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Molecular Confirmation that *Fasciola gigantica* Can Undertake Aberrant Migrations in Human Hosts▽

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Two cases of aberrant migration by the liver fluke *Fasciola gigantica* in humans are reported. In both cases, subadult worms emerged through the skin. The identity of the worms was confirmed from their DNA sequences. This uncommon human pathogen might be more likely than *F. hepatica* to undertake aberrant migrations in humans.

CASE REPORTS

Case 1. In May 2002, an 11-year-old girl from a province near Hanoi, Vietnam, complained of fever and pain in her abdominal region. On admission to Bach Mai National Hospital, her temperature was 39°C and she was diagnosed as having a liver abscess. She was transferred to Hanoi National Children's Hospital. The fever persisted throughout June and July 2002. Over this 3-month period, her weight dropped from 30 kg to 18 kg. Computed tomography (CT) scans showed two abscesses, each 2.5 cm by 4 cm, in her liver. Her eosinophilia was about 29%. Her lungs and heart were normal by X-ray photography. The patient underwent abdominal surgery for inspection of the liver for an abscess or a tumor. No lesions were observed on her liver. She was treated with antibiotics and traditional herbs. With no fever and showing good recovery, the patient was discharged at the end of July 2002.

In August 2002, she felt pain on her knee associated with a reddish, itchy, and inflamed swelling. While being treated in Hanoi Public Hospital E, a puncture on her knee was seen, and through this hole a vigorously moving, pink-colored flat fluke of 1.5 cm by 2.5 cm emerged. On the basis of its shape, the fluke was regarded as *Fasciola gigantica*, and a portion was kept in 70% ethanol for molecular identification. The spontaneous emergence of the fluke from the knee was 4 months after the appearance of the first diagnostic signals in the liver and 1 month after the appearance of the lesion on the knee. Serum collected when the fluke emerged yielded a titer of 1:12,800 by a standard enzyme-linked immunosorbent assay protocol (20) with native excreted-secreted protein antigen produced from *Fasciola gigantica* (by use of a slight modification of a previously described method [4, 11]). After the emergence of the worm, all clinical symptoms, on the knee and elsewhere, disappeared and the patient remains completely healthy.

Case 2. A 48-year-old Vietnamese woman from Quang Binh Province in central Vietnam presented to a local district hospital in February 2005 with periumbilical abdominal pain, high fever, and severe dizziness which was most pronounced in the late afternoon. The patient was examined by local doctors and diagnosed as having fascioliasis, and she was admitted to the Hue City Hospital. Taken together, the results of ultrasound scanning and serology (as described for case 1) confirmed the diagnosis of fascioliasis. She was given praziquantel (Distocide), a drug routinely prescribed for the treatment of trematode infections in Vietnam, to be taken in three courses at monthly intervals (note that triclabenzadole is now the drug of choice in Vietnam when fascioliasis is diagnosed). For the first two courses, she took two tablets (600 mg/tablet) per day for 2 days. In the third month, she took two tablets (600 mg/tablet) per day for 3 days. The patient's health improved, but her recovery was not regarded as complete. At the end of April 2005, a small red hard mass appeared on the skin of the left chest. The mass was painful and itchy, and the patient sometimes felt something moving in the mass. One month later the mass faded but another distinct red spot appeared on the skin of the right chest, and for a short time there was a tunnel linking this spot to the previous one. This spot was less painful but much harder. The patient then presented at the Hanoi Viet-Duc Hospital. Oncological examination indicated that the spot was nonmalignant. During the time of admission to the hospital throughout June and July 2005, ultrasound of the liver detected no signs of abscess. The patient was treated with cefuroxime (Zinnat; two tablets [500 mg/tablet] per day for 10 days). In October 2005, 8 months after the first signs of fascioliasis and 5 months after the first appearance of the tumor in the right chest, a very hot, red, itchy swelling appeared in the right breast not far from the previous spot in the right chest. The fever and dizziness returned. The patient was admitted to the Quang Binh provincial hospital, diagnosed as having abscess of the right breast and given prazepam (Centrax) (three vials [1 g/vial] per day for 15 days) for anxiety. The spot was punctured weekly for collection of exudate. On the second occasion on which this was done, in early December 2005, a

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TABLE 1. Primer pairs used for amplification of the entire nuclear ITS2 region and portions of the mitochondrial *nad1* and *cox1* genes[a]

Primers	Target gene	Sequence (5′→3′)	Size of amplicon (bp)
FND1F	*nad1*	TGGGGTCTGTTGCAGAGATTTGC	465[b]
FND1R	*nad1*	ATCCAATGGAGTACGGTTACA	
JB3F	*cox1*	TTTTTTGGGCATCCTGAGGTTTAT	448[b]
JB4.5R	*cox1*	TAAAGAAAGAACATAATGAAAATG	
3SF	ITS2	GGTACCGGTGGATCACTCGGCTCGTG	556[c]
BD2R	ITS2	TATGCTTAAATTCAGCGGGT	

[a] The same primers were used for sequencing of the cloned amplicons.
[b] The regions used for analysis were slightly shorter than this (see text).
[c] Includes flanking regions and portions of the 5.8S and 28S genes that were not used in analysis.

fluke about 1 cm wide and 2 cm long crawled out of the puncture site. It appeared to be a *Fasciola* species. This fluke was kept in 70% ethanol prior to molecular identification. After the emergence of the fluke, the patient felt no unusual symptoms, was kept under observation for 4 days in the hospital, and then returned home. One month later, in mid-January 2006, the patient was examined again and appeared to be in good health. The sites of the spots had faded to a dark color, and the hole where the fluke had emerged was visible only as a red point.

