultrasound. Subsequently, peritonitis appeared accompanied by increased fever and abdominal pain. Serology showed positivity (titer of 1/6,400; OD = 2.1), but coprology was negative. Additional symptoms included increased fever, swollen abdomen, nausea and vomiting, diarrhea, additional weight loss, and fluid filled abdominal cavity (ascites). This alarming picture led to surgery, in which perforation of the large intestine was found, and a 34-mm-long fluke in the abdominal cavity was collected (Figure 4A and B). Treatment with 20 mg/kg (1 tablet for 18 kg) triclabendazole led to total healing. Father and mother showed no infection at serology and coprology. Parents referred to the usual consumption of freshwater vegetables by their son.

Molecular confirmation of causal agent. Comparative nucleotide alignments with *nad1* sequences available in the

FIGURE 4. Largest ectopic gravid fasciolid specimen ever reported, surgically collected from the abdominal cavity of a 4-year-old boy, causing peritonitis after intestinal wall perforation (case 5): (A) ventral view of complete 34-mm-long *Fasciola gigantica*-like adult showing anterior shoulders and nonparallel lateral walls; (B) anterior part showing long coiled uterus full of eggs. This figure appears in color at www.ajtmh.org.

GenBank database showed high nucleotide (98–99%) and amino acid (96–99%) similarity to *nad1* sequences of *F. gigantica* from Korea, Japan, and Indonesia, but a lower rate (90% and 92%) than those of *F. hepatica* from Australia and the Americas (Table 2).

Fascioliasis reports in worldwide preschool children. In human-endemic areas and among human cases in animal-endemic areas, human infection reports become numerous from the age of 5 years, that is, from the beginning of school years. This has traditionally been linked to the risky daily walking way from home to the school in rural areas because of the opportunities for infection when children put sylvatic vegetables into the mouth or drink natural water.

The review performed has been restricted to preschool children aged 4 years or younger (Tables 3 and 4). A total of 38 past cases were found, including 3, 7, 12, and 16 cases of 1, 2, 3, and 4 years, respectively. They comprised 22 boy children, eight girl children, and eight with unspecified gender. There is an evident faster increase of infection in males than in females from 2 years onward (Figure 5).

Reports were from all continents, except Oceania, including 10 cases in Europe (three in Austria and one in each Croatia, France, Italy, Poland, Spain, Switzerland, and the United Kingdom), eight in Africa (Egypt 6, Algeria 1, and Ethiopia 1), five in Asia (China 1, India 1, Iran 2, and Turkey 1), and 15 in the Americas (Argentina 1, Bolivia 1, Cuba 1, Guatemala 1, Mexico 7, and Peru 4).

The oldest report dates from 1856 in the United Kingdom and the most recent from 2016 in Peru, including many different types of reports. This long interval explains the use of many anti-fasciolid drugs according to the different periods: emetine, dehydroemetine, entobex, bithionol, praziquantel, triclabendazole, and nitazoxanide.

In the reports in which a specific diagnosis was achieved, the causal agent was always noted to be *F. hepatica*. Among the 38 globally reported cases, only in four from Egypt and two from Iran was the worm species not defined and cannot be established because of the geographical distribution overlap of the two fasciolid species in these two countries. There were 11 cases diagnosed in the acute phase, 24 in the chronic phase, and three ectopic cases. Only in five cases was intensity assessed by egg counts, always referring to a low

FIGURE 3. Effusion of the lung membrane, pericardium, and abdomen observed at X-ray caused by *Fasciola* sp. infection in a 3-year-old boy (case 4).

TABLE 2

Comparison of mtDNA nad1 sequences (435 nucleotides and 145 amino acids) from eggs shed by Vietnamese small children with *Fasciola gigantica* from Japan, Korea, and Indochina, and *Fasciola hepatica* from Australia and the Americas

Comparisons	Number of different nucleotides	Homology rate of nucleotides	Number of different amino acids	Homology rate of amino acids
FgCase1 vs. FgCase2	0	100%	0	100%
FgCases 1 and 2 vs. FgJp	2	99.5%	2	98.5%
FgCases 1 and 2 vs. FgKor	2	99.5%	2	98.5%
FgCases 1 and 2 vs. FgIndo	4	99.1%	3	97.8%
FgCases 1 and 2 vs. FhAus	39	90.4%	11	91.8%
FgCases 1 and 2 vs. FhUS	39	90.4%	11	91.8%

FgCase1 = Vietnamese *F. gigantica* from case 1; FgIndo = Indonesian *F. gigantica*; FgJp = Japanese *F. gigantica*; FgKor = Korean *F. gigantica*; FgCase2 = Vietnamese *F. gigantica* from case 2; FhAus = Australian *F. hepatica*; FhUS = American *F. hepatica*.

burden between 5 and 48 epg, except 1,197 epg in a 4-year-old boy.

