

Epidemiological features of a newly described serotype of *Shigella boydii*

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SUMMARY

We report the clinical, microbiological, and epidemiological features of an emerging serotype, *Shigella boydii* 20. We interviewed patients about symptoms, and history of travel and visitors during the week before illness onset. Seventy-five per cent of the 56 patients were Hispanic. During the week before illness onset, 18 (32%) travelled abroad; 17 (94%) had visited Mexico. Eight (21%) out of 38 who had not travelled had foreign visitors. There were eight closely related patterns by PFGE with *Xba*I. *S. boydii* 20 may be related to travel to Mexico and Hispanic ethnicity. Prompt epidemiological investigation of clusters of *S. boydii* 20 infection may help identify specific vehicles and risk factors for infection.

BACKGROUND

In 2001, nearly 10 600 laboratory-confirmed cases of shigellosis were reported to the US Centers for Disease Control and Prevention (CDC). The *Shigella* spp. are comprised of four subgroups: A (*Shigella dysenteriae*), B (*Shigella flexneri*), C (*Shigella boydii*) and D (*Shigella sonnei*). Infection with shigellae can lead to a syndrome of bloody or watery diarrhoea, abdominal pain, and fever. In 2001, 77% of shigellosis cases in the United States were caused by *S. sonnei*. *S. boydii*, which has 19 recognized serotypes, caused 1% of shigellae infections in 2001 [1].

Between October 2000 and January 2002, the National Reference Laboratory for *Escherichia coli*

and *Shigella* at CDC received 82 isolates of *S. boydii*, cultured from stool, that were serologically distinct from *S. boydii* serotypes 1–19. These isolates were submitted to CDC by 20 US state public health laboratories (76 isolates), Australia (3), and Canada (3). Three of the 76 US isolates were submitted by the California Department of Health Services, which had detected 40 similar infections between October 2000 and January 2002, including one wound infection. The phenotypic and biochemical profiles of these isolates resemble those of other shigellae (Woodward, D., unpublished observations). The strains did not agglutinate in antisera against the previously established serotypes of *S. boydii* but did agglutinate in antisera against *S. boydii* 99-4528, a recently described serotype isolated in Canada that has been designated by the World Health Organization (WHO) Collaborating Centre for *Shigella* as the type strain for *S. boydii* 20 (Woodward, D., unpublished observations). PCR

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analysis demonstrated that all isolates contained the *ipaH* (invasion plasmid antigen H) gene, a marker for invasiveness [2].

METHODS

We requested public health officials in 11 states to assist with contacting patients diagnosed with gastrointestinal *S. boydii* 20 infection. To characterize patients' demographic characteristics, severity of illness, and history of travel before onset of symptoms, we conducted telephone interviews using a standardized questionnaire. When appropriate, we administered a Spanish version of the questionnaire. In California, six patients could not be reached for interview by telephone; their information was abstracted from forms used by local health departments for shigellosis case follow-up.

We examined the antimicrobial susceptibility and molecular fingerprinting patterns of 10 *S. boydii* 20 isolates. In order to examine the potential heterogeneity of these patterns, we selected a diverse set of isolates for testing on the basis of the patient's state of residence, reported ethnicity, travel history, and visitors to the home. We conducted antimicrobial susceptibility testing by semi-automated broth microdilution methods [3]. We performed pulsed field gel electrophoresis (PFGE) on the same 10 isolates using methods described previously [4, 5]. Relatedness of PFGE profiles was recorded as a per cent similarity by comparison of restriction band locations [6].

RESULTS

We obtained demographic and clinical information from 56 patients residing in eight states, including Arizona (5 patients), California (32), Illinois (8), Indiana (1), Oregon (2), Texas (7) and Washington (1). Patients residing in New York City (1), Massachusetts (1) and Georgia (1) could not be reached. The median age of patients was 27 years (range 1–75 years) and 63% were female. These demographic characteristics were similar to those of 49 patients with 12 specified serotypes of *S. boydii* infection reported in 2001 to the Public Health Laboratory Information System; the median age of these persons was 25 years (range 10 months to 65 years) and 51% were female. Seventy-five per cent of patients with *S. boydii* 20 infection reported Hispanic/Latino ethnicity. The dates of symptom onset ranged from November 2000 to November 2001 (Fig. 1). The most common symp-

