## Hemolytic Uremic Syndrome Due to Shiga-like Toxin Producing *Escherichia coli* O48:H21 in South Australia

Enterohemorrhagic *Escherichia coli* (EHEC) other than serotypes O157:H7 are increasingly recognized in association with hemolytic uremic syndrome (HUS) (1) and have been reported in Australia (2). While detecting strains of O157:H7 has become easier over the years, identifying the expanding number of other serotypes of EHEC also associated with HUS, with other conditions, and with healthy domestic animals is still very difficult.

Cases of HUS have been reported in Australia over a number of years. The most common serotype found was O111:H-, and Australia's recently reported first HUS outbreak (3) was caused by EHEC O111:H-. We wish to report a case of severe HUS due to serotype O48:H21, which, as far as we know, has not been previously reported as a cause of HUS. This case occurred in 1993, before surveillance of HUS had been initiated; after this case, between July and December 1994, 10 cases of HUS (from which four isolates were obtained; two were EHEC O111) were reported to the Australian Paediatric Surveillance Unit (E. Elliott, pers. comm.).

The patient in the 1993 case was an 8-year-old girl, living in a rural setting in the outskirts of Adelaide, South Australia. Her home was adjacent to a farm on which cows, sheep, and ducks were kept. A kelpie/healer cross puppy was in the house in November 1993. Also kept were a pet galah (Australian cockatoo) and pet fish. She was well until 23 December 1993, when she had diarrhea described as very smelly and watery "like the juice of tinned crab." The diarrhea became bloody on 2 January 1994 and was associated with severe abdominal pains which made the patient draw up her legs. She was having bowel movements six times a day, had become very weak, and was unable to stand. She was admitted to Adelaide Children's Hospital on 3 January 1994, and her condition progressed to anuric renal failure over the next few days. Serum biochemistry on 7 January showed a urea level of 23.3 mmol/L and creatinine level of 539 µmol/L. Her hemoglobin level fell from 157 g/L on 3 January to 86 g/L on 10 January. Her hematocrit fell from 48% to 24%, and her

platelet count fell from  $463 \times 10^9/L$  to  $47 \times 10^9/L$  on these dates, respectively. The blood film showed microangiopathic hemolytic anemia with fragmented red cells. She required hemodialysis for 3 weeks and was discharged from the hospital on 31 January 1994.

Apart from the patient's 5-year-old brother, who had loose bowel movements for 1 day on 28 December 1993, no other family members were affected. An adequate dietary history was not obtained; however, no food had been eaten from commercial food outlets.

Stool samples were collected on 4 and 5 January 1994. The samples were probed for Shiga-like toxin (SLT)-I and SLT-II genes by polymerase chain reaction (PCR), and the results were positive. Approximately 80% of lactose-fermenting colonies on MacConkey agar were also SLT positive. No sorbitol-negative colonies were observed on sorbitol-MacConkey agar. In addition to being cultured for *E. coli*, the stools were also routinely cultured for Shigella, Salmonella, Yersinia, Vibrio, and Clostridium. In addition, stained concentrates were examined for Giardia lamblia and Entamoeba histolytica with negative results. Four typical *E. coli* strains were subjected to further tests. They were typical *E. coli*, positive in the indole and ONPG tests, negative in the Voges-Proskauer, citrate, TDA, malonate, urease, gelatine, and H2S tests. The strains fermented glucose, lactose, mannitol, xylose, rhamnose, arabinose, sorbitol, sucrose, and melibiose. They did not ferment inositol, adonitol, salicin, raffinose, or amylose. They decarboxylated arginine, lysine, and ornithine. All the strains produced enterohaemolysin (4). The strains were O and H serotyped (5, 6) and found to be serotype O48:H21. Supernatant preparations were tested on Vero cells (7) and found to give typical verocytotoxic reactions in titers of  $10^3$  to  $10^4$ . The supernatants were also tested by enzyme-linked immunosorbent assay (ELISA) by using monoclonal antibodies 13C4 and 11E10 directed against SLT-I and SLT-II, respectively, and strong reactions with both antibodies were noted, confirming the presence of both SLTs.

## **Dispatches**

Stool samples taken from the patient on 8 February 1994 were negative for SLT-I and SLT-II genes by PCR and were not cultured further. Stool samples from the patient's brother and local animals were not forthcoming.

That all four *E. coli* isolates tested were of serotype O48:H21 and demonstrated identical toxigenicity by both PCR and ELISA and the fact that SLT-positive organisms were not found in the stools collected during the patient's convalescence strongly suggest that this serotype was the causative organism. The toxicity, virulence, and part of the molecular structure of the SLT-II gene derived from the EHEC O48:H21 strain reported here (and whose novel serotype was discovered by the authors) have recently been described elsewhere (8).

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