The only two cases detected in family outbreaks are surprising. The majority were detected in hospitals (28 cases) or surveys (six cases), with one case during a drug evaluation trial. Symptom onset, when noted, was of a maximum of 4 months. Eosinophilia appears as a general feature.

DISCUSSION

Parasitological analysis. The cases 1 and 2 are the youngest fascioliasis patients ever diagnosed. When considering the five infected preschool children, Vietnam becomes the third after Mexico and Egypt in the country list according to the number of such reports.

These Vietnamese preschool children are the first reported to be infected by *F. gigantica*. Egg size in cases 1 and 2 corresponds to the overlap range in areas where both fasciolid species are present: *F. hepatica*: 106.5–171.5/63.9–95.4 µm; *F. gigantica*: 150.9–182.2/85.1–106.2 µm.⁸ The 34.00-mm-long mature fluke recovered from case five indicates infection by *F. gigantica* because of its length: *F. gigantica* up to 52.3 mm; *F. hepatica* up to only 29.0 mm.⁶³ The mtDNA nad1 sequences from eggs in cases 1 and 2 demonstrate that a *F. gigantica* form was involved. It may be concluded that the infections were caused by *F. gigantica*-like worms,⁵ which agrees with previous molecular analyses of fasciolids in Vietnam.¹⁵ Egg absence did not allow for a specific diagnosis in cases 3 and 4.

The prepatent period in *F. gigantica* is at least 1–2 weeks longer than in *F. hepatica*.¹⁹ Consequently, the chronic cases 1 and 2 should have been infected at least when they were aged 5–6 months and 7–8 months, respectively. This means a very early postnatal infection during the lactating period. Three other infections by *Fasciola* spp. in lactating children were reported from Mexico,³² Egypt,³³ and Argentina.³⁴

Pathogenicity and epidemiological aspects. *Fasciola gigantica* is more pathogenic than *F. hepatica* because of its larger size.¹⁹ Worth mentioning is that cases 1 and 2 were not diagnosed during the acute phase, in which symptoms are more evident than in the chronic phase.⁶ Indeed, in human hyperendemic rural areas of developing countries, children are almost always detected in the chronic phase.¹³ Whereas egg absence indicates cases 3 and 4 to be in the acute phase, egg presence in the surgically obtained fluke indicates case 5 to be in the chronic phase.

Such early infections suggest that children in rural areas of developing countries may reach chronicity very early in their lives, a situation of concern for their development considering

complications such as anemia, lithiasis or cirrhosis, and sequelae, which are known consequences of the advanced chronicity,^{64–66} and to be worse in long-term infections because of delayed diagnosis and treatment.⁶⁷ Peritonitis diagnosed in the chronic case 5 is a complication already emphasized in children.^{68,69} Ectopic case five demonstrates that adult flukes have the capacity to abandon the liver, reach the intestine, and restart migration crossing the intestinal wall.

Other worth mentioning severe complications reported in these small children were liver abscess,⁴⁶ multiple hepatic cysts,³⁴ and neurological affection,⁴⁹ the latter even leading to death.⁵⁵

C-reactive protein followed by Cre and GGT appears as the most useful serum biomarkers. As a highly sensitive marker for liver inflammation associated with infection, CRP may also be useful to assess treatment efficacy because it returns quickly to normal values in response to therapy. C-reactive protein and GGT, besides aspartate aminotransferase and alanine aminotransferase, were also altered at the acute phase in a 3-year-old boy,⁴⁷ as usual in fascioliasis patients.¹⁹

The higher frequency of pseudo-septicemic forms in 5- to 16-year-old children than in adult subjects was highlighted in Europe,⁷⁰ including three 4-year-old children in Austria,⁴⁹ Italy,⁵⁰ and Switzerland.⁵¹ Liver fluke infection induces suppression of immune responses against concurrent pathogenic infections, not only bacteria but also protozoans and other helminths, in both acute⁷¹ and chronic phases.⁷² These coinfections are known to underlie morbidity and mortality. Suffering from such immunosuppression effects at such early ages becomes an evident problem.

