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Clinical and pathological variability of infection by enterohaemorrhagic (Vero cytotoxin producing) *Escherichia coli*

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SUMMARY The clinical and pathological features of five sporadic cases of enteric infection caused by *Escherichia coli* 0157 (enterohaemorrhagic or Vero cytotoxin-producing *E coli* showed a range of features. These included one case with pseudomembranous colitis, one with an acute exacerbation of ulcerative colitis, and three with enterocolitis. Diagnostic difficulties encountered initially in four of the five cases were finally resolved by correlating the results of microbiological with histopathological investigations.

In view of the heterogeneity of clinical and histological signs and symptoms, it is concluded that all patients with abdominal pain and diarrhoea or rectal bleeding should have early microbiological investigation.

The first reports linking Vero cytotoxin-producing *E coli* (VTEC) (mostly of serotype 0157:H7) with haemorrhagic colitis were published in 1983 and came from North America.[1] Since then, outbreaks in institutions such as homes for the elderly[2,3] and in the community[4] have been recognised. Sporadic cases in the United Kingdom and North America have also been described.[5-9] Outbreaks have been associated with the consumption of hamburgers[3-5] and unpasteurised milk,[4] and VTEC have also been found in samples of cheese, beef, pork, poultry and lamb.[7,10]

A case control study of a community outbreak in East Anglia suggested that handling vegetables, particularly potatoes, was the important risk factor.[11] Person to person spread has also been suggested as the likely route of transmission from outbreak studies,[2,4] and there has been a report of a nurse who acquired VTEC from a patient and developed haemolytic uraemic syndrome.[12]

The association between VTEC and haemolytic uraemic syndrome has been recognised since 1983.[13] Children and occasionally adults may be affected; in adults this may take the form of thrombotic thrombocytopenic purpura,[6] which reflects the probable overlap between the two syndromes. This form of haemolytic uraemic syndrome, known as the epidemic form, has prodromal symptoms similar to those of haemorrhagic colitis[7,14-16] and usually has a good prognosis. Although none of our cases showed signs of developing this complication, it is still not clear what factors predispose to the development of haemolytic uraemic syndrome following enteric infection with VTEC.

The typical clinical history of haemorrhagic colitis is of an acute self-limiting illness presenting with lower abdominal pain and watery diarrhoea, which later becomes bloodstained and may resemble frank rectal bleeding. The absence of fever or neutrophils in the stool may help to distinguish the syndrome from bacterial dysentery or inflammatory bowel disease.[1-8,15,17] Infection with VTEC may cause a severe or even fatal illness, especially in the elderly[2] and in children.[9,14] Milder forms of disease are also likely but may not come to medical attention. Few reports of the appearance of colonic or rectal biopsy specimens have been published.[6,18,19]

Material and methods

All five cases were patients admitted as emergencies to the County Hospital, Lincoln, or the City Hospital, Aberdeen. The case histories are detailed in the table.

For pathological examination the colectomy specimen from case 1 and the biopsy specimens from

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Table *Case histories of 5 patients studied*

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*
Age and sex	47 M	62 M	27 F	23 M	63 M
Date and hospital of admission	29/11/87 City Hospital Aberdeen	14/12/87 County Hospital Lincoln	03/07/87 County Hospital Lincoln	09/10/87 City Hospital Aberdeen	09/01/88 City Hospital Aberdeen
History	Abdominal pain, watery diarrhoea	Abdominal pain, vomiting, prior weight loss and diarrhoea—two months	Abdominal pain, diarrhoea	Abdominal pain, diarrhoea	Abdominal pain, diarrhoea, rectal bleeding, general malaise
Duration of symptoms before admission	1 day	5 days	4 days	3 days	6 days
Investigations	Emergency gastrograffin enema, stool culture, sigmoidoscopy	Stool culture colonoscopy and biopsy, barium enema	Sigmoidoscopy and biopsy, stool culture	Sigmoidoscopy and biopsy, stool culture	Sigmoidoscopy and biopsy, stool culture
Progress	Diarrhoea became blood stained, worsening abdominal pain and peritonism	Grossly bloody diarrhoea	Diarrhoea became blood stained, vomiting	Diarrhoea became blood stained	Gradual recovery
Clinical impression	Fulminant ulcerative colitis	Colonic carcinoma	Distal colitis	Appendicitis	Acute enterocolitis
Final diagnosis	Pseudomembranous colitis	Acute exacerbation of ulcerative colitis	Enterocolitis	Enterocolitis	Enterocolitis
Treatment	Emergency subtotal colectomy, broad spectrum antibiotics	Intravenous fluids, Salazopyrine, steroid enemas	Supportive	Intravenous fluids, antibiotics	Intravenous fluids
Follow up	Uncomplicated postoperative recovery	Slow improvement Colonic biopsy four months later—ulcerative colitis	Well 10 days later	Well 12 days later	Well 5 days later

cases 2–5 were processed routinely and stained with haematoxylin and eosin.

