Open Forum Infectious Diseases
MAJOR ARTICLE

Infectious Diseases Society of America
HIV Medicine Association
OXFORD

The Clinical Picture Caused by *Fasciola gigantica*: Analysis of 3250 Patients Along the 1995–2019 Countrywide Spread in Vietnam

Nguyen Van De,[1] Pham Ngoc Minh,[1] Thanh Hoa Le,[2] Do Trung Dung,[3] Tran Thanh Duong,[3] Bui Van Tuan,[4] Le Thanh Dong,[5] Nguyen Van Vinh Chau,[6] Pablo F. Cuervo,[7,8] M. Dolores Bargues,[7,8] M. Adela Valero,[7,8] Albis Francesco Gabrielli,[9] Antonio Montresor,[9,a] and Santiago Mas-Coma[7,8]

[1]Department of Parasitology, Ha Noi Medical University, Ha Noi, Vietnam, [2]Vietnam Academy of Science and Technology, Institute of Biotechnology, Ha Noi, Vietnam, [3]Institute of Malariology, Parasitology and Entomology, Ha Noi, Vietnam, [4]Quy Nhon Institute of Malariology, Parasitology and Entomology, Quy Nhon, Vietnam, [5]Ho Chi Minh Institute of Malariology, Parasitology and Entomology, Ho Chi Minh, Vietnam, [6]Research Direction, Ho Chi Minh Hospital of Tropical Diseases, Ho Chi Minh City, Vietnam, [7]Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Burjassot, Valencia, Spain, [8]Consorcio Centro de Investigacion Biomedica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain, and [9]Global Neglected Tropical Diseases Programme, World Health Organization, Geneva, Switzerland

Background. *Fasciola gigantica* is the causal agent of human fascioliasis, which is emerging in southern Asia and increasingly reported in Africa. Unfortunately, knowledge of the clinical picture by *F gigantica* is insufficient, because of the sporadic individual case reports, or few case series of short number of patients from areas where the 2 genetically pure fasciolid species geographically overlap and specific causality was not verified.

Methods. The clinical picture is assessed from patients examined in well-equipped hospitals of big cities in Vietnam. Records of 3250 *Fasciola*-infected patients were registered in individual cards and a database for their complete analysis was constructed. Case profile was based on typical symptoms, blood eosinophilia, serological test, imaging techniques, Kato-Katz test, and ex juvantibus confirmation. Anamnesis furnished information about place of residence, professional activities, and infection source.

Results. Symptoms, signs, and manifestations, including neurological, meningeal, neuropsychic, and ocular disorders, are analyzed according to frequency, sex, and age groups. Early patient diagnosis and treatment facilitated by radio broadcasting underlie differences between serological positivity, coprological positivity, and liver lesions and explain the absence of severe long-term complications and posttreatment sequelae.

Conclusions. This is the first sufficiently wide study of the clinical picture caused by *F gigantica*. The assessment has been made in a population without previous contact with fascioliasis and shows that *F gigantica* (and *F gigantica*–like hybrids) do not cause clinical pictures different from those caused by *Fasciola hepatica*. This clinical picture will be useful for physicians and health officers in endemic areas of Asia and Africa.

Received 18 September 2024; editorial decision 26 February 2025; accepted 01 March 2025; published online 4 March 2025

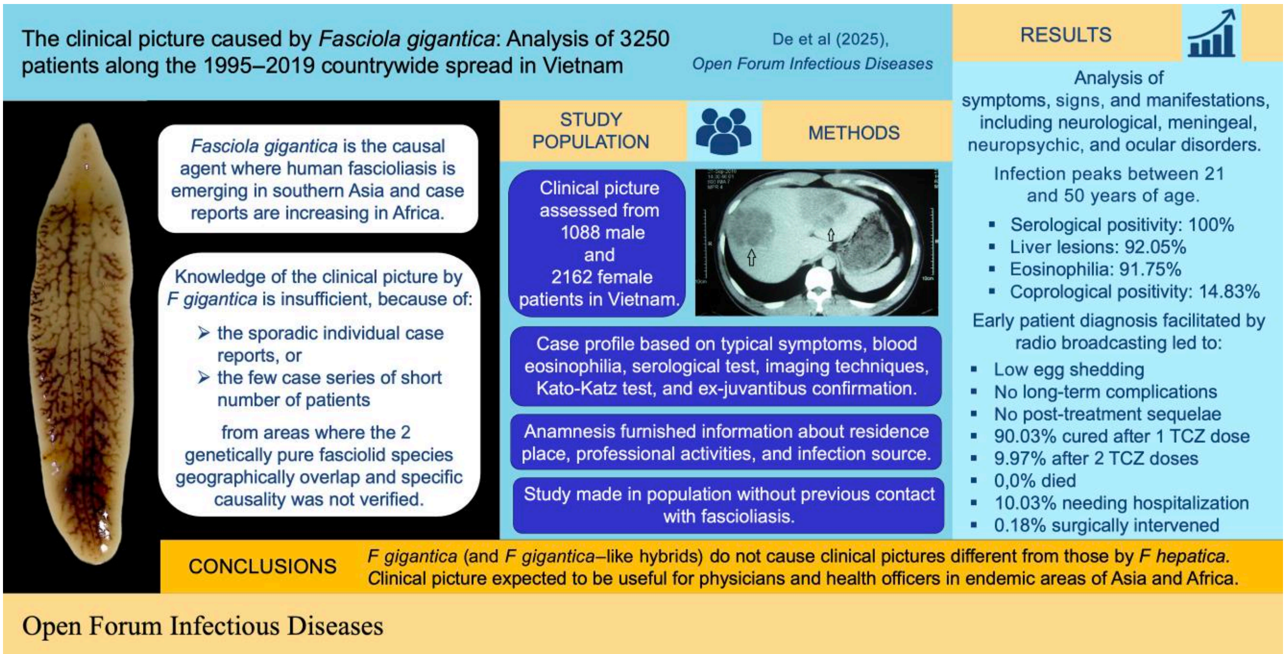
[a]Present affiliation: Ivo de Carneri Foundation, Milan, Italy.

Correspondence: Santiago Mas-Coma, PhD, Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain (S.Mas.Coma@uv.es); M. Dolores Bargues, PhD, Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain (M.D.Bargues@uv.es).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofaf116

Graphical Abstract



Keywords. *Fasciola gigantica*; neurological and ocular disorders; southern Asia and Africa; symptoms and manifestations; Vietnam.

Two species of flukes cause fascioliasis in humans: *Fasciola hepatica*, transmitted by freshwater lymnaeid snails of the *Galba/Fossaria* group in Europe, Africa, Asia, the Americas, and Oceania; and *F gigantica*, transmitted by lymnaeids of the *Radix* group only in parts, mainly warm lowlands, of Africa and Asia [1]. The absence of *Radix* in the Americas and Oceania underlies why *F gigantica* never colonized these continents [1, 2].

The World Health Organization (WHO) included fascioliasis within neglected tropical diseases [3] after considering pathogenicity [4–6], sequelae [4, 7], and immune modulation during the acute [8] and chronic phases [9]. Its impact on community development [10], increasing numbers of case reports, and description of human endemic areas throughout 1990–2010 [11] further reinforced this decision.

Fasciola hepatica is the main causal agent of fascioliasis [5] and has been considered more pathogenic than *F gigantica*, although the latter recently proved the opposite because of the bigger size of its adult stage. This confusion was due to the faster development of the adult stage of *F hepatica* inside the definitive host [12, 13]. Unfortunately, the knowledge on the clinical picture caused by *F gigantica* in humans remains insufficient when compared to *F hepatica* [5]. An aspect to be highlighted is that *F hepatica* strains showing different pathogenicity have never been reported. This is related to the absence of significant genetic and/or phenotypic lineages within fasciolid flukes due to the founder effect in an evolutionarily recent worldwide spread from the "Fertile Crescent" in Near-East Asia since only the Neolithic period [1].

Fascioliasis is a complex disease with epidemiological heterogeneity and different transmission patterns differing according to endemic areas. In recent years, human fascioliasis has emerged throughout southern and southeastern Asia—from Pakistan [14, 15] and India and Bangladesh [16, 17] in the West, up to southern China [18] and Vietnam [19] in the East. *Fasciola gigantica* is the fasciolid species that originally colonized the lowlands of this region in the past, with *F hepatica* restricted to the northern highlands (southern foothills of the Himalaya) and recent introductions by livestock importation [1].

