infected by ingesting unwashed vegetables contaminated by animal feces containing strongyloid larvae. Larvae mature into adults in the intestines.

Sporadic cases of this infection in humans have been reported in many countries (*7*). In France, several autochthonous cases were suspected, but because of their rarity and difficulty in identification, they are not commonly reported (*8*). Eggs of *Trichostrongylus* spp. can be differentiated from those of *Necator* and *Ancylostoma* spp. because they are longer, narrower, and elongated. After 6 days of culture, *T. colubriformis* nematodes can be distinguished from similar stages in *Strongyloides* and *Ancylostoma* spp. by the bead-like swelling at the tip of the tail. Except for isolation of adult worms, which are rarely found in feces, sequencing of the ITS2 region is the most accurate method for specific identification of *Trichostrongylus* spp. isolated from humans.

This familial outbreak highlights increased risk for animal parasitosis in humans in an industrialized country, which may have been caused by an increasing trend of persons using ecologic and organic farming methods. These cases confirm that hygienic recommendations for use of organic fertilizer must be disseminated on a large scale. It is also mandatory that fresh vegetables be washed carefully and thoroughly before ingestion, and only dried manure should be used as an organic fertilizer.

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Adult *Opisthorchis viverrini* Flukes in Humans, Takeo, Cambodia

To the Editor: *Opisthorchis viverrini* and *Clonorchis sinensis,* the 2 major species of small liver flukes (family Opisthorchiidae), cause chronic inflammation in the bile duct, which leads to cholangitis and cirrhosis of the liver, and are a predisposing factor for cholangiocarcinoma (*1*). Human infections with *O. viverrini* flukes are found along riverside areas of Indochina (Thailand, Lao People's Democratic Republic [PDR], and Vietnam) (*2*).

Small trematode eggs (length 20–32 μm) have been found in human fecal samples in Cambodia (*1,3,4*). During 1981–1982, two of 102 Cambodian refugees in the United States were found to be positive for *C. sinensis* (likely *O. viverrini*) eggs (*3*). Egg-positive cases were later detected in several provinces of Cambodia (*4,5*). Presence of *O. viverrini* flukes in Cambodia was verified by detection of metacercariae in freshwater fish in a lake on the border between Takeo and Kandal Provinces and by isolation of adult flukes in experimentally infected hamsters (*6*).

In May 2010, we analyzed fecal samples from 1,993 persons in 3 villages (Ang Svay Chek, Kaw Poang, and Trartang Ang) in the Prey Kabas District, Takeo Province, Cambodia, ≈45 km south of Phnom Penh, to confirm the presence of *O. viverrini* flukes among humans. We found an egg-positive rate of 32.4% for small trematode eggs. Because these eggs may be those of *Haplorchis* spp. flukes (*H. taichui*, *H. pumilio*, and *H. yokogawai*) and lecithodendriid flukes (*Prosthodendrium molenkampi* and *Phaneropsolus bonnei*) (*1*), we attempted to detect adult flukes that are responsible for these eggs.

Six of the small trematode egg–positive villagers, 1 man and 5 women (age range 16–72 years), who had occasional epigastric discomfort were selected for anthelmintic treatment, purgation, and recovery of adult worms. Fecal examination and anthelmintic treatment of villagers were approved by the Ministry of Health, Cambodia, under the agreement with the Korea–Cambodia International Collaboration on Intestinal Parasite Control in Cambodia (2006–2011). After obtaining informed consent, the villagers were treated with a single oral dose of praziquantel, 40 mg/kg (Shinpoong Pharmaceutical Co., Seoul, South Korea), and given a purgament (solution containing 30–40 g $MgSO_4$). Feces was obtained 3 or 4 times in a 2–3-hour period after purgation, pooled individually, and processed as described (*7*). Worms obtained were fixed with 10% formalin, stained with acetocarmine, and identified by morphologic features.

A total of 34 *O. viverrini* adult worms were obtained from the 6 villagers (14, 9, 5, 3, 2, and 1 from each villager, respectively). No other species of trematodes were obtained. Five worms were lanceolate and had a mean length of 9.5 mm (range 6.5–12.0 mm), a mean width of 1.5 mm (range 1.2–1.7 mm), and 2 characteristic 4–5-lobulated testes (Figure, panel A). Ten eggs in uteri were 27 μm long (range 25–29 μm) and 15 μm wide (range 13–16 μm).

To detect the source of infection, 2 freshwater fish species, *Puntioplites proctozysron* (n = 5) and *Cyclocheilichthys apagon* (n = 10), were caught in nearby Ang Svay Chek village and examined for *O. viverrini* metacercariae by using a digestion technique (*8*). A total of 50 metacercariae (Figure, panel B) were obtained from 5 *P. proctozysron* fish and fed to a hamster. Six weeks later, 42 young *O. viverrini* flukes (Figure, panel C) were isolated from the biliary tract of the hamster.

Our study identified only *O. viverrini* infections in humans in Cambodia. However, eggs of other hepatic and intestinal flukes also can be found in humans (*1*). In Thailand, Vietnam, and Lao PDR, opisthorchiids (*O. viverrini* and *C. sinensis*), heterophyids (*Haplorchis* spp., *Centrocestus formosanus*, and *Stellantchasmus falcatus*), and lecithodendriids have been found in humans (*1,7,9*). In several provinces in Lao PDR, mixed infections with *O. viverrini* and heterophyids or lecithodendriids were common (*7,9*), and the relative prevalence of each fluke species varied by locality. In Vientiane, Lao PDR, *O. viverrini* was the predominant species, whereas in Saravane Province, *H. taichui* predominated (*7*). In a mountainous area of Phongsaly Province, *H. taichui* and *H. yokogawai* worms were obtained from 10 villagers; however, no *O. viverrini* worms were detected (*10*). Thus, in Cambodia, the presence of human infections with intestinal flukes, including *Haplorchis* spp. and lecithodendriids, cannot be ruled out.

