Escherichia coli O157:H7 Infection in Colombia

To the Editor: The prevalence of *Escherichia coli* O157:H7 in Colombia is not known; we conducted a study to determine its prevalence in children with infectious diarrhea, in adult cattle, and in ground beef.

Between March 1996 and March 1997, we examined 538 children under 5 years of age with infectious diarrhea who had been admitted to either of the two children's hospitals in Bogotá. Diarrhea was defined as three or more loose stools within the previous 24 hours. One hundred and sixty-one children under 10 years of age admitted to the hospital for a medical reason other than infectious diarrhea served as controls.

Stool samples from children with and without diarrhea were placed in Stuart transport medium and sent to the laboratory within 24 hours; the samples were injected into sorbitol MacConkey agar (Oxoid Basingstoke, United Kingdom). After 24 hours of incubation at 37°C, sorbitol nonfermenting colonies were tested with 4-methylumbelliferyl-ß-glucuronide (MUG); all typical colonies of *E. coli* O157:H7 that were sorbitol-negative were confirmed as *E. coli* by biochemical tests (1,2) and were tested for agglutination with a latex test kit (Oxoid Basingstoke, United Kingdom) for detecting *E. coli* O157 and *E. coli* H antiserum H7 (Difco, Detroit, MI, USA). All human isolates were confirmed as Shiga-toxin producers by latex agglutination (Oxoid Basingstoke, United Kingdom). We used as a control strain *E. coli* O157:H7 provided by M. Karmali.

Rectal swabs from 307 healthy adult cattle from farms in Cundinamarca and Meta Departments were placed in Stuart transport medium, stored at 4°C, and transported to the laboratory within 6 hours. Swabs were injected into sorbitol MacConkey agar, and colonies that did not ferment sorbitol were characterized by standard techniques (3).

One hundred and fifty beef patties (31 cooked, 119 raw), collected in Bogotá, were examined by direct plating and enrichment culturing. Samples (1.0 g each) were serially diluted (1:10) in 0.85% NaCl solution, and 0.1 ml portions were plated in duplicate onto sorbitol MacConkey agar. Serologic and biochemical confirmation was done as mentioned above.

E. coli O157:H7 prevalence among children was 7.2%, with an age range of 0–60 months (average 21 months); diarrheal illness lasted an average of 2.5 days. Of 39 patients, eight were 6 months old or younger, five were 6 to 12 months old, 17 were 12 to 24 months old, and nine were older than 24 months. Renal failure associated with hemolytic uremic syndrome (HUS) developed in three (7.7%). Epidemiologic data were not collected regarding contaminated foods as a possible source of *E. coli* O157:H7 infection in the patients. *E. coli* O157:H7 was isolated from five (3.1%) of the 161 controls; the prevalence of *E. coli* O157:H7 was substantially higher in patients with infantile diarrhea than in controls.

All 39 strains from human cases were sorbitol-negative; five did not display MUG activity. Overall, 39 strains agglutinated strongly with antiserum O157:H7. Antimicrobial susceptibility tests were performed by the Bauer method (4). All *E. coli* O157:H7 isolated were susceptible to ciprofloxacin; 92% were resistant to ampicillin; 76% were resistant to furazolidone; and 76% were resistant to trimethoprim-sulfamethoxazole (TMP-SMZ).

E. coli O157:H7 was isolated from 20 (6.5%) of 307 rectal swabs from cattle. The strains isolated were sorbitol-negative and agglutinated strongly with antisera; five did not present activity. All strains were susceptible to ciprofloxacin; 90% were resistant to ampicillin; and 26% were resistant to TMP-SMZ. *E. coli* O157:H7 was isolated from 13 (87%) of 150 beef patties, six from raw beef, and seven from cooked beef.

Stool cultures of all patients with acute bloody diarrhea should be tested for *E. coli* O157:H7 to identify those at risk for HUS (5); however, serotyping, cytotoxicity assays, or DNA probing for *E. coli* O157:H7 are not routinely performed in Colombia.

This preliminary report suggests that *E. coli* O157:H7 is emerging as an important cause of endemic childhood diarrhea in Colombia and that the chain of contamination is present. The incidence is greatly underestimated because of limited surveillance and reporting. Further studies are needed to identify the pathogenic mechanisms of these *E. coli* O157:H7 strains and to determine the fecal carriage rate in healthy children. Data obtained will help elucidate the role of *E. coli* O157:H7 in childhood diarrhea. In addition, molecular analysis should be performed

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. cayetanenis*-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*.