Influence of colostrum deprivation and concurrent Cryptosporidium parvum infection on the colonization and persistence of Escherichia coli O157: H7 in young lambs

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Escherichia coli O157: H7 and Cryptosporidium parvum infections of man have been associated with direct contact with small ruminants. Colostrum protects neonates against gastrointestinal pathogens, and orphan lambs, which are common on petting farms, may be deprived of this protection. In a recent study, it was demonstrated that high shedding of E. coli O157: H7 by an 8-week-old goat kid was associated with coincidental C. parvum infection. Furthermore, both pathogens were co-located in the distal gastrointestinal tract. It was hypothesized that colostrum deprivation and pre-infection with C. parvum predisposed young ruminants to colonization and increased shedding of E. coli O157: H7. To test this, 21 lambs 5 weeks of age were divided into four groups as follows: (A) colostrum-deprived and inoculated with E. coli O157: H7, (B) colostrum-deprived and inoculated with C. parvum and then E. coli O157: H7, (C) conventionally reared and inoculated with E. coli O157: H7, (D) conventionally reared and inoculated with C. parvum and then E. coli O157: H7. C. parvum was detected between 8 and 12 days post-inoculation in most of the infected lambs. At 24 h post-inoculation with E. coli O157: H7, all lambs were shedding between $5 \times 10^4$ and $5 \times 10^7$ c.f.u. E. coli O157: H7 per gram of faeces. E. coli O157: H7 was shed in higher numbers in the groups pre-inoculated with C. parvum, whether conventionally reared or colostrum-deprived. Interestingly, for the colostrum-deprived lambs on day 3, a significant difference in shedding of E. coli O157: H7 was observed ($P = 0.038$), with the lambs inoculated with E. coli alone yielding higher counts than those pre-inoculated with C. parvum. From day 15 onwards, shedding of E. coli O157: H7 was highest from the colostrum-deprived C. parvum-infected lambs, then (in descending order of shedding) the colostrum-deprived lambs, the conventionally reared lambs infected with C. parvum, and the conventionally reared animals. In total, four animals were euthanized, two at 24 h and two at 96 h post inoculation with E. coli O157: H7 (two conventionally reared and two colostrum-deprived). All animals euthanized were from groups pre-inoculated with C. parvum prior to challenge with E. coli O157: H7. On examination of tissues, in three of the four animals examined, multifocal attaching and effacing lesions were observed in the caecum, colon, rectum and at the recto-anal junction, and were confirmed by immunohistochemistry to be associated with E. coli O157: H7.

INTRODUCTION

Two pathogens whose incidence in human disease has increased significantly over the last decade are Escherichia coli O157: H7 (Kaper et al., 2004) and Cryptosporidium parvum (Guerrant, 1997). Both pathogens are of zoonotic...
importance and have potential economic implications worldwide.

E. coli serotype O157:H7 infection has emerged as an important cause of human disease in the developed world. It was first recognized in the early 1980s to be associated with haemorrhagic colitis (HC), haemolytic-uraemic syndrome (HUS) and thrombocytopenic purpura (TCP) in man (Karmali et al., 1983; Riley et al., 1983). Human infection has been well documented (Smith & Scotland, 1993; Boyce et al., 1995; Swinbanks, 1996), and E. coli O157:H7, classified as belonging to the enterohaemorrhagic E. coli (EHEC) pathotype, is regarded worldwide as the leading cause of both HC and HUS (Paton & Paton, 1998).

Transmission of E. coli O157:H7 is faecal-oral (Pepin et al., 1997; Locking et al., 2001), with cattle considered to be the primary reservoir (Hancock et al., 1997; Zhao et al., 1995; Griffin & Tauxe, 1991), although sheep and goats are also recognized as significant reservoirs (Chapman et al., 1997; Heuvelink et al., 1998, 2002; Meng et al., 1998; Fegan & Desmarchelier, 1999). The transfer of E. coli O157:H7 among adult animals has been the subject of much research, with few firm conclusions; however, for young animals, colostrum deprivation is a risk factor for increased susceptibility to gastrointestinal pathogens (Kelleher & Lonnerdal, 2001), and specifically E. coli O157:H7 in cattle (Dean-Nystrom et al., 1997; Rugsbjerg et al., 2003), pigs (Dean-Nystrom et al., 2002) and goats (La Ragione et al., 2003b). Indeed, orphan lambs which are deprived of colostrum and bottle fed with milk replacer on petting farms are a known source of human infection (Chapman et al., 2000; Pritchard et al., 2000; Payne et al., 2003).