The infection source in such early infected children is a question mark, as the first deciduous teeth appear from months 6 to 9 and the last ones from months 20 to 24. Contaminated water, beverages, juices, and soups made from sylvatic vegetables, fruits, and even tubercles, as well as plant sucking, may underlie these infections.¹¹ Watercress and lettuce leaves were noted to be involved in some cases.^{32,33}

The absence of infection by *Fasciola* spp. in the parents of the Vietnamese children is not easily understandable because parents referred to their children sharing the same vegetables with them. Different immunological capacities may be involved in infections by a very low number of metacercariae. However, only two infected preschool children were reported in family outbreaks.^{53,54} The isolated preschool child infections suggest that many such infections may remain overlooked because they are undiagnosed or misdiagnosed in rural areas.

The clear dominance of males over females is surprising, as significantly higher prevalences and/or intensities in

TABLE 3
Fascioliasis reports in children aged 3 years or younger, according to the worldwide review

No.	Age/gender	Cause	Diagnosis	Epidemiological observations	Clinical observations	Treatment	Year and country (reference)
–	–	F.h./F.g.	Coprology (epg)/serology (test)/other (flake no).	Detected in/infection source	Time from symptom onset/disease phase (% eosinophilia)/complications*	Drug used	–
1	1 y 1m/M	F.h.	pos./pos. (immuno-diffusion, IHAT)/ultrasound	Hospital/lactant, watercress, and lettuce	1 m/chronic ph. (60%)/malnutrition, hydrocephaly, macrocephaly, and hepatomegaly	Praziquantel	2001, Mexico ³²
2	1 y 3m/F	n.s.	pos./pos. (IHAT)/ultrasound	Hospital/suck lettuce leaves	n.s./chronic ph. (n.s.)/n.s.	–	1995, Egypt ³³
3	1 y 7m/n.s.	F.h.	n.a./n.a./eggs from liver cyst puncture, CT scan	Hospital/lactant	n.s./chronic ph. (n.s.)/multiple hepatic cysts, severe anemia, and hepatomegaly	Metronidazole, mebendazole, and albendazole, and nitazoxanide	2014, Argentina ³⁴
4	2 y/F	F.h.	pos. (48 epg)/n.a./n.a.	Survey/n.s.	n.s./chronic ph. (n.s.)/n.s.	Triclabendazole	1999, Bolivia ¹³
5	2 y/n.s.	n.s.	pos. (24 epg)/n.a./n.a.	Survey/n.s.	n.s./chronic ph. (n.s.)/n.s.	Triclabendazole	2003, Egypt ³⁵
