Isolation of *Escherichia coli* O5:H⁻, possessing genes for Shiga toxin 1, intimin-β and enterohaemolysin, from an intestinal biopsy from an adult case of bloody diarrhoea: evidence for two distinct O5:H⁻ pathotypes

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Two typical coliforms from an intestinal biopsy from an adult case of bloody diarrhoea carried genes encoding intimin-β, stx₁, and ehxA, and produced verocytotoxin 1 and enterohaemolysin in culture. Both were biochemically typical *Escherichia coli* O5:H⁻, apart from producing urease. Such O5 isolates represent a human pathogenic *E. coli* lineage.

Case report

A 55-year-old man 3 months post autologous stem cell transplant for multiple myeloma presented to hospital with abrupt onset of bloody diarrhoea and fever (38.8°C). He was commenced empirically on intravenous ciprofloxacin and metronidazole. General stool culture for commonly encountered enteric pathogens failed to yield a possible causative agent. *Clostridium difficile* toxin was not detected. A colonoscopy on the day of admission revealed multiple mucosal areas showing a granular erythematous appearance with changes predominating on the left side of the colon. A biopsy of the macroscopically abnormal area revealed numerous bacilli adhering and effacing the surface enterocytes with scant acute inflammatory cells, minor degenerative epithelial changes and focal epithelial sloughing (Fig. 1). The appearances were diagnosed as a bacterial colitis and the possibility of an attaching and effacing *Escherichia coli* (AEEC). Once the diagnosis was known, the antibiotics were ceased as ciprofloxacin has potential adverse effects. No further antibiotics were given and the patient improved over the next 3 days, the diarrhoea ceased and he went home.

Regarding potential risk factors for exposure to Shiga toxin-producing *E. coli* (STEC), there was no recent travel history or contact with a farming environment. He worked in a food bar, where he sometimes ate cold peanuts and salami, and he had no knowledge of any reports of illness in any of the people who visited the shop around the time that his diarrhoea commenced.

The study

The biopsy material sent for microbiology was cultured for the presence of AEEC by smearing across the plates on MacConkey (MAC), sorbitol MacConkey (SMAC), and washed and unwashed sheep blood agars (WSBA and SBA). The majority of the colonies were coliform and of the same morphology, as well as a few staphylococci. The colonies all fermented lactose on MAC, sorbitol on SMAC, and were haemolytic on WSBA but not on SBA, suggesting a profuse growth of enterohaemolytic *E. coli* (Bettelheim, 1995) of similar phenotype. They were tested by the adaptation of the Denka–Seiken test (Bettelheim, 2001) and found to be verocytotoxigenic. Following standard previously described methods (Bettelheim *et al.*, 2000) on two colonies, it was found that they were typical *E. coli* apart from being anaerogenic and urease producers. They were non-motile, and O serotyping showed them to belong to serogroup O5. Supernatants prepared from overnight growth in trypticase soy broth, when tested on Vero cells, gave the typical cytopathic effects of verocytotoxins at dilutions of up to...
The presence of verocytotoxin 1 was confirmed by immunonasay using monoclonal antibodies. Verocytotoxin 2 was not found.

Faecal specimens were not available for testing by the time that the cytological observations had been made and initially E. coli had not been suspected.

A multiplex PCR for the simultaneous detection of stx1, stx2, eae and ehxA was used to examine the virulence gene profiles of two serotype O5:H^- isolates recovered from the intestinal biopsy (Paton & Paton, 1998). Both isolates were shown to possess stx1, eae and ehxA genes (data not shown). Previous studies have shown that serotype O5:H^- STEC are prevalent in the faeces of healthy, slaughter-age sheep and typically possess stx1, stx2 (formerly stx2d/OX3/O111) + ehxA, but do not possess eae (Djordjevic et al., 2001; Brett et al., 2003a; Koch et al., 2001; Ramachandran et al., 2001). O5:H^- isolates from healthy slaughter-age cattle are extremely rare and the few isolates available for examination have been shown to possess stx1/stx1 + stx2 + ehxA, a virulence profile similar to those commonly found in healthy sheep (Brett et al., 2003a, b). However, it must be noted that O5:H^- STEC may be commonly recovered from the faeces of healthy and diarrhoeagenic calves (Holland et al., 1999 and references therein). O5:H^- STEC isolated from diagnostic bovine faecal samples often possess classic stx1 + eae + ehxA and typically produce urease (Brett et al., 2003a; Hall et al., 1990; Mercado et al., 2004; Hornitzky et al., 2005). To determine the virulence gene characteristics in the two O5:H^- isolates from the patient, RFLP assays were used to subtype the stx1 and eae genes as described previously (Brett et al., 2003a; Ramachandran et al., 2003). Restriction fragment polymorphism analyses using the restriction enzymes RsaI and CfoI showed that both isolates possess a classic stx1 subtype (data not shown). Restriction fragment polymorphism analysis using the restriction enzymes AluI, RsaI and CfoI showed that both serotype O5:H^- isolates possessed Int-^beta^ (data not shown).

**Conclusions**

Although the role of STEC as causative agents of haemolytic uraemic syndrome (HUS) is well established, their role in diarrhoea is less well documented. A review of the literature suggests the presence of two distinct pathotypes of O5:H^-.

The ovine-derived clone (pathotype O) typically possesses stx1, stx2 (specifically stx2d/OX3/O111 subtypes) + ehxA, does not possess intimin (Brett et al., 2003a; Ramachandran et al., 2001) and is prevalent in the faeces of healthy, slaughter-age sheep (Djordjevic et al., 2001, 2004; Koch et al., 2001) and was the causative agent of a case of HUS in Australia (Starr et al., 1998). The bovine-derived clone (pathotype B) is typically isolated from diarrhoeagenic and healthy calves and possesses stx1 + eae (Int-^beta^ subtype) + ehxA (Wieler et al., 1996; Djordjevic et al., 2004). Unlike ovine O5:H^- STEC, the bovine-derived clone rarely possesses stx2 and typically produces urease (Brett et al., 2003b; Hornitzky et al., 2005; Mercado et al., 2004; Wieler et al., 1996). Urease-producing eae^+^ STEC O5:H^- have been associated with bovine diarrhoea (Hall et al., 1990; Mercado et al., 2004), and STEC O5:H^- are recognized as diarrhoeagenic agents in calves (Hall et al., 1990; Wieler et al., 1996) and are the causative agents in this case. O5:H^- STEC with virulence gene profiles representative of both pathotypes have been recovered from humans with HUS and other diarrhoeal diseases (Brett et al., 2003a, b; Starr et al., 1998; Beutin et al., 2004).

The role of his immune status as a risk factor for the development of STEC in this patient is unknown although it is unlikely to be of relevance with normal IgA and IgM levels. Only one previous case of STEC arising in an immunocompromised individual with AIDS has been re-
ported (Spacek et al., 2004). The lack of previous reports in immunocompromised individuals supports the notion that in many circumstances the general immune status may not be a relevant factor in the development of STEC.

Further molecular genetic studies comparing animal and human STEC O5:H− isolates are required to establish the validity of the hypothesis of two pathotypes. This study demonstrates the importance of performing detailed molecular diagnostic studies on human clinical isolates, thereby providing an invaluable indication regarding a likely source of the causative agent.

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