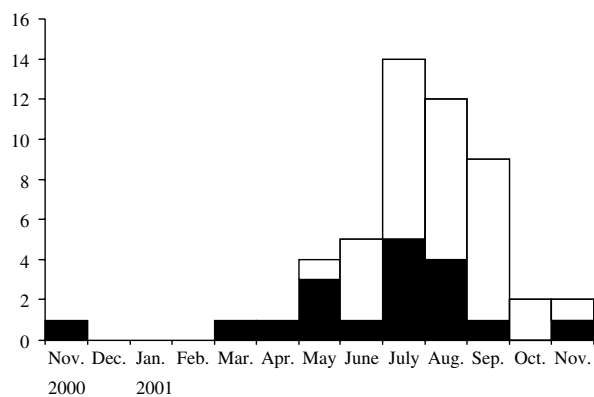


Fig. 1. Patients with *S. boydii* 20 infection, according to travel history, by month and year of onset, November 2000 to November 2001. □, Non-travellers; ■, travellers.

toms reported were diarrhoea (100%), cramps (83%), fever (81%), nausea (75%) and fatigue (77%). Bloody stool was reported by 19 (42%) out of 45 patients. The median duration of diarrhoea was 5 days (range 1–45 days). All patients sought medical assistance and 11 (21%) out of 52 were hospitalized.

Eighteen (32%) out of 56 patients [eight (21%) out of 39 Hispanic persons and 10 (59%) out of 17 non-Hispanic persons] travelled outside the United States during the 7 days before illness onset (Fig. 1). Seventeen (94%) visited Mexico and one (6%) visited the Philippines. Destinations in Mexico included Mexico City and nine different Mexican states (Fig. 2). None of the patients who travelled had taken antibiotics during the journey, either for treatment or for prophylaxis.

Among 38 patients who had not travelled outside the United States, eight (21%) reported foreign visitors during the week before illness onset and 21 (55%) denied having visitors; for nine (24%) cases, information about whether patients had foreign visitors was not available. Seven of the eight visitors were from Mexico and the eighth was from El Salvador. These visitors had brought food, including canned milk, salsa, queso fresco and other cheeses, to the homes of seven of these patients during the week before illness onset. Notably, of the 30 patients who had not travelled outside the United States and who had not had visitors from outside the country, 25 (83%) reported Hispanic ethnicity.

Isolates for antimicrobial susceptibility and PFGE testing were obtained from patients residing in six states, including Arizona (2), California (2), Illinois (3), Indiana (1), Texas (1) and Washington (1). Of the eight people who reported their ethnicity, three were



Fig. 2. Destinations of travel within Mexico by patients with *Shigella boydii* 20 infection, November 2000 to November 2001.

Hispanic, three White, one Asian Indian, and one East Asian. Two travelled to different locations in Mexico and one travelled to the Philippines during the week before illness onset. Three of the seven persons who did not travel had visitors who brought food from Mexico.

Sensitivity to amikacin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, and nalidixic acid was demonstrated for all isolates. We found resistance to ampicillin (A), streptomycin (S), sulphamethoxazole (Su), tetracycline (T), and trimethoprim (Tm). All isolates were resistant to four or more antimicrobial agents, with resistance profiles as follows: SSuTTm (1), ASSuTm with intermediate sensitivity to cephalothin (1), ASSuTTm (2), ASSuTTm with intermediate sensitivity to amoxicillin/clavulanic acid (1), and ASSuTTm with intermediate sensitivity to cephalothin (5).

Using the restriction endonuclease *Xba*I to perform PFGE analysis, we detected eight closely related patterns, differing from each other by 1–3 restriction bands, and with greater than 85% similarity in band location. *S. boydii* 20 patterns were very different from patterns of other *S. boydii* serotypes in the Shigella PFGE database housed at CDC. When isolates were digested with *Bln*I, we noted seven patterns with 90% similarity in band location, all of which were dissimilar to the other *S. boydii* serotypes in the PFGE database. Restriction analysis with the endonuclease *Not*I also yielded seven highly related patterns. No

other *S. boydii* serotypes had been restricted with *Not*I and, thus, comparison could not be performed. Eight isolates had distinct patterns by at least two of the three restriction endonucleases. The remaining two isolates were indistinguishable from each other by all three endonucleases. The two patients from whom these isolates had been obtained had symptom onset in November 2000 and March 2001; they had both travelled to Mexico, one to Mexico City and the other to Merida.