Intestinal cryptosporidiosis is caused by C. parvum, a widely distributed protozoan parasite, which infects wild animals and many domesticated ones, as well as humans, chiefly immunocompromised individuals (O’Donoghue, 1995; Griffiths, 1998). Since its first description in cattle (Panciera et al., 1971; Barker & Carbonell, 1974), C. parvum has been attributed an increasingly important role in neonatal diarrhoea syndrome in domestic livestock. A study by Munoz-Fernandez et al. (1996) showed C. parvum to be the most frequent aetiological agent involved in outbreaks of diarrhoea in lambs in Europe. Cryptosporidiosis is most common in young lambs (Foreyt, 1990), and may result in poor feed to weight conversion and occasionally death. During the incubation period and clinical course of the disease, the parasite proliferates mainly in the jejunum and the ileum. However, after the start of oocyst shedding, the lesions can spread to other parts of the small and the large intestine (de Graff et al., 1999).

C. parvum has been shown to be a common agent associated with diarrhoeal disease in young calves in Europe, and in a study by De la Fuente et al. (1999), mixed infections with other enteric pathogens were reported in approximately 50% of cases of diarrhoea. Although mixed infections are more commonly detected in clinically affected animals (Morin et al., 1980; Reynolds et al., 1986), the significance of concurrent infections is unclear, particularly with regard to the effect on colonization and shedding of E. coli O157:H7. Recently, an 8-week-old goat kid experimentally inoculated with E. coli O157:H7 shed high levels of the organism [10^6 c.f.u.(g faeces)^{-1}] and was found at post-mortem examination to have a concurrent C. parvum infection (La Ragione et al., 2005a). The E. coli O157:H7 attaching and effacing (AE) lesions and cryptosporidiosis were co-located on the mucosa of the large intestine. Also, a high incidence of C. parvum in orphan lambs in the UK has been reported, so that especially on petting farms, the risk to human health is increased (G. C. Pritchard, personal communication).

Given that neonates and young animals are protected from most gastrointestinal infections when suckling (Altmann & Mukkur, 1983), we wished to assess the longer-term impact of deprivation of colostrum and ewes’ milk upon colonization of lambs beyond weaning age with E. coli O157:H7. Additionally, we wished to assess the potential contribution of pre-infection with C. parvum in the lamb model used in these studies.

**METHODS**

**Bacteria, parasites and inocula.** A derivative of E. coli O157:H7 strain NCTC 12900 that does not possess either stx1 or stx2 verocytotoxin genes, but is eae positive (National Collection of Type Cultures, Health Protection Agency, Colindale, UK), was made resistant to nalidixic acid (Sigma) at 15 μg ml^{-1} by passage on complex medium supplemented with the antibiotic, and designated E. coli O157:H7 strain NCTC 12900 NaI (Best et al., 2005; La Ragione et al., 2005a).

Strain NCTC 12900 NaI was stored in heart infusion broth (HB; Oxoid) medium supplemented with 30% (w/v) glycerol on beads at −80 °C, and working stocks were stored at room temperature on Dorset’s egg medium.

NCTC 12900 NaI was streaked from Dorset’s egg medium onto SMAC plates (Oxoid) containing 15 μg nalidixic acid ml^{-1}, and well-isolated colonies were inoculated separately into 100 ml aliquots of LB broth (Oxoid) in 250 ml conical flasks. After incubation for 16 h at 37 °C with gentle agitation, the bacterial cells were harvested by centrifugation (3000 g for 10 min) and resuspended in PBS (0.1 M, pH 7.4). The bacterial suspensions contained approximately 1×10^8 c.f.u. ml^{-1}, as determined by serial dilution and triplicate plating on SMAC plates.