For microbiological investigation faeces were inoculated on to Sorbitol MacConkey agar (Oxoid Limited CM 813) and incubated overnight at 37°C. Non-sorbitol fermenting colonies were identified using the API 20E system (API bioMérieux UK Limited) and all identified as *E coli* were tested for agglutination with *E coli* 0157 antiserum (Public Health Laboratory Service and Difco) using slide and tube techniques. The slide test entailed emulsifying six colonies individually in a loopful of antiserum on a glass slide which was rocked and observed for agglutinatiion. A suspension of organism in saline acted as a control and isolates showing positive or equivocal results were further tested in a microtitre plate. Six colonies were picked individually into 1·5 ml nutrient broth, incubated at 37°C for five hours, and then boiled for 30 minutes. Equal volumes of diluted *E coli* 0157 antiserum in phenol saline and organism suspension were added to wells to give a final volume of 200 μl and arrange of antiserum dilution from 1/32 to 1/256. Control wells were set up with equal volumes of organism suspension and phenol saline. After gentle shaking the plate was covered and incubated at 56°C overnight. Each well was then examined for agglutination. A known *E coli* 0157 stain was included in each test. All isolates showing agglutination at 1/128 or greater in test but not control wells were sent to the Division of Enteric Pathogens, Central Public Health Laboratory for confirmation of identity and Vero cytotoxin production.

Results

PATHOLOGY

Case 1

Gross examination showed discrete plaques of yellow-green friable material up to 1 cm in diameter on the mucosal surface of the caecum and ascending colon. Distal to this, there was a confluent membrane over the mucosa. The bowel wall and serosa were indurated and congested (fig 1). Microscopical examination of the proximal colon showed focal, severe inflammation around superficial parts of disrupted crypts (fig 2). A cap of mucin, fibrin, and neutrophil polymorphs in columns streamed upwards from the crypt bases. Adjacent mucosa showed minimal inflammation and oedema and no distortion of crypt architecture. The distal colon showed almost complete mucosal ulcera-

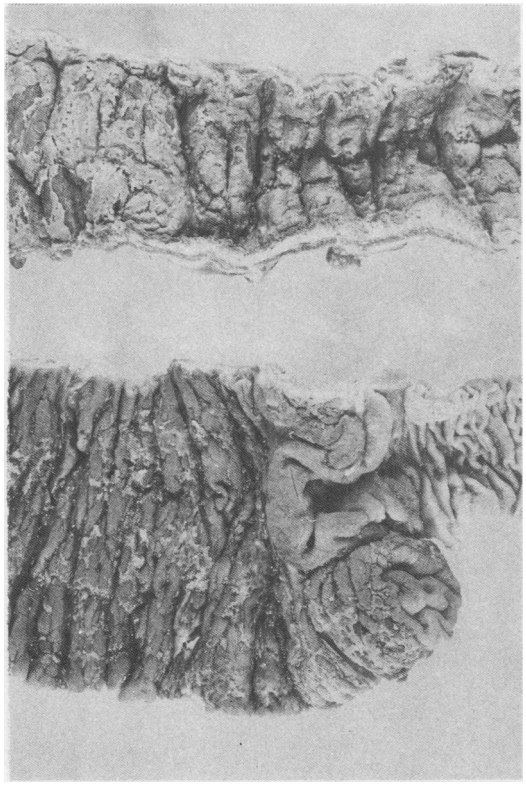

Fig 1 *Case 1: plaques over proximal colon (below); membrane over distal colon.*

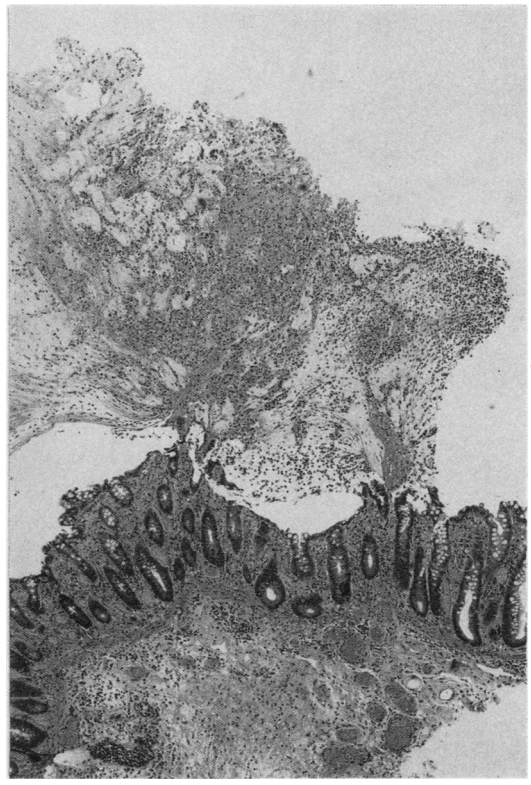

Fig 2 *Case 1: typical summit lesion of pseudomembranous colitis.*

tion covered by inflammatory slough.

