Attaching and effacing lesions in the intestines of an adult goat associated with natural infection with \textit{Escherichia coli} O145

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There have been few reports of attaching and effacing \textit{Escherichia coli} (AEEC) infections in goats. A severe outbreak was reported by Duhamel and others (1994) affecting neonatal goat kids, of which 21 of 34 born alive died after having diarrhoea. A further case (Duhamel and others 1992) involved a goat approximately two months old, with severe diarrhoea of three weeks' duration. This animal was part of a drug toxicity trial, which may have been an exacerbating factor. This short communication describes a naturally occurring infection with AEEC in an adult goat with diarrhoea.

Nine adult diary goats out of a herd of 300 had died over the course of a month with a history of milk drop, anorexia and weakness followed by death within a few days. The 10th animal, a three-year-old Saanen cross nanny which showed similar clinical signs, was submitted to the Veterinary Laboratories Agency (VLA) – Langford for examination. The animal was depressed, remained recumbent during clinical examination and had mild diarrhoea. Blood samples were collected for routine haematological and biochemical analysis. Haematological examination indicated dehydration, with a packed-cell volume of 0.53 litre/litre (reference range 0.4 to 0.8 litre/litre). The blood urea level was 94.7 mmol/litre (reference range 4.0 to 8.6 mmol/litre) and there was a raised creatinine kinase level of 1083 U/litre at 37°C (reference range 0 to 100 U/litre), consistent with muscle damage due to recumbency. The remaining parameters were within normal limits. The goat was euthanased by intravenous pentobarbitone sodium.

At postmortem examination the carcass weighed 77 kg and had a large amount of abdominal fat. No gross lesions were identified in the gastrointestinal tract. The rumen contained a large volume of pale straw-coloured fluid and a little roughage. The contents of the abomasum were fluid. The stomach and large intestines contained a moderate amount of fluid, the ileum and colon were mounted on organosilane-coated slides and incubated at room temperature with commercially available rabbit anti-045, anti-071 and anti-0145 antisera (Prolabs) at a dilution of 1/1000. These antisera were selected after preliminary analysis of the \textit{E. coli} isolates recovered from the goats. Goat anti-rabbit antiserum (Sigma), rabbit PAP (Dako) and diaminobenzidine were then applied sequentially. Sections incubated with normal rabbit serum (diluted 1/500 and 1/1000) and rabbit anti-0145 and anti-026 antisera were used as controls. For electron microscopic studies, a small area of the ileum was cut from the paraffin wax block and processed as described by Pearson and others (1989).

Bacteriological culture of the small and large intestinal contents produced a heavy, pure growth of coliforms. \textit{Salmonella} species were not isolated using specific culture techniques. The 10 randomly selected coliform colonies were tested using the multiplex PCR described by Meng and others (1997). Seven isolates possessed the \textit{eae} gene, encoding the 94 kDa outer membrane protein intimin, but none was positive for \textit{stx1} or \textit{stx2}, the genes encoding Shiga-like toxins. Five of the seven \textit{eae}-positive isolates were identified as \textit{E. coli} O145, one as \textit{E. coli} O45 and one as \textit{E. coli} O71. Following immunostaining, the five \textit{E. coli} O145 isolates were analysed further by the genetic methods of Cookson and others (2002) and each was found to possess genes encoding the \textit{β}-intimin subtype and CNF1 and CNF2 cytotoxins. There were no significant isolates following culture of heart blood, liver or brain. Clostridia-like organisms were not identified in Gram-stained smears of the small intestinal contents.