DNA sequences from the second internal transcribed spacer (ITS2; 361 bp were used in the analyses) of the nuclear ribosomal region and from portions of the mitochondrial *nad1* (456 bp) and *cox1* (402 bp) genes were obtained from the worm specimens from the two cases reported here by previously reported methods (1). The primers used for the PCR are shown in Table 1. The same gene regions were sequenced by us from a number of *Fasciola* samples collected in Vietnam and other countries (Australia, China, Indonesia, Uruguay). BLAST searches of the GenBank sequence database and comparisons with our own data clearly demonstrated that the flukes from the Vietnamese patients were pure *Fasciola gigantica*. It should be noted that hybrids between the two *Fasciola* species are known to exist in eastern Asia (1) and that their presence has recently been demonstrated in Vietnam by the use of molecular data (T. H. Le et al., data not shown). Hybrids inherit ITS2 sequences from both parents but inherit mitochondrial sequences only from the maternal species. ITS2 sequences of the two *Fasciola* species typically differ at five to six sites and by one single-base insertion/deletion. The intraspecific variation in the ITS2 region among isolates from eastern Asia is usually no greater than one site for either species (8, 9), although greater differences have been noted when specimens from other continents, especially Africa, have been considered (16). A typical ITS2 sequence for *F. gigantica* is GenBank accession number AB207149, and that for *F. hepatica* is GenBank accession number AB207148.

The mitochondrial sequences of the two species differ at many more sites than is the case for ITS2. However, there is also more intraspecific variation. For example, in the 392-bp region in which our *nad1* sequences overlap with most of those available in GenBank, the two species differ at 27 to 37 sites. Intraspecific variation in Asian *F. gigantica* strains occurs at up to 7 sites (and at up to 24 sites if the sequences of strains from southern Africa are included) and in *F. hepatica* strains at 4 sites. A typical *nad1* sequence for Asian *F. gigantica* is GenBank accession number AB207158, and that for *F. hepatica* is GenBank accession number AB207156. Taken together, ITS2 and mitochondrial sequences permit identification of both *Fasciola* species and their hybrids.

The two unrelated cases reported here resembled a case previously described from Vietnam, also in a woman (21). In all three cases, the worm had made an aberrant migration culminating in emergence through the skin at sites far from the liver or intestine. The presence of a liver abscess was suspected or confirmed. In none of the cases had the worms matured. *Fasciola* species in ectopic sites generally do not mature (13).

Fasciola species are primarily parasites of ruminants but may infect humans and can cause considerable pathology. Metacercariae form cysts on edible aquatic vegetation. Excysted metacercariae penetrate the intestinal wall and normally migrate to the liver, where they mature in the bile ducts and release eggs. In the liver they can form abscesses, often leading to a misdiagnosis of liver cancer by ultrasound or CT scanning, unless fascioliasis is suspected and appropriate serological tests are done. Some juveniles fail to localize in the liver and wander through the body (15). *Fasciola hepatica*, the better-known species, occurs in cooler climates than its more tropical congener, *F. gigantica*. However, the two species overlap in distribution in some places where human fascioliasis is common (best documented from Egypt [6, 10] and Iran [2]), and hybrids exist in eastern Asia and perhaps elsewhere (1). Distinguishing between adult *F. hepatica* and *F. gigantica* on the basis of morphology is possible, but much variation exists (10, 12). In places where hybrid populations occur, a range of morphologies that confound identification is seen. Differentiating between the two species in human infections is not possible on the basis of clinical, pathological, or immunological findings; and their eggs are very similar (12, 14). Consequently, in many places where both species occur, it is not possible to be certain whether both are responsible for human fascioliasis. In regions where only *F. gigantica* occurs, human infection with this parasite is known (e.g., in Hawaii [17] and Africa [7]). However, the extent to which human infection with *F. gigantica* actually occurs relative to the extent of exposure to infective stages (encysted metacercariae on edible aquatic vegetation) remains rather unclear. To quote Marcilla et al. (12) "the low number of records of human infection with *F. gigantica* may well be due to the lack of good tools to distinguish this species from *F. hepatica*".

There are hints in the literature that humans might be poor hosts for *F. gigantica* (17) and that this parasite undertakes ectopic migrations more often than its congener (19). However, *F. gigantica* can mature normally in humans, as demonstrated by the discovery of adult specimens during surgery or on postmortem examination (18, 19). Both species are assumed to infect humans in Egypt (6), and *F. gigantica* is thought to be the more common species infecting humans in northern Iran (2). In neither place is ectopic infection apparently frequent. A review of fascioliasis cases in Vietnam (20) noted that there had been a huge increase in the number of reported cases of human fascioliasis in that country in the past

decade. Of 500 cases diagnosed by serology and clinical signs and summarized elsewhere (1), only 2 had worms in atypical sites. Almost all Vietnamese cases have been ascribed to *F. gigantica* (5).

It is clear that more data about the relative prevalences of *F. gigantica* and *F. hepatica* in humans are needed in order to evaluate clinical findings. It is easy to distinguish between the species by molecular analyses, such as DNA sequencing (also reviewed elsewhere [14]). In this paper, we have presented the first molecular confirmation of the presence of *F. gigantica* in humans. In the future, amplification and sequencing of DNA extracted from *Fasciola* eggs from patients with patent infections should throw light on the contributions made by the two *Fasciola* species to human fascioliasis. An example has been set by the sequencing of DNA from eggs, recovered from human sputum, of *Paragonimus westermani* (3). Rapid real-time PCR assays can also be devised. Of course, the identities of immature worms in ectopic cases will remain unclear unless they emerge spontaneously or can be recovered by medical intervention.

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