Human fascioliasis evolves according to 4 infection phases [4, 5, 20]: (1) incubation period: between ingestion of the metacercarial cysts and symptom onset; (2) invasive, migratory, or acute phase: 3- to 4-month-long period corresponding to fluke juvenile migration through intestinal wall, abdominal cavity, and liver parenchyma up to biliary canals, usually showing pronounced symptomatology, which facilitates early detection; (3) latent period: asymptomatic egg-shedding persons with unexplained eosinophilia usually diagnosed in epidemiological surveys; and (4) biliary, chronic, or obstructive phase: up to 13.5-year-long period with egg-producing flukes inside the biliary canals and/or gallbladder. Almost all patients are diagnosed in the acute or chronic phases, given the difficulties in determining the incubation or latent period. Time elapsed since symptom onset and egg finding in stools allow differentiation between these phases.

The large magnitude of the recent countrywide spread of human fascioliasis in Vietnam has no precedent [19]. A multidisciplinary assessment has characterized the epidemiology and transmission pattern [20] of the 1995–2019 spread by analyzing the progressive evolution of the disease in the 63 provinces [19]. The initial implementation of radio broadcasting, leading rural and urban patients to health centers for early cost-free diagnosis and treatment after symptom onset, pronouncedly facilitated the outbreak follow-up since its beginning.

In Vietnam, *F gigantica* has been demonstrated to be able to cause epidemic and endemic situations similar to *F hepatica* and shows how human fascioliasis may reach worrying infection rates in areas where children are not the most affected group [19], despite the possibility for their very early infection [21]. Human-guided livestock movements from an initial outbreak area to all provinces were the way used by fasciolids and lymnaeid vectors to spread. Neither climate influences linked to monsoon regime changes nor fasciolid hybridization after foreign livestock importation played a role in the spreading outbreak [19]. It should be highlighted that we molecularly verified, by DNA sequencing, that the fasciolids infecting both patients and livestock in Vietnam are genetically pure *F gigantica* in its majority and fasciolid hybrids in a small minority, and that *F hepatica* is absent [19].

Our study aims to characterize the clinical picture caused, and pathogenicity induced, by *F gigantica* infections in Vietnam, by analyzing 3250 serologically positive patients diagnosed in well-equipped hospitals. It furnishes valuable information about the infection by *F gigantica* (and *F gigantica*–like hybrids), because such a quantity of patients from the same epidemiological scenario could never be clinically analyzed before, not even for the human infection by *F hepatica*.

METHODS

Patients and Analytical Procedures

The clinical picture is assessed from patients examined in well-equipped hospitals of big cities, including Ha Noi University Hospital, Ha Noi; National Institute of Malariology, Parasitology and Entomology (NIMPE), Ha Noi; Quy Nhon Institute of Malariology, Parasitology and Entomology, Quy Nhon; and Ho Chi Minh Hospital of Tropical Diseases, Ho Chi Minh City. Records of *Fasciola*-infected patients were registered in individual cards and a database for their complete analysis was constructed. The years from 2020 onward were excluded to avoid bias due to a potential impact of the coronavirus disease 2019 pandemic.

Case Profile and Diagnostic Techniques

Case profile was based on:

- Typical symptoms: Facilitated diagnosis in the acute and chronic phases [4–6].
- Blood eosinophilia: A common, early-appearing disorder [4, 5].
- Serological test: The enzyme-linked immunosorbent assay (ELISA) based on a Vietnamese *F gigantica* excretory/secretory protein showed 100% sensitivity and 97.67% specificity [22].
- Imaging techniques: Ultrasonography, computed tomography, and magnetic resonance allowed for hepatic abnormality detection suggestive of fascioliasis [5, 6, 23].
- Kato-Katz test: Coprological technique for fasciolid egg search [11].
- Ex juvantibus confirmation: Posttreatment symptom disappearance with the anti-fasciolid specific triclabendazole [24].

Anamnesis was performed to obtain information about the place of residence (rural/urban), professional activities (vegetable cultivation/livestock management), and potential infection source (vegetables/natural water drinking) [25].

It should be highlighted that serological tests do not differentiate between *F gigantica* and *F hepatica* [11]. All available commercial ELISAs have been developed using *F hepatica*. That is why the NIMPE in Ha Noi decided to use a new ELISA directly developed from local Vietnamese *F gigantica*, in a way to obtain a higher sensitivity whenever possible. Despite the verified usefulness demonstrated in the initial local evaluation of the new test, WHO requested us to perform a mission to Vietnam to reevaluate and validate the Vietnamese test and assess the spreading outbreak of human fascioliasis [19]. The evaluation was made by comparison with previously validated diagnostic tests, including 2 serological tests (CL1 and MM3-SERO), a coprodiagnostic monoclonal antibody MM3 test, and the coprological Kato-Katz test [19].

Statistical Analyses

Statistical analyses were done using GraphPad Prism version 9.5.1 for MacOS (GraphPad Software, San Diego, California). The fraction of total cases was displayed as percentage (%) and 95% confidence intervals (assessed with the Wilson/Brown method). The χ^2 test and Fisher exact test were used to analyze the percentage of cases, symptoms, and disorders by sex and age. A P value of <.05 was considered significant (2-sided when applying Fisher exact test).

Ethics

Patient data collection and analyses were approved by the ethical committees, in agreement with the principles expressed in the Declaration of Helsinki: the Institutional Review Board for Ethics in Biomedical Research, Ha Noi Medical University, Ministry of Health, Ha Noi, Vietnam (approval number 4018/HMUIRB, 26 December 2018); and the Comité Etico de Investigación en Humanos de la Universidad de Valencia, Valencia, Spain (approval number H1496156195013, 1 June 2017).

Table 1. Diagnostic Tools Used in 3250 Patients With Serologically Positive Fascioliasis in Vietnam, by Sex

Diagnostic Tool	Male (n = 1088)	% Male (95% CI)	Female (n = 2162)	% Female (95% CI)	*P* Value
ELISA for *Fasciola*	1088	33.48 (31.87–35.12)	2162	66.52 (64.91–68.15)	≤.001[a]
Liver lesions by US/CT/MRI	1016	31.26 (31.18–34.37)	2005	61.69 (60.01–63.35)	≤.001[a]
Eggs detected in stool	168	5.17 (4.46–5.98)	314	9.66 (8.69–10.73)	≤.001[a]
Ectopic fascioliasis	3	.09 (.03–.27)	6	.18 (.08–.40)	.50

Abbreviations: CI, confidence interval; CT, computed tomography; ELISA, enzyme-linked immunosorbent assay; MRI, magnetic resonance imaging; US, ultrasound.
[a]Significant differences in the comparison between the proportions of female and male patients.

Table 2. Diagnostic Tools Used in 3250 Patients With Serologically Positive Fascioliasis in Vietnam, by Age Group

	Age Group, y											
Diagnostic Tool	0–5	6–10	11–15	16–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	Total
ELISA for *Fasciola*[a]	21	80	138	375	461	478	467	436	402	322	70	3250
Liver lesions by US/CT/MRI[a]	20	78	130	351	437	436	429	416	365	291	68	3021
Eggs detected in stool[a]	7	21	34	49	67	66	63	65	55	48	7	482
Ectopic fascioliasis	…	1	1	…	2	2	1	2	…	…	…	9

Abbreviations: CT, computed tomography; ELISA, enzyme-linked immunosorbent assay; MRI, magnetic resonance imaging; US, ultrasound.
[a]Significant differences in the comparison between the proportions of patient age groups. See *P* values obtained in the analysis of age groups in Figure 2.

RESULTS

Comparison of Diagnostic Tools

The low number of patients shedding fasciolid eggs compared to serologically positive subjects, and/or showing liver lesions, should be highlighted (Tables 1 and 2). Serological positivity, coprological positivity, and liver lesion detection showed significant differences when comparing between the proportions of female and male patients and also age groups (Figures 1 and 2). Throughout the initial years of the outbreak, the number of patients suspected of presenting with a malignant hepatic tumor was worryingly high, showing a 34% peak of cases in 2005–2010. Such cancer-misdiagnosed patients consequently underwent unnecessary surgery and/or radiotherapy.

Nonsignificant *P* values in sex and age group comparisons were obtained when analyzing cases of ectopic fascioliasis due to insufficient sample size (Figures 1 and 2). Such ectopic cases were detected in a very low number of patients (Tables 1 and 2), and concerned worms moved to knee (1 case), mammary gland (1), chest (4), and colon (3).