The C. parvum isolate used in these studies was from a field case of diarrhoea in a calf, confirmed by standard Veterinary Laboratories Agency (VLA) testing protocols to have been associated with C. parvum. The inocula employed to dose lambs were made directly from the faeces of this calf by resuspension in PBS (0.1 M, pH 7–4) and centrifugation to remove faecal debris. Oocysts were suspended in PBS at 1×10^8 oocysts ml^{-1} and stored at 4°C until required.

**Animals.** Nine cross-bred lambs from multiple births (leaving at least one lamb with its mother) were separated from their mothers immediately after birth and placed into two groups of three and six animals, respectively (groups A and B). These animals were bottle fed with milk replacer. A further 12 neonatal lambs were allowed to suckle, and after weaning at approximately 4 weeks of age, were separated from their mothers and placed in two groups (C and D).
of five and seven animals, respectively. Each animal was identified with duplicate ear tags encoding a unique four-digit identification number. The animals were housed indoors in groups which allowed oral-faecal contact, and were provided with standard rations and water ad libitum. At 4 weeks of age and prior to inoculation with E. coli O157:H7 or C. parvum, faeces were taken per rectum from each animal and cultured for E. coli O157:H7. The same samples were tested for cryptosporidia by a modified Ziehl–Neelsen (mZN) method and by the indirect fluorescent antibody test (IFAT). At 5 weeks of age, the lambs in groups B and D were dosed orally with 5 × 10^6 and 1 × 10^7 cryptosporidia oocysts, respectively. At 6 weeks of age all groups (A–D) were dosed orally with 1 × 10^6 c.f.u. E. coli O157:H7. All inocula were delivered in a 10 ml volume using a worming gun (Novartis Animal Health) ensuring that the whole inoculum was delivered directly to the pharynx. Faecal samples were taken per rectum by digital insertion. Lambs euthanized for necropsy and at the end of the study (day 39) were euthanized by intravenous barbiturate overdose. All procedures were conducted under the jurisdiction of Home Office licence 70/5441 granted under the Animals (Scientific Procedures) Act (1986).

**Preparation and necropsy procedures for animals studied post-mortem.** Two colostrum-deprived lambs [tagged 1458 (lamb 1) and 1470 (lamb 2), both group B] and two conventionally reared ('conventional') lambs [tagged 1486 (lamb 3) and 1489 (lamb 4), both group D] that had been challenged with both C. parvum and E. coli O157:H7 were euthanized and necropsed as described previously (Wales et al., 2001a, b). Briefly, one lamb from each group was examined 24 h (1 and 3) and 96 h (2 and 4) after challenge with E. coli O157:H7. These animals were euthanized with a barbiturate overdose, and tissue samples were collected immediately thereafter. Tissue samples were collected from the rumen, duodenum, jejunum, ileum, caecum, ascending colon, spiral colon and recto-anal junction (RAJ), and from six sites excised at approximately 2 cm intervals measured from the RAJ along the rectum toward the distal colon, as described previously (Wales et al., 2001a).

**Bacteriological examination.** Faecal samples taken prior to oral inoculation were examined for the presence of E. coli O157:H7 by previously described methods (Wales et al., 2001a, b; 2005; Woodward et al., 2003). Briefly, faeces (1 g) was resuspended in 9 ml buffered peptone water (BPW; Oxoid), incubated at 37 °C for 6 h statically, and E. coli O157:H7 organisms were recovered by O157-specific immunomagnetic separation (IMS; Dynal) and plated onto CT-SMAC plates (Oxoid).

To detect the inoculated strains, previously described methods were followed (Wales et al., 2001a, b; 2005; Woodward et al., 2003; La Ragione et al., 2005a). Briefly, faeces (1 g) was resuspended in 9 ml BPW by vortexing, and serial dilutions were plated directly onto SMAC plates containing 15 μg nalidixic acid ml⁻¹. Additionally, dilutions were retained overnight at 4 °C and, if there were no direct counts of confirmed E. coli O157:H7, the highest dilution was incubated at 37 °C for 6 h and samples were then plated on SMAC plates containing 15 μg nalidixic acid ml⁻¹. The serogroup of bacteria recovered by these processes was verified by E. coli O157-specific latex agglutination (Oxoid). Tissue samples were processed as described above.