6	2 y/M	F.h.	pos./n.a./n.s.	Hospital/Ethiopian adoptee in Norway	n.s./chronic ph. (13%)/diarrhea, and colicky abdominal pain	Triclabendazole	2005, Ethiopia ³⁶
7	2 y 1m/M	F.h.	n.a./n.a./6 flukes from tumor	n.s./weaning at 18 months	2 m/ectopic case (n.s.)/tumor at occiput	n.a.	1856–1857, United Kingdom ³⁷
8	2 y 6m/M	F.h.	neg./n.a./liver biopsy	Hospital/unboiled well water and vegetarian family	10 d/acute ph. (n.s.)/cirrhosis, hepatomegaly, splenomegaly, and sibling died from liver disease	Corticosteroids	1980, India ³⁸
9	2 y 6m/n.s.	F.h.	pos./n.a./n.a.	Trial/n.s.	n.s./chronic ph. (n.s.)/n.s.	Nitazoxanide	2003, Peru ³⁹
10	2 y 9m/M	F.h.	neg./pos. (Arc2)/n.a.	Hospital/n.s.	4 d/acute ph. (15%)/hepatomegaly, and vomiting	n.s.	2005, Peru ⁴⁰
11	3 y/M	F.h.	neg./pos. (IDR)/histological examination	Hospital/living close to farm	3 d/acute ph. (46.4%)/pronounced swelling of the right submaxillary and jugular region	Entobex and dehydroemetine	1965, France ⁴¹
12	3 y/M	F.h.	pos./pos. (IFT, IHAT, and ELISA)/increased biochemical parameters	Hospital/n.s.	3 w/transit acute to chronic ph. (24%)/exanthema	dehydroemetine	1981, Austria ⁴²
13	3 y/n.s.	F.h.	pos./pos. (counterimmunoelectrophoresis)/n.a.	Hospital/n.s.	3 m/chronic ph. (pos. %)/hepatomegaly	Bithionol	1988, Egypt ⁴³
14	3 y/n.s.	F.h.	pos./pos. (counterimmunoelectrophoresis)/n.a.	Hospital/n.s.	3 m/chronic ph. (pos. %)/hepatomegaly	Dehydroemetine	1988, Egypt ⁴³
15	3 y/M	F.h.	?/?/finding in bile	Hospital/?	n.s./chronic ph. (? %)/?	?	1992, Mexico (Gomez PhD, 1992)
16	3 y/n.s.	n.s.	pos./pos. (IHAT)/ultrasound	Hospital/n.s.	n.s./chronic ph. (n.s.)/ascites	n.s.	1995, Egypt ³³
17	3 y/n.s.	n.s.	pos./pos. (ELISA CL-1)/n.a.	Survey/n.s.	n.s./chronic ph. (n.s.)/none	n.s.	2002, Iran ⁴⁴
18	3 y/n.s.	n.s.	pos. (48 epg)/n.a./n.a.	Survey/n.s.	n.s./chronic ph. (n.s.)/n.s.	Triclabendazole	2003, Egypt ³⁵
19	3 y/M	F.h.	pos./n.a./n.a.	Survey/beverages	n.s./chronic ph. (n.s.)/n.s.	n.s.	2004, Peru ⁴⁵
20	3 y/M	F.h.	pos./pos. (FasciD)/ultrasound, CT, and liver biopsy	Hospital/natural freshwater	1 w/chronic ph. (56%)/liver abscess	Triclabendazole	2011, Cuba ⁴⁶
21	3 y/M	F.h.	neg./pos. (IgG immunoblot)/ultrasound and CT scan	Hospital/n.s.	10 d/acute ph. (25%)/hepatomegaly, splenomegaly, ascites, and pleural effusion	Triclabendazole	2016, Peru ⁴⁷
22	3 y 6m/F	F.h.	pos./pos. (IgG)/n.a.	Hospital/watercress	2 m/chronic ph. (n.s.)/hepatomegaly	Dehydroemetine	1992, Mexico ⁴⁸