Typical Symptoms and Manifestations

Symptoms, signs, and other manifestations in the aforementioned 3250 patients are noted in Table 3. When comparing their frequency according to sex, their proportion in female patients proved to be significantly higher than in male patients, which evidently agrees with the higher number of fasciolid infections in females (Table 3, Figure 1). Nonsignificant *P* values in this sex comparison were only obtained for anemia, bile duct bleeding, pericardial effusion, pancreatitis, and lung tumor, all detected in a very low number of patients.

In the analysis according to age group (Table 4, Figure 2), significant *P* values were found in the majority of manifestations, excepting anemia, bile duct bleeding, pericardial effusion, pancreatitis, and lung tumor, for which the comparison test was not applicable due to sample size deficit (Figure 2).

Among patients presenting with liver abscess, a 40-year-old woman suffered from upper stomach ache, against which she was unsuccessfully treated during 1 month. Extreme suffering one night led her to emergency assistance in Ha Noi University Hospital where she underwent surgery for intestine caseation and peritonitis. Surgeons removed 2 m of intestine and cleaned the abdominal cavity. Blood examination at this occasion included parasitological tests. A positive ELISA with *Fasciola* antigen (titer 1:6400, optical density = 3.8) and 31% eosinophilia led to specific treatment with 20 mg/kg triclabendazole, after which the patient was cured in 1 month.

Neurological, Meningeal, Neuropsychic, and Ocular Disorders

The high number of patients presenting with neurological, meningeal, and/or neuropsychic disorders (Table 5) and those with ocular disorders (Table 6) are worth mentioning. Delusional disorders and delirious condition, altered consciousness, vigilance disorders, temporo-spatial disorientation, confusion, and amnesia are worryingly high. Diplopia, nystagmus, and vision reduction and the absence of amaurosis should be highlighted.

Females show a significantly higher risk to suffer from cephalalgias (headache, migraines), vertigo and nightmares, delusional or delirious condition, insomnia, and vigilance disorders ($P \leq .001$). No significant sex differences appear in other neurological, meningeal, neuropsychic, and ocular disorders (Figure 3).

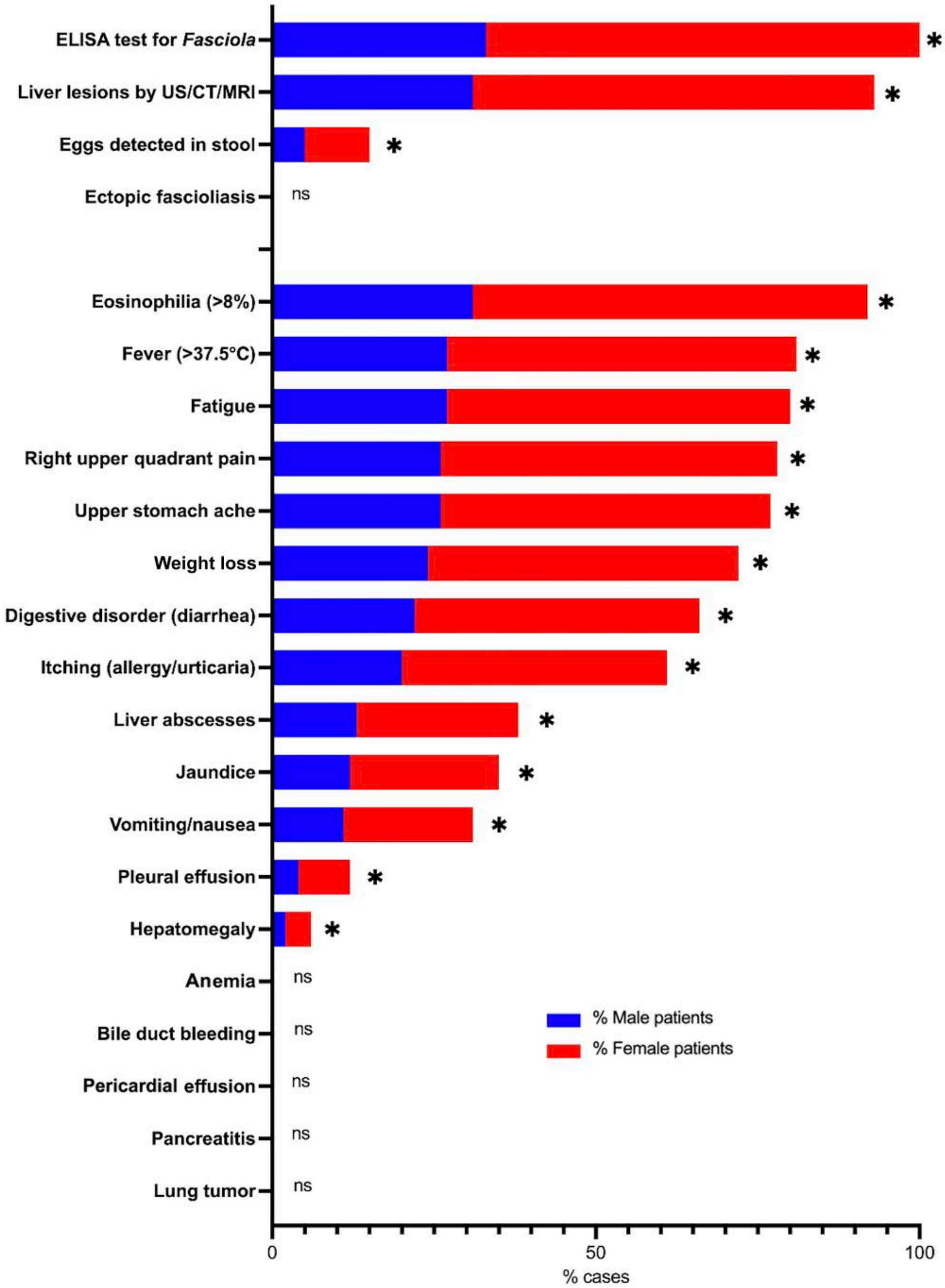


Figure 1. Proportions of symptoms and manifestations in 3250 fascioliasis infected patients in Vietnam and their comparison according to sex. Bars are proportions of infected patients (% cases). *Statistically significant differences when comparing sex proportions in each symptom and manifestation (Fisher exact test, 2-sided $P < .025$); no significant differences were detected in the comparison between the proportions of symptoms and manifestations (χ^2 test, $P > .996$). Abbreviations: CT, computed tomography; ELISA, enzyme-linked immunosorbent assay; MRI, magnetic resonance imaging; ns, not significant; US, ultrasound.

Significant *P* values were detected between age groups in most of the neurological, meningeal, neuropsychic, and ocular disorders (Figure 4). In the remaining disorders, the comparison test was not applicable due to sample size deficit.

Anamnesis and Treatments

Among 3250 patients, 64.95% lived in rural areas, whereas 35.05% resided in urban areas and consumed vegetables from noncontrolled city markets. Most patients were linked to field activities in vegetable cultivation (64.03%) or livestock management (38.71%). Anamnesis of the great majority (92.68%) suggested infection by plant consumption and only sporadically by water drinking (0.49%).

All the patients were cured after treatment with triclabendazole: 90.03% after 1 dose and 9.97% after 2 doses. In all patients, eosinophilia, positive serology, and liver lesions