The appearances in the proximal colon were typical of types 1 and 2 lesions, pathognomonic of pseudomembranous colitis, and those in the distal colon, of the type 3 lesion.[20 21]

Case 2

Colonic biopsy specimens showed glandular architecture distortion and atrophy with numerous crypt abscesses (fig 3). No granulomata were seen and there was no evidence of malignancy. A further biopsy specimen taken four months later showed similar appearances, consistent with active ulcerative colitis.

Cases 3, 4, and 5

Colonic and rectal biopsy specimens showed similar features with neutrophil polymorphs in the lamina propria, some extending into surface and crypt epithelium (fig 4). No glandular architectural abnormalities or granulomata were identified. The appearances were consistent with an acute infective aetiology. No amoebae were identified in any case.

MICROBIOLOGY

Stool cultures were performed soon after admission in each case (in the first few days of the acute illness, therefore). A Vero cytotoxin producing *E coli* 0157 was isolated from the stool in all cases. No salmonella, shigella, campylobacter, aeromonas or yersinia species were isolated. Cryptosporidium ova, cysts, and parasites were not seen. *Clostridium difficile* was not found in case 1, nor was toxin detected. In case 2 stool culture six weeks after the acute illness was negative for *E coli* 0157.

Discussion

The morphological features of VTEC infection reported to date mostly show non-specific changes of varying severity, consistent with an infective aetiology.[18] Histological changes of infective enterocolitis, as seen in our cases 3, 4, and 5, are not specific for the organism involved. Similar features can be seen in infections due to many pathogens, including shigellae.

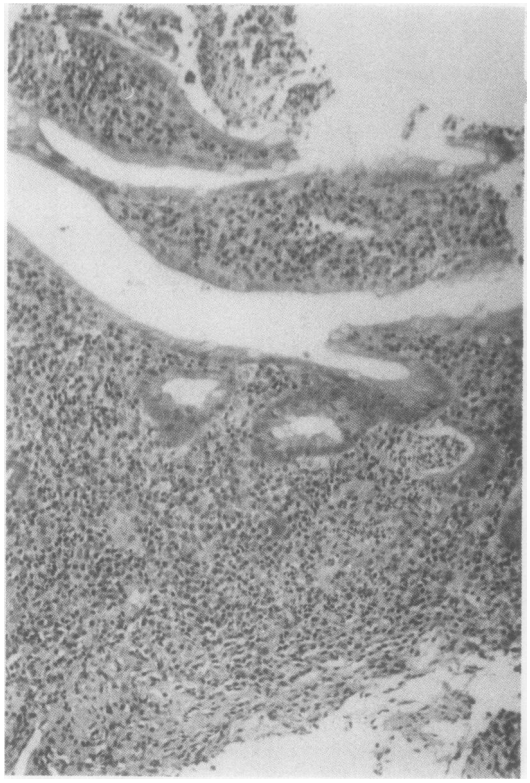

Fig 3 *Case 2: severe active ulcerative colitis.*

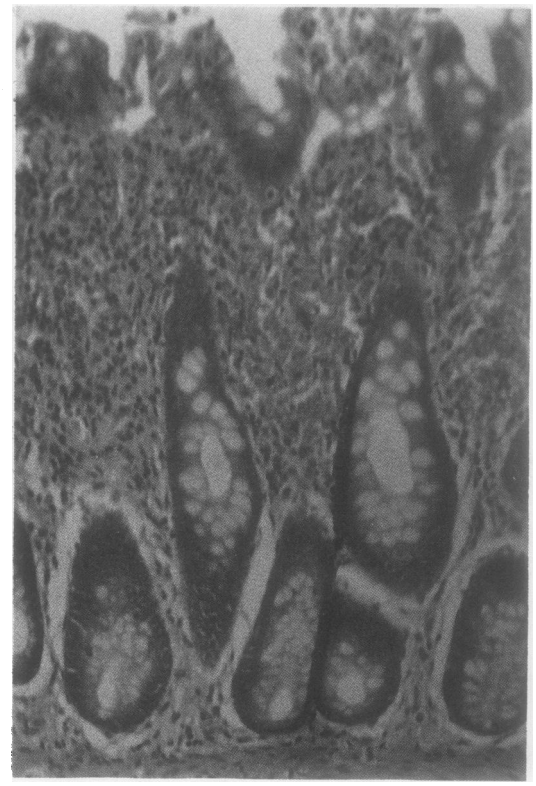

Fig 4 *Case 3 (4 and 5 similar): Mild acute inflammation consistent with infective proctitis.*