**Parasitological studies.** Tissues and faeces were examined for the presence of cryptosporidia by both the mZN method and the IFAT. For the mZN stain, the methods were essentially as described previously (Henriksen & Pohlenz, 1981). Briefly, a smear of fresh faeces or a deep tissue scrape was prepared on a microscope slide. Fixed slides were then immersed in cold carbol fuchsin, rinsed in water, decolourized in 5 % sulphuric acid, and rinsed in water. The preparation was then counter-stained in 5 % malachite green, rinsed in tap water, air-dried, and examined at a magnification of ×400. For the IFAT, a fresh faecal sample or deep tissue scrape was smeared within the well of a 4-well microscope slide, fixed by carefully applying 50 μl methanol to each well, and then allowed to air dry. Once dry, 30 μl anti-Cryptosporidium immunofluorescent antibody (IFA) (TCS Water Biosciences) was applied, and the slide placed in a humidified staining chamber and incubated for 45 min at 37 °C. Excess IFA stain was carefully aspirated from each well and replaced with distilled water for approximately 1 min. Excess water was aspirated and the smear air-dried. Once dry, 20 μl UV-compatible mounting fluid was added and a cover slip placed over the preparation. The cover slip was then sealed with DPX mountant. Preparations were examined using a fluorescence microscope fitted with a 450–490 nm excitation filter and 20 × or 40 × objectives. C. parvum was identified as apple-green epifluorescent, slightly ovoid to spherical oocysts, 4–6 μm in diameter.

**Pathological studies**

**Light microscopy.** At necropsy, tissues were placed immediately into 10 % neutral buffered formalin at room temperature and left to fix for at least 24 h. Trimed tissues were processed routinely to paraffin wax. Sections were cut at 4 μm and stained with haematoxylin and eosin (HE). Selected tissues were also stained using the Giemsa method.

**Immunohistochemistry.** Essentially, methods were as described previously (La Ragione et al., 2005a; Wales et al., 2001b). Briefly, tissue blocks were fixed in 10 % neutral buffered formalin, processed to wax and sectioned at 4 μm. Sections were rehydrated prior to assembly into the Shandon QuanSera staining system. Slides were washed with 2 × sodium chloride Tris-buffered saline [0.005 M Tris-buffered saline (TBS), pH 7.6, 1:7 % NaCl] before incubation at room temperature with normal goat serum (Vector Laboratories). E. coli O157:H7-specific polyclonal antibody (VLA, Weybridge), was then applied (1:1000 and 1:5000 diluted in 2 % normal goat serum). E. coli antigens were visualized following incubation with biotinylated goat anti-rabbit IgG. Sections were counter-stained in Meyer's haematoxylin.

**Transmission electron microscopy.** Essentially, methods were as described previously (La Ragione et al., 2005a). Briefly, glutaraldehyde-fixed tissues were sectioned at 1–2 μm in thickness. Tissues were then washed in 0–1 M phosphate buffer, post-fixed in 1 % osmium tetroxide, dehydrated by immersion in a series of alcohol solutions increasing to 100 % alcohol, and placed in propylene oxide prior to embedding in araldite resin. The resin was polymerized at 60 °C for 48 h. Ultrathin sections at 70–90 nm thickness were prepared on copper grids and stained with uranyl acetate and lead citrate.

**Confocal microscopy.** After de-waxing and rehydration, tissues were placed in PBS prior to preparation for examination by confocal microscopy. Briefly, tissues were permeabized in PBS containing 0·1 % Triton X-100, followed by detection of E. coli using a FITC-labelled affinity-purified antibody to E. coli O157:H7 produced in goats. The sections were then washed thoroughly using PBS and mounted in Vectashield containing 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories). Images of the FITC-labelled E. coli were obtained by confocal laser scanning microscopy using a Leica TCS SP2 AOBS confocal system attached to a Leica DM IRE2 microscope equipped with Ar-Kr laser excitation (488 nm) and a diode laser (405 nm). An oil-immersion objective lens (×63, numerical aperture 1·32) was used, and imaging parameters were selected to optimize resolution.