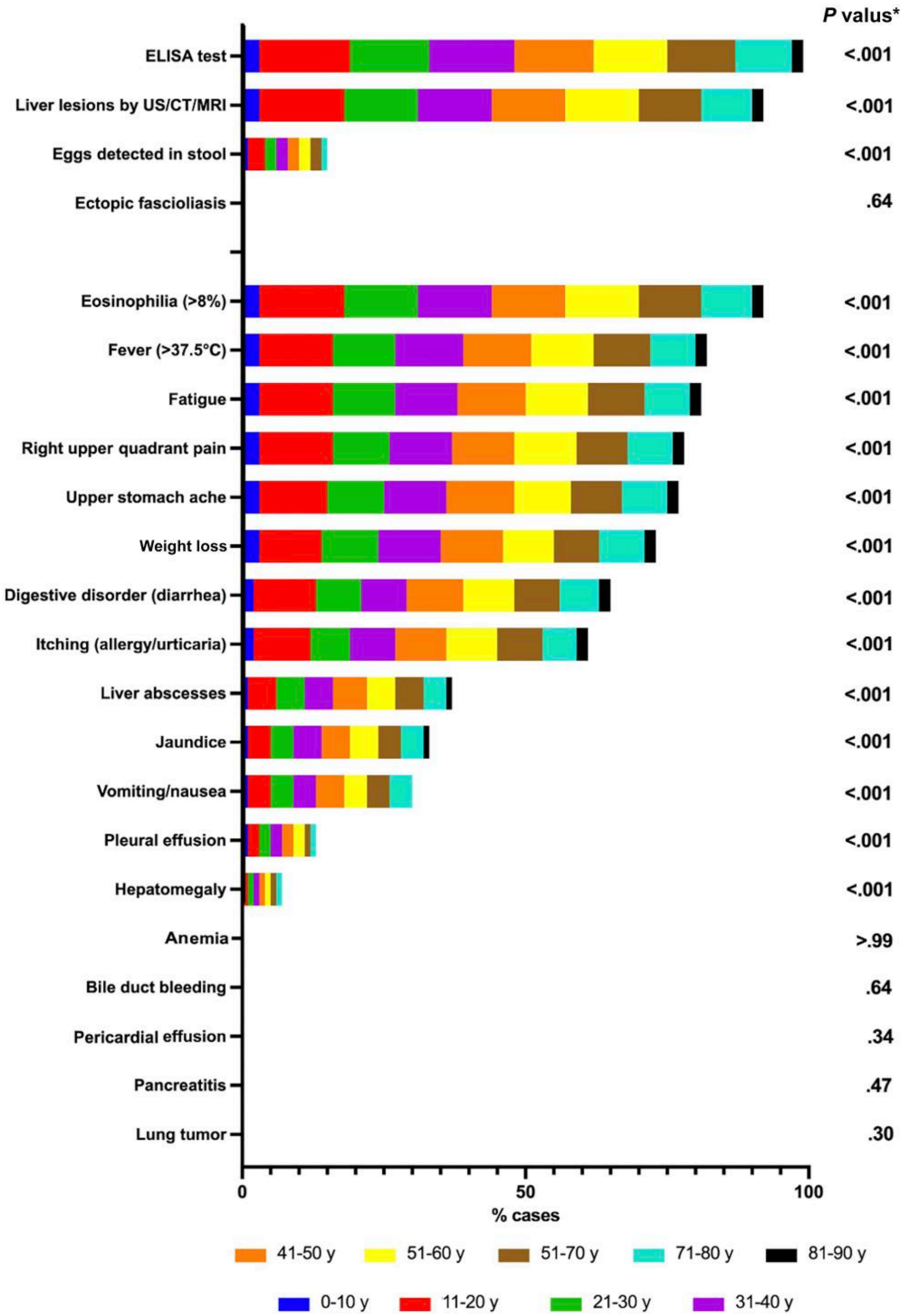


Figure 2. Proportions of symptoms, signs, and manifestations in 3250 fascioliasis infected patients in Vietnam and their comparison according to age. Bars are proportions of infected patients (% cases). *P values obtained when comparing age groups in each symptom, sign, and manifestation (χ^2 test, $P < .05$); no significant differences were detected in the comparison between the proportions of symptoms and manifestations (χ^2 test, $P > .676$). Abbreviations: CT, computed tomography; ELISA, enzyme-linked immunosorbent assay; MRI, magnetic resonance imaging; US, ultrasound.

disappeared after 6 months, while itching and fecal eggs disappeared in a maximum of 3 months. No patient was in need of a third dose. The only secondary effect was light headache, which disappeared quite rapidly. Most patients showed a posttreatment increasing weight, in several cases up to even 10 kg. No patient died, although 10.03% were hospitalized for an average of 8 days, and 0.18% received surgical intervention.

Table 3. Typical Symptoms, Signs, and Manifestations in 3250 Patients With Serologically Positive Fascioliasis in Vietnam, by Sex

Symptoms, Signs, and Manifestations	Male (n = 1088)	% Male (95% CI)	Female (n = 2162)	% Female (95% CI)	*P* Value[a]
Eosinophilia (>8%)	1005	30.92 (29.36–32.53)	1977	60.83 (59.14–62.50)	≤.001[b]
Fever (>37.5 °C)	886	27.26 (25.76–28.82)	1765	54.31 (52.59–56.01)	≤.001[b]
Fatigue	875	26.92 (25.43–28.47)	1737	53.45 (51.73–55.16)	≤.001[b]
Right upper quadrant pain	847	26.06 (24.58–27.60)	1676	51.57 (49.85–53.28)	≤.001[b]
Upper stomach ache	832	25.60 (24.13–27.13)	1652	50.83 (49.11–52.55)	≤.001[b]
Weight loss	786	24.18 (22.74–25.69)	1565	48.15 (46.44–49.87)	≤.001[b]
Digestive disorder (diarrhea)	719	22.12 (20.73–23.58)	1423	43.78 (42.09–45.50)	≤.001[b]
Itching (allergy/urticaria)	665	20.46 (19.11–21.88)	1318	40.55 (38.88–42.25)	≤.001[b]
Liver abscesses	421	12.95 (11.84–14.15)	825	25.38 (23.92–26.91)	≤.001[b]
Jaundice	385	11.85 (10.78–13.00)	736	22.65 (21.24–24.12)	≤.001[b]
Vomiting/nausea	351	10.80 (9.78–5.23)	634	19.51 (18.18–20.91)	≤.001[b]
Pleural effusion	145	4.46 (3.80–34.37)	276	8.49 (7.58–9.50)	≤.001[b]
Hepatomegaly	76	2.34 (1.87–2.92)	135	4.15 (3.52–4.90)	≤.001[b]
Anemia	8	.25 (.12–.49)	12	.37 (.21–.64)	.50
Bile duct bleeding	7	.22 (.10–.44)	11	.34 (.19–.61)	.48
Pericardial effusion	2	.06 (.01–.22)	4	.12 (.05–.32)	.68
Pancreatitis	2	.06 (.01–.22)	3	.09 (.03–.27)	>.99
Lung tumor	2	.06 (.01–.22)	2	.06 (.01–.22)	>.99
P value	…	…	…	…	>.996[c]

Patients may manifest >1 symptom.

Abbreviation: CI, confidence interval.

[a]Analyzed with 2-sided Fisher exact test.

[b]Significant differences in the comparison between the proportions of female and male patients.

[c]Analyzed with χ^2 test; statistically significant when $P < .05$.

Table 4. Symptoms, Signs, and Manifestations in 3250 Patients With Serologically Positive Fascioliasis in Vietnam, by Age Group

	Age Group, y											
Symptoms, Signs, and Manifestations	0–5	6–10	11–15	16–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	Total
Eosinophilia (>8%)[a]	19	77	129	345	432	428	426	412	362	285	67	2982
Fever (>37.5 °C)[a]	18	78	127	294	348	381	377	352	340	271	65	2651
Fatigue[a]	19	75	128	291	345	371	375	350	328	264	66	2612
Right upper quadrant pain[a]	18	74	126	282	334	365	373	347	291	251	62	2523
Upper stomach ache[a]	17	71	121	283	335	361	375	323	287	246	65	2484
Weight loss[a]	15	67	100	261	322	345	358	289	275	255	64	2351
Digestive disorder (diarrhea)[a]	16	65	98	254	268	270	321	277	271	241	61	2142
Itching (allergy/urticaria)[a]	14	54	87	236	243	256	297	288	251	205	52	1983
Liver abscesses[a]	10	32	49	122	153	175	185	176	158	141	45	1246
Jaundice[a]	11	29	46	91	146	151	172	163	145	135	32	1121
Vomiting/nausea[a]	9	31	44	86	121	146	151	142	121	118	16	985
Pleural effusion[a]	6	23	27	45	54	60	59	58	43	40	6	421
Hepatomegaly[a]	3	6	12	23	29	32	30	26	24	21	5	211
Anemia	1	2	2	3	3	4	1	2	1	2	1	22
Bile duct bleeding	…	1	1	2	4	3	2	2	2	1	…	18
Pericardial effusion	1	1	…	1	…	2	1	…	…	…	…	6
Pancreatitis	…	…	…	…	1	1	2	1	…	…	…	5
Lung tumor	…	…	…	…	…	2	1	1	…	…	…	4

Patients may manifest >1 symptom.

[a]Significant differences in the comparison between the proportions of patient age groups. See *P* values in Figure 2.

DISCUSSION

Diffusion through radio about symptoms proved useful by leading patients to health centers for cost-free diagnosis and treatment after symptom onset [19]. This explains the early diagnosis in the majority of patients and the disagreement between the lower number of coprologically positive compared to serologically positive patients.

