

to establish the connection between the strains isolated from different sources in Colombia.

Our findings suggest that the risk for *E. coli* O157:H7 infection in Colombia is high; therefore, more active screening and surveillance would enhance case detection, epidemiologic understanding of *E. coli* O157:H7 infection and HUS, and could lead to more specific therapeutic interventions.

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### Autofluorescence and the Detection of *Cyclospora* Oocysts

**To the Editor:** From May through July 1997, we searched for the seasonally occurring *Cyclospora cayetanensis*, along with other coccidia and microsporidia, in fecal samples from 385 patients. The samples, in 10% formalin for evaluation of coccidia and microsporidia, were initially processed by a routine formalin-ethyl acetate concentration method; the parasite was detected in 18 patients (1,2). The resulting sediment was examined as follows. A drop of sediment was placed on a slide, cover-slipped, and examined microscopically as a wet mount at 200x and 400x magnification and subsequently at 200x magnification by epifluorescence with a 330 to 380 nm UV filter. Four smears were also prepared and stained by routine trichrome (2), modified trichrome (3), auramine-rhodamine (4),

and Kinyoun acid-fast (5) procedures. All wet mount and stained preparations were evaluated by at least two trained persons.

Of the 385 fecal samples examined, 18 were positive for *C. cayetanensis*. The positive samples were from eight states, which encompassed northeastern (Rhode Island, New York, Massachusetts, Pennsylvania), midwestern (Wisconsin), western (Oregon, California), and southern (Florida) sections of the United States.

In 12 of 18 patients, the organisms were detected without much difficulty in wet mounts as round or partially collapsed nonrefractile bodies; however, in the other six, repeated wet preparations were needed to detect the organisms. When the same wet mounts were examined with epifluorescence microscopy, oocysts were easily discerned in all samples, even the six in which repeated wet preparations and stains were needed. While the trichrome procedures were ineffective, the auramine-rhodamine and Kinyoun stains gave varied results. The autofluorescence technique, however, was distinctly superior to the wet mount and staining procedures.

Extensive outbreaks of diarrhea caused by *C. cayetanensis* were reported in 1997 from different parts of the United States (6-8), and several procedures have been used to confirm the diagnosis in clinical samples. While the organisms are large enough to be seen in direct wet mounts, they are frequently caught up in mucus or covered by debris, so they are difficult to detect. Autofluorescence in *C. cayetanensis* oocysts makes them easily visible in clinical samples (1,9) with the use of a 330 to 380 nm UV filter; this feature enhanced their detection at least twofold over the direct wet mount, especially when the wet mount and stained slides contained few oocysts. (The same wet mount preparation can be used for the epifluorescence procedure.)

The 18 patients with cyclosporiasis were ages 2 to 71 years, which indicates that the infection was not specific to any age group. Twelve of the 18 cases were in women. Massachusetts had 11, the largest number of *C. cayetanensis*-positive patients. Of the 18, 16 were adults; the other two were children with a coexisting parasite (*Dientamoeba fragilis*). In one instance, three members of the same family were infected, the parents with only *C. cayetanensis*, the son with *D. fragilis* and *Blastocystis hominis*.

Because *C. cayetanensis* is a seasonal diarrheal agent, fecal samples from persons with persistent unexplained explosive diarrhea during the summer should be carefully evaluated for this infection. Stool specimens should be fixed in 10% formalin and examined with autofluorescence microscopy for enhanced detection.

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### Partnerships for Detecting Emerging Infectious Diseases: Nepal and Global Influenza Surveillance

**To the Editor:** With new influenza strains emerging each year, identification of circulating strains by coordinated global surveillance is crucial to vaccine development for the coming year (1-3). Approximately 110 laboratories in 80 countries voluntarily participate in the World Health Organization (WHO) influenza surveillance

network (4). Comprehensive surveillance is especially important in Asia, since new influenza strains often originate there. To participate in influenza global surveillance, countries need not rely on their own laboratory capability. Clinical specimens from patients thought to have influenza can be sent to designated laboratories around the world for analysis. A unique partnership has led to the expansion of the WHO global influenza surveillance network to Nepal.

The U.S. Army Medical Component - Armed Forces Research Institute for Medical Sciences (AFRIMS) (5) in Bangkok, Thailand, is well situated to assist with surveillance in Asia. Scientists at AFRIMS have conducted medical research in collaboration with Nepali colleagues for more than 20 years. Several studies have been conducted in collaboration with the CIWEC Clinic Travel Medicine Center (a travel medicine clinic that serves the diplomatic, aid, and tourist communities in Nepal). The clinic has approximately 5,000 patient visits per year, of which half are drawn from the 2,500 expatriates in Nepal and half from the 200,000 non-Indian tourists who visit Nepal annually.

A protocol was developed for a pilot influenza surveillance program. The staff of the CIWEC Clinic was responsible for volunteer recruitment, clinical evaluation, and specimen collection. Febrile upper respiratory infections were defined as temperature  $\geq 100^{\circ}\text{F}$  ( $37.8^{\circ}\text{C}$ , oral or equivalent) and cough or sore throat of  $\leq 72$  hours duration. Other symptoms, such as streptococcal pharyngitis, were excluded. No age or gender restrictions were included. Volunteers had to have been in Nepal for the 5 days preceding illness. Only the first patient in any single household with similar symptoms within days of other household members was asked to participate.

The AFRIMS field station in Kathmandu (locally known as the Walter Reed/AFRIMS Research Unit - Nepal or WARUN) was responsible for shipping specimens collected by the CIWEC Clinic to AFRIMS, Thailand. Since dry ice was not available in Kathmandu, dry ice and shipping containers were sent by AFRIMS, Thailand for use by WARUN. Shipments from WARUN were then sent back to AFRIMS, where specimens were repacked in dry ice and sent for testing at the central laboratory of the U.S. Air Force's Project Gargle (6) in San Antonio, Texas. Project Gargle has been testing viral respiratory