Order after age and year of report. d = days; F.h. = *Fasciola hepatica*; F.g. = *Fasciola gigantica*; IDR = intradermal reaction test; IFT = indirect fluorescence test; IHAT = indirect hemagglutination test; MF = male/female; pos./neg. = positive/negative result in diagnosis; m = month(s); n.s. = not specified; y = year(s); w = week(s); ? = information could not be obtained; * = only complications important or unusual in fascioliasis noted.

TABLE 4
Fascioliasis reports in 4-year-old children, according to the worldwide review

No.	Age/gender	Cause	Diagnosis	Epidemiological observations	Clinical observations	Treatment	Year and country (reference)
–	–	F.h. F.g.	Copology (epg)/serology (test)/other (flake no).	Detected in/infection source	Time from symptom onset/disease phase (% eosinophilia)/complications*	Drug used	
23	4 y/M	F.h.	pos./n.a./surgery, and liver biopsy	Hospital/n.s.	1 m/chronic ph. (47%)/neurological affection, and hepatomegaly	n.a.	1927, Austria ⁴⁹
24	4 y/M	F.h.	pos./n.a./radiology	Hospital/field plants into mouth	3 m/chronic ph. (23%)/pronounced slimming, hepatomegaly, coinfections with <i>A. lumbricoides</i> and <i>T. trichiura</i> , and caquexia	Emetine clorhydrate	1936, Italy ⁵⁰
25	4 y/M	F.h.	pos./n.a./n.a.	Hospital/n.s.	3 m/transit acute to chronic ph. (14%)/severe colics, abscess, hepatomegaly, coinfections with <i>A. lumbricoides</i> and <i>E. vermicularis</i>	Emetine clorhydrate	1938, Switzerland ⁵¹
26	4 y/M	F.h.	pos. (1,197 epg)/pos. (IDR, precipitation reaction, complement fixation)/liver biopsy, radiology, and surgery	Hospital/fresh aquatic plants directly from field	3 m/chronic ph. (71%)/hepatomegaly, and very serious condition	Emetine clorhydrate	1957, Mexico ⁵²
27	4 y/M	F.h.	pos. (5 epg)/n.a./n.a.	Family outbreak/watercress from city market	n.s./chronic ph. (n.s.)/asymptomatic	Emetine	1959, Mexico ⁵³
28	4 y/F	F.h.	neg./pos. (complement fixation test and IDR)/n.a.	Family outbreak/watercress	2 m/acute ph. (34%)/only slight abdominal pain	Entobex and dehydroemetine	1963, Algeria ⁵⁴
29	4 y/M	F.h.	n.a./n.a./autopsy (2 flukes in liver)	Hospital/n.s.	2.5 m/n.s./convulsions, blindness, severe brain affection, and death	n.a.	1967–68, Guatemala ⁵⁵
30	4 y/M	F.h.	neg./pos. (hemagglutination)/hepatic biopsy	Hospital/grass field with sheep	2 w/transit acute to chronic ph. (45%)/hepatomegaly, frequent vomiting, and symptom relapses	Dehydroemetine	1977, Poland ⁵⁶
31	4 y/F	F.h.	pos./pos. (IFT, IHA, and ELISA)/increased biochemical parameters	Hospital/n.s.	n.s./transit acute to chronic ph. (17%)/exanthema	Dehydroemetine	1981, Austria ⁴²
32	4 y/M	F.h.	pos./pos. (IFT)/ultrasound, 1 immature fluke from cutaneous abdominal fistula	Hospital/play with sheep and goat	3 w/ectopic and chronic case (60%)/hepatomegaly, anemia, and blood transfusion	Praziquantel	1990, Croatia ⁵⁷
33	4 y/F	F.h.	?/?/finding in bile	Hospital/?	n.s./chronic ph. (? %)/?	?	1992, Mexico (Gomez PhD, 1992)
34	4 y/F	F.h.	pos./n.a./n.a.	Survey	n.s./chronic ph. (n.s.)/edema on feet and face, and coinfection with <i>Giardia intestinalis</i>	Metronidazole, albendazole, and triclobandazole	1998, Turkey ⁵⁸
35	4 y/M	F.h.	neg./pos. (hemagglutination test and ELISA)/CT scan	Hospital/familiar orchard irrigated from nearby pond	1 m/acute ph. (62.8%)/hepatomegaly, and hydrocele	Bithionol	1999, Spain ⁵⁹
36	4 y/M	F.h.	neg./n.a./ultrasound, CT scan, 1 adult by choledocotomy	Hospital/no ingestion of freshwater plants	n.s./chronic ph. (2.5%)/Oddi sphincter stenosis, jaundice, and obstruction of choledocuss	Praziquantel	2002, Mexico ⁶⁰
37	4 y/M	F.h.	neg./n.a./ultrasound, CT scan, and juvenile worm from subcutaneous track	Hospital/raw water and raw watercress	4 m/ectopic case (44%)/creeping eruption, hepatomegaly	n.a.	2010, China ⁶¹
38	4 y/F	n.s.	neg./pos. (ELISA)/n.s.	Hospital/watercress	n.s./acute ph. (n.s.)/n.s.	Triclobandazole	2012, Iran ⁶²

Order after age and year of report (continuation of Table 3). d = days; F.h. = *Fasciola hepatica*; F.g. = *Fasciola gigantica*; M/F = male/female; m = month(s); pos./neg. = positive/negative result in diagnosis; n.s. = not specified; n.a. = not applied; IDR = intradermoreaction test; IFT = indirect fluorescence test; IHA = indirect hemagglutination test; y = year(s); w = week(s); ? = information could not be obtained; * = only complications important or unusual in fascioliasis noted.

FIGURE 5. Relationship of case numbers with age, according to the worldwide data reviewed: progressive increase of total preschool children (including reports with unspecified gender) and faster increase of frequency in males than in females from the age of 2 years. Five Vietnamese cases here reported included. This figure appears in color at www.ajtmh.org.

females have repeatedly been highlighted in developing countries,^{5,8,13} and also in schoolchildren in western Europe.⁷⁰ Differentiation appearing from the age of 2 years may perhaps be linked to a higher activity and more precocious curiosity of males in sucking or putting things into the mouth.