**Statistical analyses.** The sensitivity of detection by direct plating was approximately 500 c.f.u.(g faeces)⁻¹. Samples positive by enrichment were considered to have up to 500 c.f.u.(g faeces)⁻¹, and those samples in which no organisms were detected were given an
arbitrary value of 1 to avoid the use of a zero value giving results to infinity. t tests were used to compare the mean counts transformed to log_{10}(count + 1) at each time point.

RESULTS

Clinical findings

All animals in all study groups remained clinically normal throughout the experiment, with no evidence of pyrexia or diarrhoea. However, a change in faecal consistency was observed for the animals in groups B and D challenged with *C. parvum* between 5 and 8 days after oral dosing, whereby the faeces became smooth and sticky in consistency (*E. coli* O157 : H7 inoculated at day 7).

Faecal shedding of *C. parvum*

Prior to experimental inoculation with *C. parvum*, all animals were confirmed to be free from *C. parvum* by analysis of faeces by mZN and IFAT. In both the conventional and colostrum-deprived lambs, *C. parvum* was detected in the faeces 24 h after inoculation in all animals except 1493 (group D), which remained negative throughout the study. In addition, *C. parvum* was only detected on day 1 post-inoculation in lamb 1487 (group D). In all other lambs, the organism persisted for between 8 and 12 days, with the exceptions of lambs 1492 and 1496 (both group D), in which *C. parvum* was detected in the faeces 39 days post-inoculation.

Faecal shedding of *E. coli O157 : H7*

All faeces samples collected from the animals prior to the experimental procedure were negative for *E. coli* O157 : H7, as assessed by IMS.

The individual animal faecal-shedding data are shown in Fig. 1. At 24 h after oral inoculation, only two animals (1487 and 1492, both group D) were not shedding *E. coli* O157 : H7, although by 72 h after oral inoculation, all

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![Fig. 1. Persistence of *E. coli* O157 : H7 NCTC12900 Na+ as assessed by faecal shedding in 6-week-old lambs. (A) Colostrum-deprived and inoculated with *E. coli* O157 : H7, (B) colostrum-deprived and inoculated with *C. parvum* and then *E. coli* O157 : H7, (C) conventional and inoculated with *E. coli* O157 : H7, (D) conventional and inoculated with *C. parvum* and then *E. coli* O157 : H7.](image-url)
animals in all groups were shedding in the range $10^3$–$10^8$ c.f.u. (g faeces)$^{-1}$.

For the colostrum-deprived lambs (groups A and B) on day 3, a significant difference in shedding was observed ($P=0.038$) in the counts of *E. coli* O157:H7, whereby the counts from the lambs inoculated with *E. coli* O157:H7 alone were higher than those from lambs pre-inoculated with *C. parvum*. Thereafter, the *E. coli* O157:H7 counts were consistently lower for the lambs inoculated with *E. coli* O157:H7 alone compared to those for lambs pre-inoculated with *C. parvum*.

For the conventional lambs (groups C and D) on day 3, a significant difference in shedding ($P=0.019$) was observed, with lambs inoculated with *E. coli* O157:H7 alone having higher counts than those pre-inoculated with *C. parvum*. Thereafter, the *E. coli* O157:H7 counts were consistently lower for the lambs inoculated with *E. coli* O157:H7 alone compared to those from lambs pre-inoculated with *C. parvum*.

From day 15 onward, the numbers of animals shedding in each group were low, and the statistical analysis indicated that the differences between the groups were not significant. However, the shedding frequency at and beyond day 15 (expressed as the number of positive faecal samples over the number of faecal samples examined per test group) was 0.16 for group C (conventional), 0.33 for group D (conventional plus *C. parvum*), 0.46 for group A (colostrum-deprived) and 0.625 for group B (colostrum-deprived plus *C. parvum*).