Table 5. Neurological, Meningeal, and Neuropsychic Disorders in 3250 Patients With Serologically Positive Fascioliasis in Vietnam, by Sex and Age Group

	Sex		Age Group, y											
Neurological, Meningeal, and Neuropsychic Disorders	M	F	0–5	6–10	11–15	16–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	Total
Cephalalgias (headache, migraines)[a,b]	221	430	10	25	36	65	97	98	99	86	61	55	19	651
Vertigo, nightmares[a,b]	120	222	5	12	18	38	51	52	49	45	33	29	10	342
Delusional disorders, delirious condition[a,b]	34	61	2	3	5	12	15	14	13	12	9	8	2	95
Insomnia[a,b]	55	97	2	5	6	19	22	24	23	20	17	11	3	152
Sleepiness[b]	75	86	3	5	7	20	23	24	25	21	18	12	3	161
Altered consciousness[b]	21	31	1	2	3	8	9	11	6	5	4	2	1	52
Vigilance disorders[a,b]	41	70	2	3	5	15	16	19	18	14	10	8	1	111
Temporo-spatial disorientation[b]	28	33	1	3	4	8	11	12	6	8	5	2	1	61
Confusion, amnesia[b]	35	51	2	3	5	12	13	15	12	11	8	3	2	86
Character disorders (instability)	14	21	1	2	4	5	5	5	4	3	2	2	2	35
Irritability[b]	29	46	2	3	6	12	13	12	11	7	6	2	1	75
Paresis	6	9	…	1	1	1	3	2	3	2	1	1	…	15
Paralysis (of limbs)	…	1	…	…	…	…	…	…	…	…	…	1	…	1
Walking problems and movement disorders	1	1	…	…	…	…	…	…	…	…	…	1	1	2
Paraesthesia (of limbs)	2	1	…	1	…	1	…	…	…	…	1	…	…	3
Speech disorders (dysphasia, dysarthria, aphasia)	1	…	…	…	…	…	…	…	…	…	…	1	…	1
Loss of senses (anosmia, ageusia)	…	2	…	…	…	…	1	…	…	…	…	…	1	2
Convulsions, epilepsy	1	…	…	1	…	…	…	…	…	…	…	…	…	1
Babinski sign	1	…	…	…	…	…	…	…	…	…	…	1	…	1
Hoffmann sign	2	2	…	…	…	1	…	1	…	…	1	1	…	4
Kernig sign	2	4	1	2	1	…	…	…	…	…	…	1	1	6
Meningitis symptoms	2	4	1	2	1	…	…	…	…	…	…	1	1	6
Mental disorders (phobias, pronounced fear)	1	3	…	…	…	1	1	…	1	…	1	…	…	4
Alternating depression/euphoria episodes	2	1	…	…	…	1	…	1	…	1	…	…	…	3
Psychiatric or neuropsychic manifestations	1	3	…	…	…	1	1	…	1	…	1	…	…	4

Patients may manifest >1 symptom.

Abbreviations: F, female; M, male.

[a]Significant differences in the comparison between the proportions of female and male patients. See *P* values in Figure 3.

[b]Significant differences in the comparison between the proportions of patient age groups. See *P* values in Figure 4.

Hence, the clinical picture should be considered for the assessment of the acute phase, but only with caution for the chronic phase. Most patients shedding eggs were probably at the beginning of the chronic phase, that is, patients manifesting first symptoms at the end of the acute phase. Consequently, this explains why disorders typical of the advanced chronic phase of human fascioliasis are absent or rare in Vietnam.

Symptom onset is from a few days to 6 weeks after infection; juvenile flukes reach the liver from the sixth day and migrate in the hepatic parenchyma at least for 5–6 weeks, and the adult begins egg production 3–4 months after infection [11]. In *F gigantica* infections, these phases are delayed and eggs in stools appear 1–2 weeks later than in *F hepatica* infection [13]. Serology and coprology detect infection from 2 weeks and 3.5–4.5 months after infection, respectively [11]. Thus, liver lesion detection in 92.05% and positive coprology in only 14.83% of the ELISA-positive patients indicates that most patients were diagnosed in the initial acute phase. In Vietnam, length/width (in μm) of eggs shed by humans is 150.9–182.2/85.1–106.2 [13], which perfectly fits egg size of 137.2–191.1/73.5–120.0 of genetically pure *F gigantica* shed by humans in different world regions [11]. This is similar when dealing with eggs shed by animals (length/width): 156.2–182.8/90.6–114.9 in Vietnam [13] and 129.6–204.5/61.6–112.5 in different world regions [11]. Sporadic patients infected by fasciolids producing no eggs because of insufficient adaptation to the human host [11] should, however, not be ruled out.

An experimental long-term follow-up of serum biochemical markers as morbidity indicators demonstrated that *F gigantica* is more pathogenic than *F hepatica*, mostly due to its bigger size [13]. Unfortunately, present knowledge of the clinical picture caused by *F gigantica* is insufficient for this purpose, because of including only sporadic individual case reports, or a very few case series that include either a very short number of patients or concern areas where the 2 genetically pure fasciolid species geographically overlap and the specific causality was not verified. Examples are clinical studies in Egypt [26] and

Table 6. Ocular Disorders in 3250 Patients With Serologically Positive Fascioliasis in Vietnam, by Sex and Age Group

	Sex		Age Group, y											
Ocular Disorders	M	F	0–5	6–10	11–15	16–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	Total
Blurred vision	5	7	…	…	…	1	2	1	2	1	2	2	1	12
Sudden bilateral reduction of the visual acuity	3	5	…	…	…	1	1	…	1	…	1	2	2	8
Eye-roll sign	1	…	…	…	1	…	…	…	…	…	…	…	…	1
Retrocular pain and unilateral exophthalmos	5	10	…	1	1	1	2	1	3	…	2	3	1	15
Transitory or intermittent diplopia	5	9	…	1	1	3	2	1	2	1	1	2	…	14
Unilateral concentric narrowing of the field of vision	2	3	…	…	…	1	…	1	…	1	…	1	1	5
Disorders of the visual accommodation	1	3	…	…	1	…	1	…	1	…	…	1	…	4
Nystagmus	3	4	…	1	1	2	1	…	1	…	1	…	…	7
Visual hallucinations	1	1	…	…	…	…	…	…	…	…	…	1	1	2
Visual deformation and image superimposition	1	1	…	…	…	…	…	…	…	1	1	…	…	2
Partial to almost complete vision loss	…	2	…	…	…	…	…	…	…	…	…	1	1	2
Bulge-sticking pain	4	6	…	1	1	1	1	2	1	…	1	1	1	10
Photophobia	5	6	…	1	1	1	2	2	2	1	1	…	…	11

Patients may manifest >1 symptom.

Abbreviations: F, female; M, male.

[a]Significant differences in the comparison between the proportions of female and male patients. See *P* values in Figure 3.

[b]Significant differences in the comparison between the proportions of patient age groups. See *P* values in Figure 4.

southern China [18]. In the latter endemic area, despite *F gigantica* having been highlighted to be the only fasciolid species present, 2 photographs of flukes recovered from patients illustrating morphologically typical *F hepatica* specimens pose doubts about this specific diagnosis [27]. Thus, the 14 cases included in an old series from Hawaii offered the only so far verified *F gigantica*–caused clinical picture [28], as *F hepatica* never colonized this archipelago [1].

The occurrence of ectopic cases agrees with previous reports molecularly proving *F gigantica* to also be involved in such presentations in Vietnam [29].

Symptoms, signs, and manifestations and their frequency in Vietnamese patients fit the typical clinical picture of human fascioliasis [4–6]. Bleeding, pancreatitis, and lung tumor are severe complications. Unusual colic manifestations were only observed in 2 patients with colon tumors and in 1 with colon perforation caused by ectopic worms found during surgery.

The high proportion of fatigue and pleural effusion may be considered exceptions. Fatigue may be related to infection by numerous juveniles during the acute phase. Unfortunately, serological techniques are useless for quantitative diagnosis [11]. Pleural effusion by *F gigantica* was already noted in Hawaii [28]. An excess of serous fluid within the pleural space is known to be caused by liver affection and infections. In fascioliasis, lithiasis and cirrhosis are long-term–appearing disorders [30–32], absent in Vietnam because of early patient diagnosis. Pulmonary manifestations in fasciolid infection are rare and mainly include Loeffler type infiltrates and pleural hemorrhage, but may be frequent in patients presenting with neurological and/or ocular conditions [11].