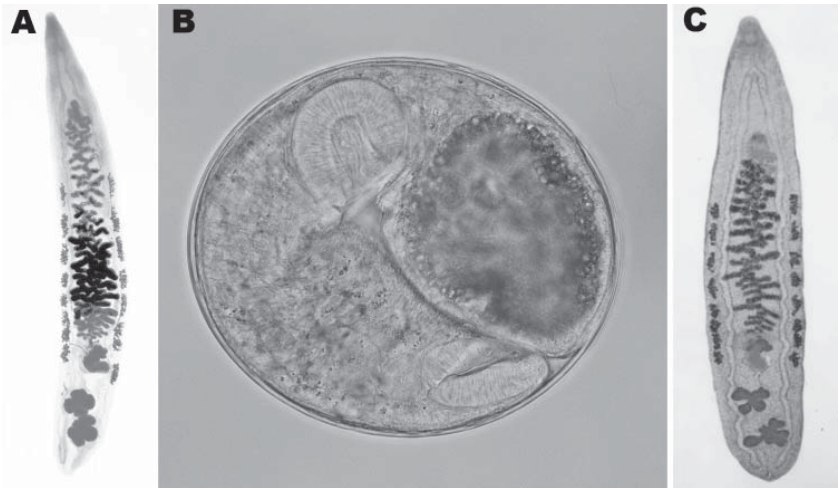

Figure. A) Adult *Opisthorchis viverrini* liver fluke (length 12.0 mm) isolated from a human after chemotherapy and purgation in Takeo Province, Cambodia, showing the characteristic morphology of the two 4–5-lobulated testes. B) Metacercaria of *O. viverrini* fluke (diameter 0.22 mm) detected in a freshwater fish (*Puntioplites proctozysron*). C) Young adult *O. viverrini* fluke (length 5.5 mm) isolated 6 weeks after experimental infection of a hamster with metacercariae from *P. proctozysron* fish. Original magnification levels ×8 (A), ×120 (B), ×9 (C). A color version of this figure is available online (www.cdc.gov/EID/content/17/7/1302-F.htm).

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Easy Test for Visceral Leishmaniasis and Post–Kala-azar Dermal Leishmaniasis

To the Editor: Diagnosis of visceral leishmaniasis (VL), fatal if untreated, is complex because the symptoms are the same for many fever-associated ailments. Despite limitations, diagnosis remains based on finding *Leishmania* amastigotes in spleen and/or bone marrow aspirates (*1*). Sophisticated laboratory methods, although sensitive, are costly. The immunochromatographic strip test that uses recombinant K39 antigen (rK39), although satisfactory in India, is less sensitive in Africa, Latin America, and Mediterranean regions (*2*). Post–kala-azar dermal leishmaniasis (PKDL), a sequel to VL in India and Africa, is often confused with other skin diseases (*3,4*). Diagnosis of VL in dogs in Latin America and Mediterranean countries remains confusing because of rampant asymptomatic infections and elevated antibodies against *Leishmania* spp. (*5*).

Earlier we reported the diagnostic potential of *L. donovani* (MHOM/IN/83/AG83) promastigote membrane antigens (LAg) (*3,6*). Here we report applicability of LAg-based ELISA and dipstick systems even at primary health centers. Using randomized sampling, we tested samples from 122 kala-azar patients from India, 20 PKDL patients from India, and 40 VL patients from Brazil. VL was confirmed by finding parasites in aspirates. Serum samples were collected before chemotherapy was given. PKDL was diagnosed as described (*3*). Control samples were collected from 24 healthy persons from non–disease-endemic areas in India; 15 healthy persons from disease-endemic areas in India; 20 healthy persons from disease-endemic areas in Brazil; and 21 persons with Hansen disease, 7 with filariasis, 4 with tuberculosis, 1 with lymphoma, 1 with leukemia, 2 with virus-induced fever, and 5 with malaria. Consent was obtained from all human donors. This study was approved by Ethical Committee on Human Subjects at Indian Institute of Chemical Biology and the Ethical Board for Human Subjects and Animal Experimentation of the Federal University of Piauí.

We developed a diagnostic ELISA with modifications of our previous method (*6*). Microtiter plates were coated with 2.5 μg LAg at pH 7.5 (100 μL/well) and kept at 4°C overnight, after which they were blocked with 1% bovine serum albumin, dried, and stored at 4°C as precoated plates. The assay performed at room temperature took ≈2.5 h. Test and control serum samples (1:1,000 dilution, 100 μL/well) were applied to the plates for 45 min and shaken occasionally. Horseradish peroxidase–conjugated goat anti-human immunoglobulin (Ig) G (Genei, Bangalore, India) was applied at 1:5,000 (100 μL/well) for 45 min. Color development with orthophenylenediamine (Sigma-Aldrich, St. Louis, MO, USA) was allowed for 5–10 min. Positive results were determined by comparing colors with those on a card previously prepared for positive and negative wells. ELISA, performed for the VL and PKDL patients from India, was 100% sensitive (percentage of patients with confirmed disease and positive