**Post-mortem findings**

**Bacteriological and parasitological findings.** There were differences in the numbers of *E. coli* O157:H7 strain NCTC 12900 NaI recovered from tissues in individual animals (Fig. 2). *E. coli* O157:H7 was not detected in the duodenum of any animal examined, or the jejunum of animals 1 (group B), 3 and 4 (group D). *E. coli* O157:H7 was only recovered from the ileum and rumen of animals 2 and 3. By contrast, in the large intestine, *E. coli* O157:H7 was detected at all sites examined in all four animals, and in high numbers [$5 \times 10^6$ c.f.u. (g tissue)$^{-1}$] in tissues from the rectum and spiral colon of animal 2 (group B). The *E. coli* O157:H7 counts for the caecum of animal 1 (group B) were particularly high [$1 \times 10^7$ c.f.u. (g tissue)$^{-1}$] and numerous AE lesions were observed in these tissues (see below). The overall numbers of *E. coli* O157:H7 recovered from the GIT were notably higher (by

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Fig. 2. Counts of *E. coli* O157:H7 NCTC 12900 NaI from tissues collected post-mortem from the GIT of lambs 1 and 3 (day 1) and lambs 2 and 4 (day 4) after oral inoculation with *E. coli* O157:H7. A. colon, ascending colon. S. colon, spiral colon.
approximately two orders of magnitude) in the colostrum-deprived lambs compared to the conventional lambs.

In lamb 1 (colostrum-deprived plus C. parvum, group B), necropsied 24 h after E. coli O157 : H7 inoculation, cryptosporidia were detected in the rumen, duodenum, jejunum, ileum, caecum, ascending colon, spiral colon and proximal rectum. Cryptosporidia were found in the rumen, duodenum and spiral colon of lamb 3 (conventional plus C. parvum, group D) necropsied 24 h after E. coli infection. Cryptosporidia were not detected in tissues collected at necropsy from animals 2 (colostrum-deprived plus C. parvum, group B) and 4 (conventional plus C. parvum, group D) necropsied 96 h after E. coli infection.

Gross and histopathological findings. Gross pathological changes were not observed post-mortem in any of the four animals examined. On histopathological examination, significant changes were confined to the caecum, rectum and RAJ of animals 1 (colostrum-deprived plus C. parvum, 24 h, group B), 2 [colostrum-deprived plus C. parvum, 96 h post-infection (p.i.), group B] and 4 (conventional plus C. parvum, 96 h p.i., group D). Most of the mucosa at all sites appeared normal. However, where scattered foci of closely adherent bacteria were seen in the caecum, rectum (Fig. 3) and RAJ, some epithelial cells associated with bacteria had rounded up. AE lesions were not identified in animal 3 (conventional plus C. parvum, group D, examined 24 h p.i.); however, E. coli O157 : H7 cells were identified by immunohistochemistry (IHC) in the lumen of the caecum and rectum of this animal. Cryptosporidia were not observed on the mucosal surface in any animal.

Sections of small intestine (duodenum, jejunum and ileum) were less well preserved than the large intestine, and some artefactual separation of the epithelium had occurred. C. parvum was only identified in the ileum of animal 1 (colostrum-deprived, 24 h, group B) (Fig. 5).

Small numbers of coccidia were identified in the ascending colon of animal 3 (conventional plus C. parvum, 24 h, group D). Abnormalities were not detected in the rumen of any animal.

Immunohistochemistry and confocal microscopy. Multifocal, variable-sized colonies of E. coli O157 : H7 organisms were identified in three of the four animals examined (1, 2 and 4) (Figs 3 and 5). E. coli O157 : H7 colonies were identified most frequently in the rectum, although E. coli O157 : H7 colonies were also identified in the caecum and RAJ. Lesions tended to involve one, two or up to several adjacent epithelial cells. Lesions identified in the mucosa at the terminal rectum were found on
both lymphoid-associated and non-lymphoid-associated epithelium.

Transmission electron microscopy. AE lesions associated with bacteria were confirmed on the intestinal mucosa (Fig. 6). Cryptosporidia were observed in the ileum of one animal (1, colostrum-deprived plus C. parvum, group B) (Fig. 7), confirming the light-microscopic findings.