The low frequency of anemia is surprising. Mild to moderate anemia usually appears in long-term infections, especially in cases of heavy parasitic burdens and also reinfections [33, 34]. The rarity of anemic patients should be due to the early diagnosis of patients, still in the initial acute phase and without having been reinfected.

The absence of lithiasis is also worth mentioning, because fasciolid infections are usually detected with imaging techniques [21]. Lithiasis has been reported to be frequent in patients infected by *F hepatica* [31]. However, lithiasis should a priori not be considered a difference between infections by *F gigantica* and those by *F hepatica*, because this is another disorder appearing in advanced chronic infection [30].

In Vietnam, neurological, meningeal, neuropsychic, and ocular disorders are noteworthy. Mainly *F hepatica* but sporadically also *F gigantica* have been involved in patients presenting with such disorders [4]. All these manifestations had already been reported in fascioliasis patients elsewhere before [4]. Nothing indicates *F gigantica* to be different from *F hepatica* in its capacity to induce such disorders in humans. Ingestion of highly metacercariae-contaminated vegetables should underlie such disorders, which occur more frequently in the acute than in the chronic phase and are among the initial, when they are not the first, symptoms to appear [4, 35].

The number of Vietnamese patients presenting with such disorders agrees with prevalences known in other countries. Indeed, such disorders are pronouncedly more frequent during the acute phase [4], which agrees with the early patient diagnosis. Similarly as elsewhere, mild neurological and meningeal

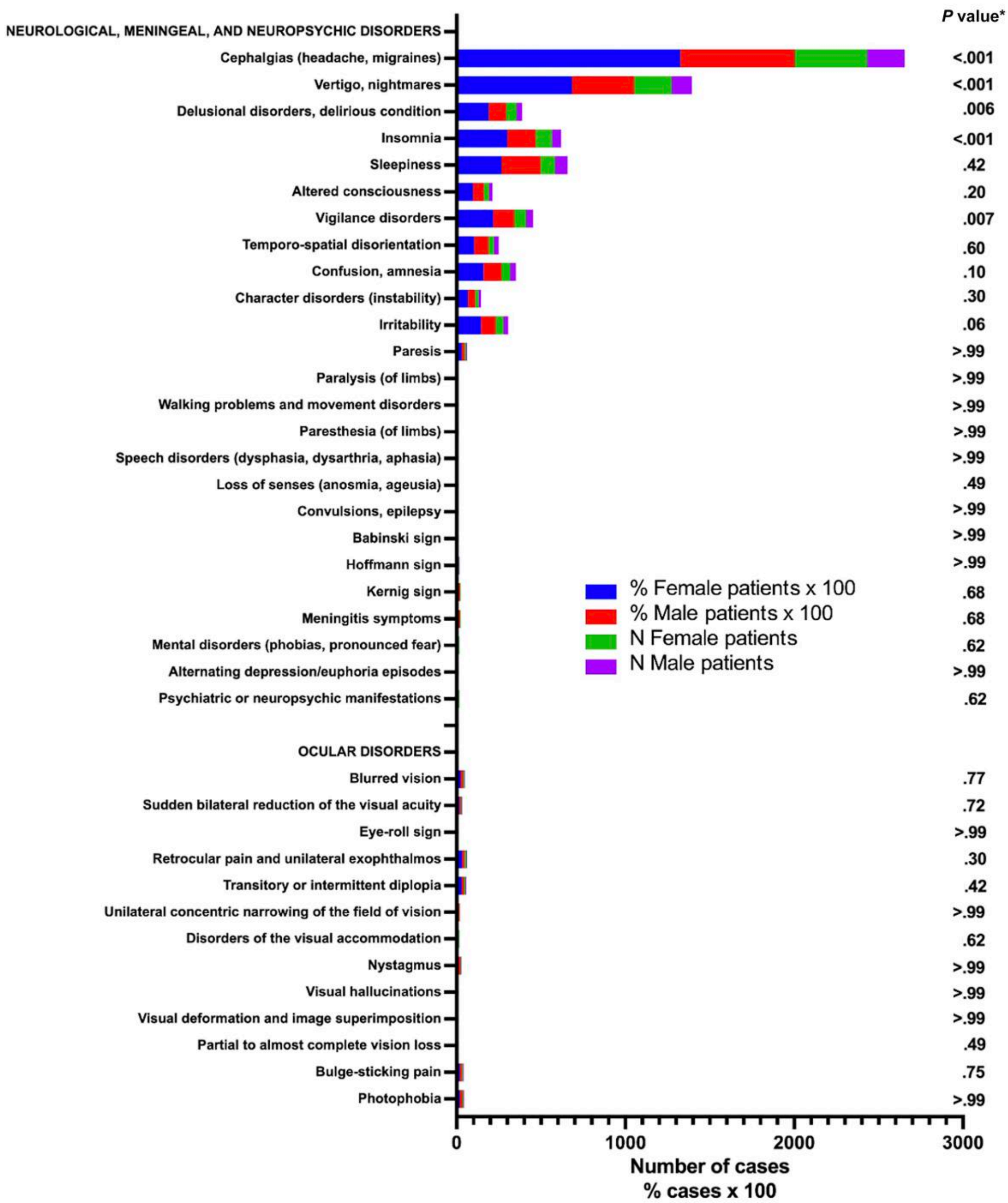


Figure 3. Proportions of neurological, meningeal, neuropsychic, and ocular disorders in 3250 fascioliasis infected patients in Vietnam and their comparison according to sex. Bars are proportions of infected patients (% of cases). **P* value obtained when comparing sex groups in each neurological, meningeal, neuropsychic, and ocular disorder (χ^2 test), noted in the right margin; no significant differences were detected in the comparison between the neurological, meningeal, and ocular disorders (χ^2 test, $P = .88$); statistically significant when $P < .05$.

manifestations are the most frequent, whereas severe disorders known to worsen with time and giving rise to sequelae in the long term are rare.

Neurofascioliasis or intracranial infection by *Fasciola* and ophthalmofascioliasis or direct eye affection by migrating flukes are rare but not sporadic, whereas neurological, meningeal, and neuropsychic manifestations, and ocular disorders caused at distance by liver-infecting flukes, may be frequent [4]. Impressive clinical pictures differing between patients, puzzling polymorphism complexity, and changing manifestation multifocality along the evolution of the disease in the same patient are disconcerting and easily lead to