Type 2 mucosal lesions of pseudomembranous colitis, as seen in case 1, have been reported twice previously in VTEC infection.[6 19] Pseudomembranous colitis is usually associated with prior use of broad spectrum antibiotics and the isolation of *C difficile* or its toxin, but case reports exist which suggest that a range of organisms besides *C difficile* may cause pseudomembranous colitis, including other clostridia and staphylococci.[22-28]

The diagnosis of ulcerative colitis in case 2 seems well substantiated in view of the appearances of the biopsy specimen, the subsequent course, and response to treatment. Isolation of *E coli* 0157 from the stool accompanied a pronounced deterioration in the patient's state of only a few days duration; a later stool culture was negative for the organism. It would therefore seem reasonable to attribute this acute exacerbation to enteric infection.

Cases 3, 4, and 5 had similar clinical findings, which, in retrospect, are in keeping with previous descriptions of haemorrhagic colitis.[1-8 15 17] Only in case 5, however, was acute enterocolitis diagnosed initially. Case 3 was thought to have proctocolitis due to chronic inflammatory bowel disease, based on the sigmoidoscopic appearance, but recovery was complete after 10 days. A previous report describes cases of VTEC infection in one of which the endoscopic appearances were suggestive of ulcerative colitis.[18] An earlier study of five children who developed haemolytic uraemic syndrome describes their initial diagnoses as ulcerative colitis, based on sigmoidoscopic appearances.[29] In the four who had biopsies, however, the histological description was non-specific.

Case 4 had such severe abdominal pain that appendectomy was considered soon after admission. Wider knowledge of the condition of haemorrhagic colitis should lessen such diagnostic difficulties.

The importance of studying morphological patterns lies not only in diagnosis and management but also in the insights into pathogenesis that they may provide. VTEC produce at least three toxins, all cytopathic to Vero cells in culture[17]: VTI (or Shiga-like toxin I) which is indistinguishable from Shiga toxin produced by strains of *Shigella dysenteriae* type I;VT2 (or Shiga-

like toxin II), which although structurally similar to Shiga toxin is not neutralised by anti-Shiga or anti-VTI toxins; and a Shiga-like toxin II variant. The relative importance of these toxins in the pathogenesis of VTEC infections, including haemolytic uraemic syndrome, is still uncertain.

Animal experiments with VT alone have shown that there is a direct toxic effect on the absorptive epithelial cells of the intestinal villus in rabbits.[30] This may contribute to the diarrhoea observed in VTEC infection. In gnotobiotic piglets enteric infection with *E coli* 0157 causes a diarrhoeal illness, although without blood in the stool.[31] The colon shows luminal exudation and surface ulceration similar to the confluent lesions of pseudomembranous colitis seen in our case 1. Interestingly, the effects in the piglets are the same whether the strain of *E coli* 0157 used produces high or low concentrations of toxin. The mechanism of bacterial action seems to be by mucosal adherence and brush border dissolution, similar to enteropathogenic *E coli*, but then progresses until bacteria are seen to multiply in the lamina propria after destroying the surface and glandular epithelium.

It therefore seems that the bacteria directly damage the intestine in addition to producing toxins which may then have more distant effects once they have reached the bloodstream. The severity of clinical enterocolitis and the triggering factors for haemolytic uraemic syndrome probably depend not only on individual susceptibility, which may be related to age, but also on the relative proportions of toxins produced by the particular infecting organisms. Studies of haemolytic uraemic syndrome have implicated endothelial damage, possibly mediated by free radicals, reduced vascular prostacyclin production, abnormalities of platelet function, red cell haemolysis and reduced fibrinolysis.[14 32] These changes are thought to be toxin-mediated.

The changes of pseudomembranous colitis have also been attributed to the action of toxin, usually a cytopathic endotoxin produced by *C difficile* and neutralised in vitro by antitoxin to *Clostridium sordellii*.[26] One possible mechanism suggested is of local ischaemia due to capillary microthrombi in the lamina propria preceded by a local Schwartzman reaction.[20] Toxin-mediated endothelial damage would therefore be common to both pseudomembranous colitis and haemolytic uraemic syndrome (the latter being more widespread) and may explain the association of both syndromes with VTEC infection.

The clinical presentations of our five cases show considerable variation. Although each case showed some similarities to the history of haemorrhagic colitis as described in case reports, four caused diagnostic problems, at least initially. The duration of bacterial shedding may be short, particularly in adults.[5] Microbiological investigation should therefore be carried out early in all patients with abdominal pain associated with diarrhoea or rectal bleeding.

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