The very low epg amounts in cases 1 and 2 agree with infections in Bolivia¹³ and Egypt.³⁵ This suggests that high burdens in schoolchildren of hyperendemic areas may in some cases be the consequence of fluke accumulation by long-term reinfections started very early in life.⁷³

Although several reports on infected small children are from developed countries where human infection by *Fasciola* spp. continues to be diagnosed,⁷⁴ countries where more infected preschool children were reported agree with those known to present human-endemic areas, for example, Mexico,⁷⁵ Egypt where the highest human prevalence in Africa has been reported,⁷⁶ and Peru.^{45,77} Bolivia should be highlighted because of only one such case,¹³ despite presenting the highest human hyperendemic area known,^{5,8,11} suggesting a possible overlook of preschool infections. In Peru, there is indeed reference to unpublished observations on fascioliasis in children from an age of 2 years.⁴⁰ Fascioliasis is a neglected non-reportable infection,⁷⁴ which explains why many such child cases are never published or, when reported, usually with very restricted diffusion.^{33,34,37,54,55}

Diagnosis and treatment. A serological test and a coprological analysis are recommended for the detection of infection during the acute and the chronic phases in so small children. Typical fascioliasis symptoms may be overlooked, mainly when the infection is by only one or two flukes, which appears to be the most usual situation in these children. A high sensitivity coproantigen detection test may also be very useful in the chronic phase of these extreme low-burden infections,⁷⁸ even before the beginning of egg appearance in stools.¹⁹ Right upper quadrant and abdominal pain, fever, weight loss, and eosinophilia, accompanied by alteration of the biochemical markers (at least liver function tests, CRP, and

Cre),¹⁹ and liver observation through ultrasound (or computerized tomography [CT] scan if available), may help. Anamnesis including parent's information about suspicious infection sources may be crucial in guiding to a faster and correct diagnosis.¹¹ The modern trend for "natural feeding habits" by prioritizing the consumption of non-treated, fresh, raw/green fruits, and vegetables, when not also natural water, becomes risky for small children if used for beverages, juices, and soups.¹¹

Lack of symptomatic cure, disease relapses, insufficient efficacy despite long treatment courses, and need for retreatments after egg reappearance in stools have been highlighted in preschool children treated with different drugs.^{42,43,56} Only triclabendazole appears to avoid such problems, undoubtedly because of its unique action on both intra-tissularly migrating juveniles and fluke adults.⁷⁹ The present work fills the information gap related to the usefulness and applicability of triclabendazole for the treatment of early postnatal infections and preschool-age child infections. In the smallest Vietnamese children, a 10-mg/kg single dose post-prandially administered was sufficient for the disappearance of clinical symptoms and eggs in feces in 1 month, and to obtain a subsequent evident gain in weight. Reappearance of eggs in stools 2 months after the first 10 mg/kg dose was, however, reported in a 4-year-old girl in Turkey.⁵⁸ This girl responded well to a second 10 mg/kg dose, eggs disappeared, and she remained free of complaints during a whole year follow-up. The aforementioned short 2-month period suggests triclabendazole to have not been initially efficient against a migratory fluke originated in a reinfection. The drug was well tolerated with the exception of mild temporary right upper abdominal pain.⁵⁸ Similarly, no serious side effects were reported in other small children treated with triclabendazole, for example, a 2-year-old Ethiopian boy in Norway,³⁶ a 2-year-old girl in Bolivia,¹³ 2- and 3-year-old children in Egypt,³⁵ a 3-year-old boy in Cuba,⁴⁶ and a 3-year-old boy in Peru.⁴⁷ In the three 3- and 4-year-old Vietnamese children, a 20-mg/kg dose (1 tablet for 16, 15, and 18 kg, respectively) was also well tolerated, no side effects were observed, and led to total healing.

In addition, it should here be remembered that, when in the chronic phase, egg counts (epg) should be assessed by means of a quantitative coprological diagnostic test, such as the Kato-Katz.⁸ Children with an intensity of infection of ≥ 400 epg should be hospitalized before receiving treatment. This is justified by the consideration that individuals infected with a large number of worms are more likely to develop both systemic and mechanical adverse events (e.g., hepatic colic) following treatment and might, therefore, require specialist medical attention and care.⁸⁰ A lower threshold of ≥ 300 epg was requested in Bolivia where intensities in children are known to reach very high levels.¹³ For very small children aged 4 years or younger, this threshold may be even reduced as precautionary measure. The finding of a 1,197 epg in a 4-year-old Mexican boy presenting with a very serious condition is worrying.⁵² It should also be considered that a coproantigen test is not useful for this purpose because concentration of coproantigens in feces does not correlate with epg.⁸¹

One unavoidably wonders about how many such early infections may remain undiagnosed or misdiagnosed. Family doctors and pediatricians should consider them in areas where fascioliasis is a public health problem, and also in livestock hyperendemic rural areas.

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