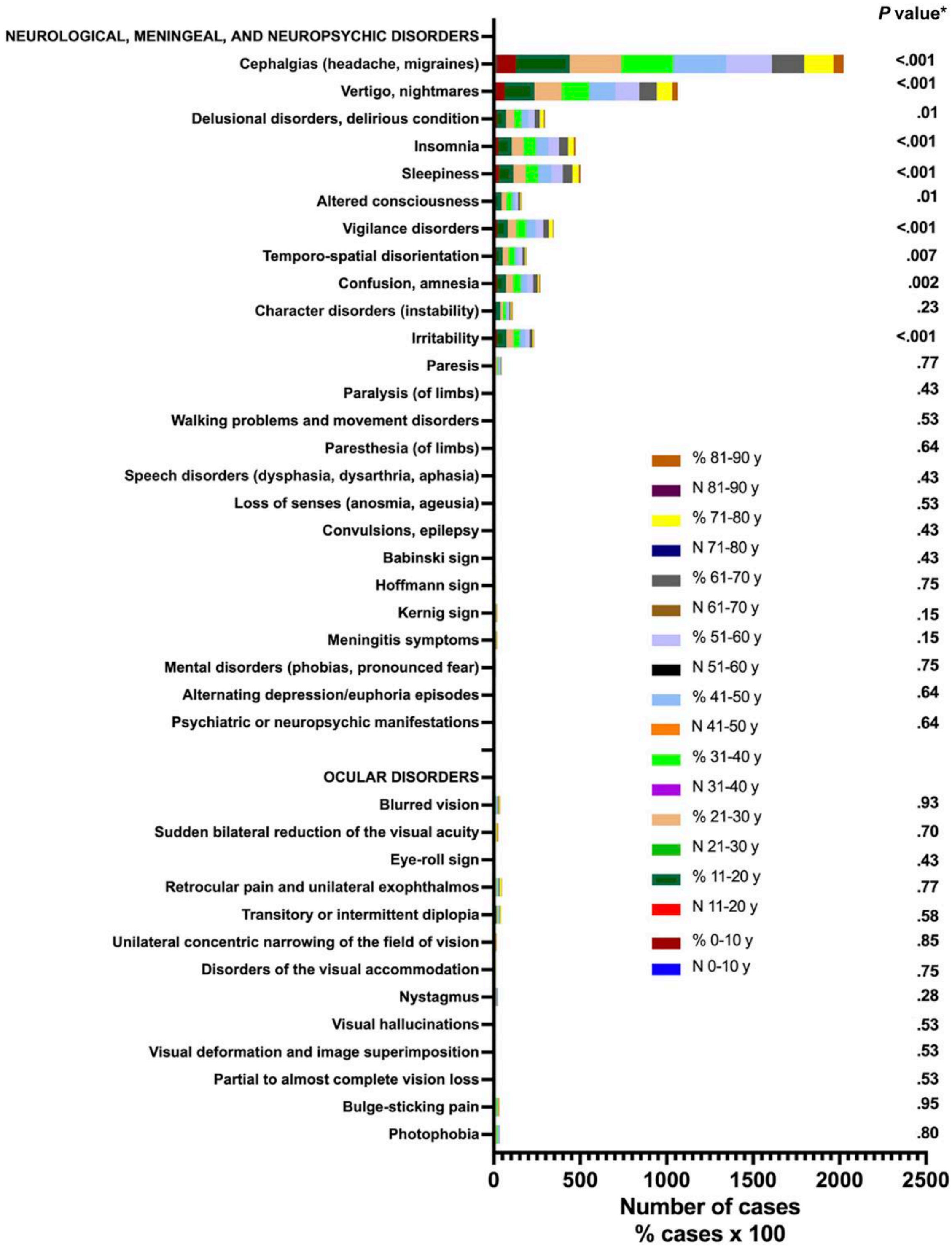


Figure 4. Proportions of neurological, meningeal, neuropsychic, and ocular disorders in 3250 fascioliasis infected patients in Vietnam and their comparison according to age groups. Bars are proportions of infected patients (% of cases × 100). **P* value, obtained when comparing age groups in each neurological, meningeal, neuropsychic, and ocular disorder (χ^2 test), noted in the right margin; no significant differences have been detected in the comparison between the neurological, meningeal, and ocular disorders (χ^2 test, $P = .14$); statistically significant when $P < .05$.

misdiagnosis, mainly in low-income regions [4]. Moreover, posttreatment sequelae and mortality have been emphasized in such neurological patients [4].

Interactions between diverse *Fasciola* infection situations and alterations of the fibrinolytic system and the plasma kallikrein-kinin system (also known as contact system) explain the complexity, heterogeneity, and timely variations of neurological disorders [35]. Numerous *Fasciola*-secreted plasminogen-binding proteins enhancing plasmin generation may underlie blood-brain barrier leakage by the proinflammatory peptide bradykinin, whether by many juveniles in the acute phase [36], or breakage of encapsulating formations including adults in the chronic phase [35].

The proportions of patients living in rural or urban areas, activities related to vegetables/livestock, and plant/water infection sources fit this epidemiological scenario recently described for the transmission areas in Vietnam [19].

Anamnesis about symptom onset before hospitalization from 307 patients throughout 1997–2000 (49%, 26%, 22%, and 3% of 1, 2, 3–4, and ≥5 months, respectively) [37] agrees with the general early diagnosis of the patients facilitated by radio broadcasting.

Treatments evidence the usefulness of triclabendazole, highly efficient in all disease phases, against *F gigantica* and hybrids and the absence of resistance to this drug in Vietnam [38].

CONCLUSIONS

This is the first sufficiently wide study of the clinical picture caused by *F gigantica*. The assessment has been made in a population without previous contact with fascioliasis [19] and shows that *F gigantica* (and *F gigantica*–like hybrids) do not cause clinical pictures different from those by *F hepatica*.

Early patient detection and treatment should be highlighted, because of avoiding severe long-term complications and also posttreatment sequelae, which mainly appear in patients having been treated late [4, 7, 31]. Combining radio broadcasting facilitating the passive detection of symptomatic patients and their treatment, thanks to the WHO triclabendazole donation program, is a helpful strategy [19].

The clinical picture assessed in Vietnam may be useful for physicians and health officers of south and southeastern countries of Asia where fascioliasis cases caused by *F gigantica* (and *F gigantica*–like hybrids) are at present emerging [16], but also in Africa where human infection is increasingly being reported [39, 40].

In these 2 continents including areas where *F gigantica* and *F hepatica* overlap, care should be taken with diagnostic techniques that are not able to differentiate between the 2 “pure” fasciolid species and also between them and nuclear ribosomal DNA (rDNA) admixed and/or mitochondrial DNA (mtDNA) introgressed hybrids. This is the case with serological tests [11] and also several molecular methods that are insufficient for hybrid detection. The report of a female patient infected in a trip to Vietnam and diagnosed to be infected by *F hepatica* in France, by using serology and the technique of recombinase polymerase amplification (RPA) for *F hepatica* DNA detection in stool and pus aspirate, is such an example [41]. The RPA technique is only based on nuclear rDNA ITS-1, whose sequence in *F gigantica* and *F hepatica* differs only in 5 polymorphic nucleotide positions. In southern Asia, including from India up to Vietnam, hybrid specimens are originated by crossbreeding of autochthonous *F gigantica* with *F hepatica* introduced by imported livestock. The consequent offspring include admixed hybrids, which show a very wide heterozygotic variability in these aforementioned 5 positions, whose detection needs nucleotide sequencing and cloning for single-nucleotide polymorphism analysis [16, 17, 19, 42]. The RPA technique is, therefore, useless for the detection of admixed hybrids, and also introgressed hybrids because of not analyzing mtDNA, and to differentiate them from the “pure” fasciolids [43].

Notes

Acknowledgments. Studies of this article have been performed within the framework of the global initiative of WHO against human fascioliasis. Authors acknowledge the collaboration by staff from the WHO Western Pacific Region Office and the National Institute of Malariology, Parasitology and Entomology in Ha Noi, Institute of Malariology, Parasitology and Entomology in Quy Nhon, and Ho Chi Minh Hospital of Tropical Diseases.

Author contributions. Study concept and design: N. V. D. and S. M.-C. Participated in patient diagnosis: N. V. D., P. N. M., D. T. D., T. T. D., B. V. T., L. T. D., and N. V. V. C. Local and international coordination, protocols, and logistics: N. V. D., T. H. L., A. M., and S. M.-C. Analysis and interpretation of data: S. M.-C., N. V. D., P. F. C., M. D. B., and M. A. V. Drafting of the manuscript: S. M.-C. and N. V. D. Critical revision of the manuscript for important intellectual content and for final approval: N. V. D., T. H. L., P. F. C., M. D. B., M. A. V., A. F. G., A. M., and S. M.-C. Obtained project funding: N. V. D., M. D. B., A. M., and S. M.-C. Principal investigator: S. M.-C.

Data availability. Datasets generated for this study are available on request to the corresponding author.

Financial support. This work was supported by the National Foundation for Science and Technology Development in Vietnam (grant number 114/QD-HDQL-NAFOSTED). Spanish collaboration was funded by the CIBER de Enfermedades Infecciosas Project CB21/13/00056, Instituto de Salud Carlos III, Ministry of Science and Innovation, and European Union–NextGenerationEU, Madrid, Spain; the PROMETEO Program, Programa of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain (project numbers 2016/099 and 2021/004); and Project of the Marie Skłodowska-Curie Actions program (Horizon Europe), European Commission, Brussels, Belgium (grant number 101062347).

Potential conflicts of interest. All authors: No potential conflicts of interest.

Disclaimer. The funders had no influence on the design or conduct of the study and were not involved in data collection or analysis, the writing of the manuscript, or the decision to submit it for publication.

Patient consent. All patients individually sought diagnosis and treatment in the hospitals of the authors. Individual consents given at hospitals participating in the present study and waived by the Institutional Review Board for Ethics in Biomedical Research, Ha Noi Medical University, Ministry of Health, Ha Noi, Vietnam, for the present general study (Approval number 4018/HMUIRB, 26 December 2018).

References

1. Mas-Coma S, Valero MA, Bargues MD. Human and animal fascioliasis: origins and worldwide evolving scenario. Clin Microbiol Rev **2022**; 35:e0008819.
2. Bargues MD, Mangold AJ, Muñoz-Antoli C, Pointier JP, Mas-Coma S. SSU rDNA characterization of lymnaeid snails transmitting human fascioliasis in South and Central America. J Parasitol **1997**; 83:1086–92.