DISCUSSION

It is established that E. coli O157 : H7 colonizes the intestinal tract of ruminants, often leading to persistent infection (Cray & Moon, 1995; Brown et al., 1997; Dean-Nystrom et al., 1999; Woodward et al., 2003; La Ragione et al., 2005a; Cookson et al., 2002). In a conventional 6-week-old lamb model, it has been previously reported that the intimin of E. coli O157 : H7 is a significant factor for colonization and persistence, but that AE lesions, for which intimin is essential, are small and extremely sparse in infected animals (Woodward et al., 2003). Interestingly, in the present study, the frequency, density and distribution of E. coli O157 : H7-induced AE lesions observed in the animals was higher than previously reported.

We have shown previously (Wales et al., 2001b; Woodward et al., 2003) that in conventional lambs of this age, faecal shedding of E. coli O157 : H7 after oral inoculation declines gradually from 10^7 to 10^3 c.f.u. (g faeces)^−1 over the first 10–14 days, and thereafter faecal shedding is intermittent. Similar findings were found in the present study for early sampling times (days 1, 3, 8 and 11 or 12). The experimental differences between this study and others that may have contributed to the observed outcomes were the deprivation of colostrum and ewes’ milk, and the pre-inoculation with C. parvum.

It has been demonstrated in neonatal calves that colostrum is highly protective against E. coli O157 : H7 challenge and that AE lesions are readily induced in the distal GIT if colostrum is withheld (Dean-Nystrom et al., 1997). In conventional, 6-day-old lambs, it has been shown that small and sparse AE lesions are induced by E. coli O157 : H7 (Wales et al., 2001b). Given these data, it seems reasonable to assume that neonatal lambs deprived of colostrum would be more susceptible to colonization by E. coli O157 : H7 and extensive AE lesion formation. Of concern, and relevant to this study, was the issue of longer-term sequelae for lambs deprived of colostrum and ewes’ milk, such as the hand-reared orphan lambs that are frequently found on petting farms. The lamb model used in this study therefore attempted to mimic this scenario. The experimental evidence indicated that E. coli O157 : H7-induced AE lesions were detected readily in the distal GIT of lambs deprived of colostrum and ewes’ milk, and that faecal shedding of E. coli O157 : H7 organisms was greater than for conventional animals of the same age. By comparison, in a previous study, in which 6-week-old conventional lambs were challenged with the same E. coli O157 : H7 strain, and in which a similar number of tissues were examined (Woodward et al., 2003), only two lesions were identified, in a single animal, one in the caecum and one in the rectum. The present data support the concept that colostrum is essential for the longer-term health of the GIT, even beyond weaning (Butler, 1979; Logan et al., 1974).

Following the incidental observation of high faecal shedding of E. coli O157 : H7 and readily identified multifocal AE lesions associated with C. parvum in the large intestine of an experimentally orally inoculated 8-week-old goat kid (La Ragione et al., 2005a), this raised the question of whether C. parvum has an effect on E. coli O157 : H7 colonization and shedding in the lamb model used in this study. Concurrent infections with two or more enteropathogens, including cryptosporidia in association with E. coli, in naturally infected diarrhoeic and asymptomatic calves and goats, have been described previously (Moon et al., 1978; Janke et al., 1990; de la Fuente et al., 1999; Gunning et al., 2005).

Fig. 6. Lamb 4 (conventional plus C. parvum, group D) 4 days p.i. with E. coli O157 : H7, rectum. AE E. coli (arrows) are attached to the surface of epithelial cells. Transmission electron micrograph; bar, 0.5 μm.