Histological examination of the duodenum and jejunum revealed moderate separation of the villous epithelium from the lamina propria, associated with early autolytic changes, but the villi were otherwise normal. In the ileum, severe stunting and fusion of villi, with flattening of the mucosa and an irregular epithelial surface, was observed (Fig 1). Adherent bacteria were present as multifocal colonies of variable size on the mucosal surface. In the colon, a few areas of focal, irregular surface epithelium were seen, of which were associated with adherent bacteria similar to those observed in the ileum. Examination of the kidneys revealed a marked ectasia of the distal convoluted tubules, with many of them containing oxalate casts. Sparse neutrophilic foci were present in a few tubules. Immunostained ileum and colon sections demonstrated bacterial expression of \textit{E. coli} O145 antigen (Fig 2), but \textit{E. coli} O45 and O71 staining was not identified. Electron microscopy of the ileum confirmed that these bacteria were associated with attaching-effacing (AE) lesions (Fig 3).
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In the present case, enteritis associated with AEEC was considered to be the cause of the clinical signs in the goat. It may also have played a role in the disease involving the other nine goats which had died on the same farm. The main changes in the kidneys were most likely due to prerenal kidney failure, possibly associated with dehydration (I. Roberts, personal communication). The changes in the uterus were considered to be incidental.

To the authors' knowledge, this is the first report of naturally occurring AEEC in an adult goat. Enteric colibacillosis is regarded as a disease of newborn animals (Radostits and others 1997). However, reports of diarrhoea associated with AEEC in cattles or other ruminants (Pearson and others 1989) and in experimental infections (Tominaga and others 1989) have been mentioned. Enteritis associated with AEEC has been described in goats (Duhamel and others 1992, Drolet and others 1994), presumably occurring organism on bacteriological examination, with adherent bacteria stained with O145 antiserum. Immunoperoxidase. Bar=20 μm

FIG 2: Attaching-effacing lesion in the ileum of an adult goat, with adherent bacteria stained with O145 antiserum. Immunoperoxidase. Bar=20 μm

FIG 3: Ileum of an adult goat in which bacteria are intimately attached to the enterocyte surfaces and the microvilli are effaced. Uranyl acetate and lead citrate. Bar=1 μm

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To the authors' knowledge, this is the first report of naturally occurring AEEC in an adult goat. Enteric colibacillosis is regarded as a disease of newborn animals (Radostits and others 1997). However, reports of diarrhoea associated with AEEC in cattle aged eight months (Pearson and others 1999) and one year (Wada and others 1994) indicate that older animals may be affected. The incidence of AEEC-associated diarrhoea in older animals may be higher than current evidence suggests, since demonstration of the characteristic lesions requires well-preserved intestinal tissues to be obtained at postmortem examination within a few minutes of euthanasia.

In previous reports of naturally occurring AE lesions in goats (Duhamel and others 1992, Drolet and others 1994), and in experimental infections (Tominaga and others 1989), only the large intestine was involved. Thus, the involvement of the small intestine in the present case extends the recognised distribution of naturally occurring AEEC in goats. The present report is also the first involving caprine AE lesions in which the identity of the organism involved has been confirmed on the mucosa, by immunohistochemistry.

Of the three putative AEEC serogroup isolates (O45, O71 and O145) from the affected animal, only E coli O145, the most frequently detected organism on bacteriological examination, formed AE lesions. Thus, the presence of putative AEEC in the intestines or faeces may not always equate with the presence of intimate association and AE lesion formation. It may be that the presence of one AEEC, intimately associated with the mucosa, can interfere with the adhesion of another. In the present study, it is therefore possible that E coli O145 may have excluded the adhesion of E coli O45 and O71. Alternatively, E coli O45 and O71 may have been present at intestinal sites not examined in the study.

Recently, Orden and others (2003) identified putative AEEC, that is, E coli with the genetic potential to form AE lesions, in a study of farmed ruminants from Spain. The prevalence of such AEEC in healthy animals was between 33·5 and 40·6 per cent of isolates tested, and in diarrhoeic animals the prevalence was significantly higher, between 66·7 and 100 per cent. From that study, putative goat AEEC were shown to belong to 19 different serogroups, of which O3, O55, O112, O121, O145 and O153 were detected frequently. Orden and others (2003) did not detect O71 in goats, but did detect this serogroup in healthy cattle and sheep and in diarrhoeic lambs. The AEEC O145 isolates identified by Orden and others (2003) possessed the intimin eae γ subtype, unlike the eae-β subtype harboured by the isolates described in the present study. These data suggest pathogenic diversity within AEEC of serotype O145.

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