DISCUSSION

We describe the epidemiological characteristics of an emerging serotype of *S. boydii*, which was first detected in 1999 in Canada, and 2001 in Australia and the United States. It is important to identify unique serotypes for several reasons. Specific serotypes can have unique clinical and epidemiological features that are not shared by other serotypes of the same species. For example, *Shigella dysenteriae* type 1, which produces the shiga toxin, is the cause of large deadly epidemics of dysentery in parts of the developing world whereas other serotypes of *S. dysenteriae* cause a relatively milder illness. Additionally, in the case of pathogens such as *S. flexneri*, for which vaccines are under development, infection with or immunity to one serotype does not necessarily confer immunity to newly emerging serotypes.

Our study suggests that *S. boydii* 20 is related to travel outside the United States, particularly to Mexico, and is more common among persons of Hispanic/Latino ethnicity. Since Hispanic Americans have been reported to access health-care services at lower rates than non-Hispanic whites, we do not believe that the finding of a Hispanic predominance among patients with *S. boydii* 20 infection is a reflection of increased care-seeking behaviour in this ethnic group [7, 8]. Forty-six per cent of US cases of *S. boydii* 20 were associated with travel, either by the patient (32%) or by a visitor to the patient's home (14%). Most travel by patients or visitors was to or from Mexico but, interestingly, one patient had travelled to the Philippines. Travel to countries other than Mexico, including Cuba, Ethiopia, Guatemala and India, has been reported by Canadian patients with *S. boydii* 20 infection (Woodward, D., unpublished observations). The association between international travel and shigellosis has long been recognized, with a nationwide study in 1985–1986 in the United States linking 20% of *Shigella* infections to travel abroad [9]. The transmission of *S. boydii* 20 infection to international travellers is probably related to the ingestion of contaminated food and water. Specific vehicles are not easily identified, since consumption of common foods, such as salsa, are often similar between ill and well travelling companions. Additionally, outbreaks among travellers may not be detected because returning travellers disperse to their home states before seeking medical attention and having stool cultured.

Recent data from the Foodborne Diseases Active Surveillance Network (FoodNet) demonstrates that Hispanic persons have nearly three times the incidence of laboratory-confirmed *Shigella* infections of non-Hispanic whites [10]. We found that, among those who had not travelled outside the United States and who did not have visitors from abroad, 83% reported Hispanic or Latino ethnicity. In this group, transmission may have occurred by person-to-person contact or by consumption of contaminated foods. In previous outbreaks of shigellosis among closely knit ethnic and religious groups, illness was most common among young children and person-to-person contact was the described mode of transmission [11]. Since the median age of patients with *S. boydii* 20 infection was 27 years, person-to-person transmission is less likely. Transmission of *S. boydii* 20 among persons who did not travel abroad could be related to specific foods. Foods such as queso fresco and cilantro, which are commonly consumed by Hispanic persons in the

United States, have been implicated in outbreaks of foodborne illness from bacterial pathogens such as *Salmonella* and *Listeria* [12–14].

Antimicrobial resistance is extremely common among shigellae isolates. Among 344 shigellae isolates tested in 2001 by the National Antimicrobial Resistance Monitoring System: Enteric Bacteria (NARMS), 95% were resistant to at least one antimicrobial agent and 71% were multiply resistant. All *S. boydii* 20 isolates tested were resistant to at least four antimicrobial agents. Given the high prevalence of resistance to multiple antimicrobial agents and the rapidity with which shigellae can acquire resistance to additional antimicrobial agents, drug susceptibility testing should be performed on all *S. boydii* 20 isolates. Prompt notification of antimicrobial susceptibility results may aid physicians in appropriate antimicrobial use.

This investigation was limited to a convenience sample of those patients who could be reached by telephone for interview or who had been contacted by their local health department following their diagnosis of shigellosis. Since many interviews took place months, and occasionally, more than a year after the onset of illness, some patients could not remember details about their illness or about exposures before symptom onset. Despite these limitations, we gained important information on demographics and history of travel and visitors from patients with *S. boydii* 20 infection.

In 2002, a total of 19 isolates consistent with *S. boydii* 20 were forwarded to CDC. No specific vehicles for transmission and no discrete outbreaks were identified in this investigation. The variety of travel destinations reported by patients, the multiple patterns detected by PFGE, and the diversity of antimicrobial susceptibility profiles are consistent with multiple sources of *S. boydii* 20 infections. If cases of *S. boydii* 20 are found to be clustered in time or space, prompt epidemiological investigation may help to identify specific risk factors or food vehicles for the infection. By promptly submitting isolates for serotyping to reference or public health laboratories, clinical laboratorians and clinicians play a key role in the detection of emerging pathogens, such as *S. boydii* 20.

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