3. World Health Organization. Ending the neglect to attain the sustainable development goals. A road map for neglected tropical diseases 2021–2030. 2020. Available at: https://www.who.int/publications/i/item/9789240052932. Accessed 23 July 2020.
4. Mas-Coma S, Agramunt VH, Valero MA. Neurological and ocular fascioliasis in humans. Adv Parasitol **2014**; 84:27–149.
5. Chen MG, Mott KE. Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. Trop Dis Bull **1990**; 87:R1–38.
6. Arjona R, Riancho JA, Aguado JM, Salesa R, Gonzalez-Macias J. Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. Medicine (Baltimore) **1995**; 74:13–23.
7. Rondelaud D, Dreyfuss G, Vignoles P. Clinical and biological abnormalities in patients after fasciolosis treatment. Méd Mal Infect **2006**; 36:466–8.
8. Dalton JP, Robinson MW, Mulcahy G, O'Neill SM, Donnelly S. Immunomodulatory molecules of *Fasciola hepatica*: candidates for both vaccine and immunotherapeutic development. Vet Parasitol **2013**; 195:272–85.
9. Girones N, Valero MA, Garcia-Bodelon MA, et al. Immune supression in advanced chronic fascioliasis: an experimental study in a rat model. J Infect Dis **2007**; 195:1504–12.
10. Angles R, Buchon P, Valero MA, Bargues MD, Mas-Coma S. One Health action against human fascioliasis in the Bolivian Altiplano: food, water, housing, behavioural traditions, social aspects, and livestock management linked to disease transmission and infection sources. Int J Environm Res Publ Health **2022**; 19:1120.
11. Mas-Coma S, Bargues MD, Valero MA. Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitology **2014**; 141:1918–46.
12. Valero MA, Panova M, Mas-Coma S. Phenotypic analysis of adults and eggs of *Fasciola hepatica* by computer image analysis system. J Helminthol **2005**; 79: 217–25.
13. Valero MA, Bargues MD, Khoubbane M, et al. Higher physiopathogenicity by *Fasciola gigantica* than by the genetically close *F. hepatica*: experimental long-term follow-up of biochemical markers. Trans R Soc Trop Med Hyg **2016**; 110: 55–66.
14. Afshan K, Fortes-Lima CA, Artigas P, Valero MA, Qayyum M, Mas-Coma S. Impact of climate change and man-made irrigation systems on the transmission risk, long-term trend and seasonality of human and animal fascioliasis in Pakistan. Geospat Health **2014**; 8:317–34.
15. Qureshi AW, Tanveer A, Mas-Coma S. Epidemiological analysis of human fascioliasis in northeastern Punjab, Pakistan. Acta Trop **2016**; 156:157–64.
16. Bargues MD, Artigas P, Varghese GM, et al. Human fascioliasis emergence in southern Asia: complete nuclear rDNA spacer and mtDNA gene sequences prove Indian patient infection related to fluke hybridization in northeastern India and Bangladesh. One Health **2024**; 18:100675.
17. Ahasan SA, De Elías-Escribano A, Artigas P, et al. Wide variation of heterozygotic genotypes of recent fasciolid hybrids from livestock in Bangladesh assessed by rDNA internal transcribed spacer region sequencing and cloning. One Health **2023**; 17:100614.
18. Chen JX, Chen MX, Ai L, et al. An outbreak of human *Fascioliasis gigantica* in Southwest China. PLoS One **2013**; 8:e71520.
19. De NV, Minh PN, Le TH, et al. A multidisciplinary analysis of over 53,000 fascioliasis patients along the 1995–2019 countrywide spread in Vietnam defines a new epidemiological baseline for One Health approaches. One Health **2024**; 19: 100869.
20. Mas-Coma S. Human fascioliasis: epidemiological patterns in human endemic areas of South America, Africa and Asia. Southeast Asian J Trop Med Publ Health **2004**; 35(Suppl 1):1–11.
21. De NV, Le TH, Agramunt VH, Mas-Coma S. Early postnatal and preschool age infection by *Fasciola* spp.: report of five cases from Vietnam and worldwide review. Am J Trop Med Hyg **2020**; 103:1578–89.
22. Nguyen TGT, Le TH, De NV, et al. Assessment of a 27-kDa antigen in enzyme-linked immunosorbent assay for the diagnosis of fasciolosis in Vietnamese patients. Trop Med Int Health **2010**; 15:462–7.
23. Bächler P, Baladron MJ, Menias C, et al. Multimodality imaging of liver infections: differential diagnosis and potential pitfalls. RadioGraphics **2016**; 36: 1001–23.
24. Gandhi P, Schmitt EK, Chen CW, Samantray S, Venishetty VK, Hughes D. Triclabendazole in the treatment of human fascioliasis: a review. Trans R Soc Trop Med Hyg **2019**; 113:797–804.
25. Mas-Coma S, Bargues MD, Valero MA. Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. Parasitology **2018**; 145:1665–99.
26. Ibrahim N, Khalek EMA, Makhlouf NA, et al. Clinical characteristics of human fascioliasis in Egypt. Sci Rep **2023**; 13:16254.
27. Huang L, Li F, Su H, Luo J, Gu W. Emerging human fascioliasis: a retrospective study of epidemiological findings in Dali, Yunnan province, China (2012–2021). Med Sci Monit **2023**; 29:e940581.
28. Stemmermann GN. Human infestation with *Fasciola gigantica*. Am J Pathol **1953**; 29:731–59.
29. Le TH, De NV, Agatsuma T, et al. Molecular confirmation that *Fasciola gigantica* can undertake aberrant migrations in human hosts. J Clin Microbiol **2007**; 45: 648–50.
30. Valero MA, Santana M, Morales M, Hernandez JL, Mas-Coma S. Risk of gallstone disease in advanced chronic phase of fascioliasis: an experimental study in a rat model. J Infect Dis **2003**; 188:787–93.
31. Mera y Sierra R, Agramunt VH, Cuervo P, Mas-Coma S. Human fascioliasis in Argentina: retrospective overview, critical analysis and baseline for future research. Parasit Vector **2011**; 4:104.
32. Marcos LA, Bussalleu A, Terashima A, Espinoza JR. Detection of antibodies against *Fasciola hepatica* in cirrhotic patients from Peru. J Helminthol **2009**; 83:23–6.
33. Valero MA, Girones N, Garcia-Bodelon MA, et al. Anaemia in advanced chronic fasciolosis. Acta Trop **2008**; 108:35–43.
34. Valero MA, Perez-Crespo I, Chillon-Marinas C, et al. *Fasciola hepatica* reinfection potentiates a mixed Th1/Th2/Th17/Treg response and correlates with the clinical phenotypes of anemia. PLoS One **2017**; 12:e0173456.
35. Gonzalez-Miguel J, Valero MA, Reguera-Gomez M, et al. Numerous *Fasciola* plasminogen-binding proteins may underlie blood-brain barrier leakage and explain neurological disorder complexity and heterogeneity in the acute and chronic phases of human fascioliasis. Parasitology **2019**; 146:284–98.
36. Serrat J, Becerro-Recio D, Torres-Valle M, et al. *Fasciola hepatica* juveniles interact with the host fibrinolytic system as a potential early-stage invasion mechanism. PLoS Negl Trop Dis **2023**; 17:e0010936.
37. Hien TV, Dung TTK, Chi NH, Danh PH, Hanh PT. Fascioliasis in Vietnam. Southeast Asian J Trop Med Publ Health **2001**; 32(Suppl 2):48–50.
38. Fairweather I, Brennan GP, Hanna REB, Robinson MW, Skuce PJ. Drug resistance in liver flukes. Int J Parasitol Drugs Drug Resist **2020**; 12:39–59.
39. Periago MV, Valero MA, Artigas P, et al. Very high fascioliasis intensities in schoolchildren from Nile Delta governorates, Egypt: the Old World highest burdens found in lowlands. Pathogens **2021**; 10:1210.
40. Dermauw V, Muchai J, Al Kappany Y, Castaneda ALF, Dorny P. Human fascioliasis in Africa: a systematic review. PLoS One **2021**; 16:e0261166.
41. L'Ollivier C, Eldin C, Lambourg E, Brouqui P, Lagier JC. Case report: first molecular diagnosis of liver abscesses due to *Fasciola hepatica* acute infection imported from Vietnam. Am J Trop Med Hyg **2020**; 102:106–9.
42. Le TH, De NV, Agatsuma T, et al. Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola hepatica* and *Fasciola gigantica* in Vietnam. Int J Parasitol **2008**; 38:725–30.
43. Mas-Coma S, Valero MA, Bargues MD. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol **2009**; 69:41–146.