Fig. 7. Lamb 1 (colostrum-deprived plus C. parvum, group B) 1 day p.i. with E. coli O157 : H7, ileum. Cryptosporidia (arrows) are attached to the surface of epithelial cells. Transmission electron micrograph; bar, 1 μm.
2001). In natural and experimental infections in goats and
lambs, C. parvum causes severe clinical disease, often with
high morbidity and mortality (Tzipori et al., 1981; Angus
et al., 1982; Ortega-Mora et al., 1993; Koudela & Bokova,
1997; Johnson et al., 1999; Castro-Hermida et al., 2002), and
severe lesions are often induced in the posterior jejunum and
ileum (Koudela & Jiri, 1997). However, asymptomatic
carriage of cryptosporidia has been described in adult goats
(Noordeen et al., 2002; La Ragione et al., 2005a). In the
present study, lambs challenged with C. parvum remained
asymptomatic, but two animals shed cryptosporidia for up
to 39 days post-inoculation. On microscopical examination
of the four lambs examined post-mortem, cryptosporidia
were only found associated with the mucosa of the ileum
in one lamb, and not in the large intestine where E. coli
O157: H7-induced AE lesions were detected. It is well
documented that the formation of E. coli O157: H7 AE
lesions is the result of actin polymerization (Shaner et al.,
2005; Knutton et al., 1989), and similarly C. parvum utilizes
actin polymerization to initiate infection (Elliott & Clark,
2000; Elliott et al., 2001). This may explain the opportunistic
co-localization that has been described previously between
E. coli O157: H7 and C. parvum in a goat (La Ragione et al.,
2005a). The absence of cryptosporidia in the large intestine
may indicate that the model used in this study was not
appropriate, especially with regard to the timing of bolus
infection with E. coli O157: H7, to reproduce the incidental
finding observed in the goat study (La Ragione et al., 2005a).
However, the relative frequency with which O157: H7-
induced AE lesions were observed in one conventional
animal inoculated with C. parvum prior to E. coli O157: H7
in this study may indicate a predisposition within that
animal to colonization by E. coli O157: H7.

The shedding profiles of E. coli O157: H7 NCTC 12900,
irrespective of the study group, were broadly similar to each
other, and consistent with shedding profiles shown in pre-
vious studies (Wales et al., 2001b). The numbers of E. coli
O157: H7 organisms declined over the first 10–14 days after
inoculation, followed by intermittent shedding thereafter
for up to 18 days. With the exception of two time points
soon after oral inoculation (day 3), at which the lambs
inoculated with E. coli O157:H7 alone shed more E. coli
O157: H7 than those pre-dosed with C. parvum, there were
no statistically significant differences between groups.
However, a trend was shown which suggested that colostrum
deprivation and prior C. parvum challenge increased the
number of E. coli O157: H7-positive faecal samples com-
pared to the conventional animals. The duration of E. coli
O157: H7 shedding, however, was not increased. It may be
hypothesized that the cellular immune responses evoked by
C. parvum act in the early stages of E. coli O157: H7
infection, hence the observation of lower initial shedding of
E. coli O157: H7 in the C. parvum pre-dosed groups.

Recent studies in cattle reported by Naylor et al. (2003)
suggest a preferred site of colonization and AE lesion
formation by E. coli O157: H7 to be the terminal rectum.
Bach et al. (2005) have linked this observation with a ‘super
shredder’ status, such that these may act as sentinel animals
within a herd. Sheng et al. (2004) have confirmed the
tropism for E. coli O157: H7 colonization of the terminal
rectum of cattle by rectal inoculation studies. These authors
however, do not cite any data on the colonization of sheep
following rectal administration of the inoculum. In the
present study, the E. coli O157: H7-induced AE lesions were
not associated specifically with lymphoid tissue in the ter-
ninal rectum, as has been described for cattle (Naylor et al.,
2003). Therefore, data from this and previous studies (Wales
et al., 2001b, 2005) suggest that there are significant differ-
ences between cattle and sheep with regard to tissue tropism
during colonization.

Collectively, the data produced in this study suggest that
lambs are predisposed to E. coli O157: H7 colonization by
colostrum deprivation, but the effects of concurrent infec-
tion with C. parvum are less clear. The number of animals
in the study was a limiting factor, and trends, rather than
statistically confirmed differences between treatment
groups, were observed. These trends need to be examined
further before firm conclusions can be drawn. However,
the potential public health implications of the findings of
this study are of particular importance. Lambs on petting
farms are frequently orphans which may not have received
adequate colostrum (La Ragione et al., 2005b). A con-
sequence may be greater susceptibility to infection with
C. parvum, E. coli O157: H7 or other gastrointestinal patho-
gen. It is widely accepted that ruminants are the primary
source of E. coli O157: H7 and C. parvum infections for
humans, and so a greater understanding of the relationship
between these two enteropathogens may lead to the develop-
ment of effective intervention strategies.

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